www.koreaphysiol.org
ISSN 1226-4512

The Korean Physiological Society

Physiology

The Stem of Life Sciences

KPS 2015

Oct, 21~23
Pusan National University, Yangsan

• Pflügers Archiv Symposium
2015. 10. 21
Oceanus Arthall - Haeundae, Busan

• KPS-PSJ Joint Symposium
2015. 10. 22
Moam Hall, Pusan National University Hospital

The 67th Annual Meeting of The Korean Physiological Society

Physiology - The Stem of Life Sciences
The Korean Physiological Society

The 67th Annual Meeting of The Korean Physiological Society

Pflügers Archiv Symposium

Cardiovascular physiology: Application and Translation

October 21 , 2015
OCEANUS ARTHALL Haeundae, Busan

Korean Physiological Society Pflügers Archiv - European Journal of Physiology College of Medicine, Busan Paik Hospital, Cardiovascular Metabolic Disease Center, Inje University, Korean Society for Ion Channel Research Ischemic/Hypoxic Disease Institute, Seoul National University

ISSN 1226-4512
Volume 19, Supplement I

THE KOREAN JOURNAL OF

Physiology & Pharmacology

The 67th Annual Meeting of The Korean Physiological Society

21-23 | 10 | 2015 KPS 2015
Pusan National University, Yangsan

Physiology
The Stem of Life Sciences

October 2015

Official Journal of
The Korean Physiological Society and
The Korean Society of Pharmacology

The Korean Journal of

Physiology & Pharmacology *(Korean J Physiol Pharmacol)*

http://www.kjpp.net/
ISSN 1226-4512(Print)
ISSN 2093-3827(Online)

An Official Publication Founded in 1997
The Korean Physiological Society and The Korean Society of Pharmacology

Scope and Policy

The Korean Journal of Physiology and Pharmacology (Korean J Physiol Pharmacol, KJPP) is the official journal of both the Korean Physiological Society and the Korean Society of Pharmacology. The journal is published bi-monthly in English. Submission of any original paper in the physiological and pharmacological sciences and on the interactions of chemicals with biological systems is invited. KJPP does not publish work on the actions of crude biological extracts of unknown chemical composition (e.g. unpurified and unvalidated) or unknown concentration. All papers accepted for publication in KJPP will appear simultaneously in the print Journal and online.

This Journal is Indexed in

- SCI-extented (SCI-E) Journal listed by ISI.
- PubMed (www.ncbi.nlm.nih.gov/sites/entrez), Pub Med Central (www.ncbi.nlm.nih.gov/pmc) by NLM, SCOPUS (www.scopus.com), and KoreaMed (www.koreamed.org) and KoMCI (www.komci.org) by KAMJE.

All communications should be addressed to:

The Editorial Office and the Publisher

- Physiology 1209, 14 Teheran-ro 83-gil, Gangnam-gu, Seoul 06169, Korea.
  Tel: 82-2-568-8026, Fax: 82-2-568-8051, E-mail: physiology@koreaphysiol.org
- Pharmacology 208, Hyunil TowerOfficetel, 87, Seongmisan-ro, Mapo-gu, Seoul 03978, Korea.
  Tel: 82-2-326-0370, Fax: 82-2-326-0371, E-mail: head@kosphar.org

Published Bimonthly Annual Institutional Subscription Rate: U.S. $50.00. Personal Subscription Rate: U.S. $30.00. Prices include postage and insurance and are subject to change without notice. Circulation number of print copies is 350 per issue.

Printed on acid-free paper effective with Volume 19, Supplement I, 2015.
Printed by MEDrang Inc. (Tel. 82-2-325-2093, Fax. 82-2-325-2095, E-mail. info@medrang.co.kr)

This journal was supported by the Korean Federation of Science and Technology Societies (KOFST) Grant funded by the Korean Government.

2015 대한생리학회 임원명단

고 문 강두희 강복순 김광진 김기순 김기환 김명석 김용근 김우겸 김 전
김종규 김종환 김중수 남숙현 민병일 박양생 박춘식 박형진 배선호
문창현 신홍기 양일석 엄대용 엄융의 윤평진 이상호 이석강 이승일
이원정 이종흔 이진옥 이중우 조경우 채의업 하종식 홍승길

자문 위원 나흥식 박재식 박병림 방효원 서창국 이종은 조양혁

회 장 박재식 **차기 회장** 박병림

이 사 장 나흥식 **차기이사장** 방효원

기금위원장 조양혁 **총무 이사** 박중진

교육 이사 안덕선 **정보 이사** 전제열

국제 이사 전병화 **기획 이사** 한 진

기획 위원 김재호 박규상 송대규 우선희
이무열 이은희 전주홍

간행 이사 강동묵 **학술 이사** 김성준

부편집장 김상정 김성준 안동국 이지희 **학술 위원** 곽효범 강동묵 박규상 배영민
오석배 우선희 임채헌 진영호

이 사 강다원 강동묵 강봉균 강창원 공인덕 곽지연 구용숙 권성춘 권혁일
김경년 김동욱 김민선 김보경 김상정 김선희 김성주 김성준 김세훈
김양인 김영미 김용운 김원재 김의용 김재호 김정훈 김종연 김진혁
김창주 김형찬 나승열 나창수 나흥식 남택상 류판동 박경표 박규상
박명규 박병림 박사훈 박소라 박원균 박재식 박종성 박지호 박진봉
방효원 배영민 배재훈 배혜란 백은주 서덕준 서상원 서석효 서인석
서창국 송대규 신동민 신형철 안덕선 안도환 안동국 양훈모 연동수
염재범 염철호 오석배 오우택 우재석 윤신희 윤영욱 이경림 이덕주
이무열 이배환 이상목 이상진 이석호 이수환 이영만 이영호 이윤렬
이장헌 이종은 이지희 이호섭 임인자 임중우 임채헌 장석종 장연진
전병화 전양숙 전제열 정동근 정성우 정승준 정진섭 정창섭 정한성
조성일 조양혁 조영욱 천상우 최장규 한 진 한상준 한인옥 한재희
한호재 한희철 호원경 홍성근

감 사 우선희 임인자

Acknowledgement

Supported by

This work was supported by the Korean Federation of Science and Technology Societies (KOFST) grant funded by the Korean Government,
PNU BK21Plus Biomedical Science Education Center
and Convergence Stem Cell Research Center

Supported by

Scitech Korea Inc.
Eppendorf Korea Ltd.
KOS, Inc.
Centers for Disease Control and Prevention
신한금융그룹

Contents

Invitation (초대의 글)

대한생리학회 회원 여러분, 안녕하십니까?

유난히도 무더웠던 여름이었습니다. 건강하게 잘 지내셨는지요?

그간 지난 5월 21일 경주에서 열린 제23회 기초의학학술대회를 비롯하여 분과학회 등에 참석하여 주신 회원 여러분들께 지면을 통하여 감사의 말씀을 드립니다.

오는 10월 21일(수)~23일(금)에는 부산대학교에서 제 67회 생리학회 학술대회가 개최됩니다. 기조강연, 다양한 주제의 심포지엄 그리고 포스터 세션 등 풍성한 연구 정보들이 여러분을 기다리고 있습니다.

부산과 양산에서 열리는 이번 학술대회에 적극적으로 참여함으로써, 자신들의 연구결과의 발표는 물론 동료들의 연구발표도 경청함으로써 지식정보의 공유와 함께 개인 간의 긴밀한 교제를 통하여 우정을 돈독히 하는 기회가 될 것으로 생각합니다. 부디 많이 참석하시어 풍성한 학회가 되도록 하여 주시기 바랍니다.

그리고 학술대회를 준비하기 위해 애쓰신 이사님들 그리고 부산의대 관계자 여러분을 비롯하여 도움을 주신 많은 분들께 감사 드립니다.

끝으로 회원 여러분의 건강과 가정의 행복이 함께하기를 기원합니다.

대한생리학회 회 장 **박 재 식**
대한생리학회 이사장 **나 흥 식**

Schedule (일정표)

Pflügers Archiv Symposium - Cardiovascular physiology: Application and Translation

Wednesday, October 21

부산 해운대 오션어스아트홀 (OCEANUS ARTHALL, HAEUNDAE)

Time	Contents	
09:00-11:50	Registration	
11:50-12:00	Welcome Message and Introduction	Yung E Earm
	Session 1	**Chairs:** *Tong Mook Kang, Kyu Sang Park* **Pannel:** *JinKun, Yin Hua Zhang*
12:00-12:30	Mitochondrial DNA causes spreading necrosis in the heart	*James Downey (USA)*
12:30-13:00	Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial reactive oxygen species	*Thomas Krieg (UK)*
13:00-13:15	Coffee Break	
	Session 2	**Chairs:** *Jae Ho Kim, Byeong Hwa Jeon* **Pannel:** *Dawon Kang, Nari Kim*
13:15-13:45	Enlargement of myocardial infarct size by chronic kidney disease: a novel mechanism of disruption of Akt-GSK3beta/p70S6K signaling	*Tetsuji Miura (Japan)*
13:45-14:15	Dual roles of reactive oxygen species in myocardial ischemia/reperfusion injury and protection	*Huang-Tian Yang (China)*
14:15-14:30	Coffee Break	
	Session 3	**Chairs:** *Dae Kyu Song, Sun-Hee Woo* **Pannel:** *Sung Ryul Lee, Jae Hong Ko*
14:30-15:00	Physiological roles of unconventional eNOS expressed in the smooth muscle of skeletal and pulmonary arteries	*Sung Joon Kim (Korea)*
15:00-15:30	Zinc, zinc transporters and cardioprotection	*Zhelong Xu (China)*
15:30-15:45	Coffee Break	
	Session 4	**Chairs:** *Chae Hun Leem, Eun Hui Lee* **Pannel:** *Young Min Bae, Jae Boum Youm*
15:45-16:15	The role of TRPM4 in cardiac function and excitability	*Rudi Vennekens (Belgium)*
16:15-16:45	Bicarbonate permeation through anion channels	*Min Goo Lee (Korea)*
	Session 5	**Chairs:** *Moo Yoel Lee, Suhn Hee Kim* **Pannel:** *Ju Hyun Nam, Hyun Jin Kim*
16:45-17:15	Orai1 in ER/PM junctions	*Shmuel Muallem (USA)*
17:15-17:45	Endosomal and lysosomal chloride/proton exchange by CLC proteins: surprising roles in physiology and pathology	*Thomas Jentsch (Germany)*
17:45-18:00	Coffee Break	
	Session 6	**Chairs:** *Hyoweon Bang, Suk Hyo Suh* **Pannel:** *Seung-Kyu Cha, Hyoung Kyu Kim*
18:00-18:30	A short tribute to Pflügers Archiv (The European Journal of Physiology) and a sojourn to an endothelial anion channel	*Bernd Nilius (Belgium)*
18:30-18:40	Closing Remarks	
19:00	Pflügers Dinner	

67th Annual Meeting of the Korean Physiological Society

Thusday, October 22

부산대학교병원 (양산): 모암홀 (A), 모암홀 옆 컨퍼런스 룸 (B),어린이병원 새싹홀 (C)

Time	Contents		
08:30-09:20	Registration (등록 및 포스터 설치, 4th Floor Lobby, Moam Hall)		
09:20-09:30	Opening remarks (개회사)		
09:30-11:30	Hall A	Hall B	Hall C
	Symposium I Diverse scientific approach for resolve obesity epidemic	**Poster Oral Presentation I** **O-1~O-9** abstracts selected	**Symposium II** Mechanosensation, nanoscience and optical modulation
11:30-12:30	Lunch		자문위원회 (advisory committee)
12:30-13:00			이사회 (Board members meeting)
13:00-13:30	Poster session (Lobby)		
13:30-14:45		Hall B **Poster Oral Presentation II** **O-10~O-15** abstracts selected	
15:00-17:00	Hall A	Hall B	Hall C
	Symposium III Model-based analysis of physiological system (KPS-PSJ Joint Symposium)	**Symposium IV** Exercise physiology	**Symposium V** Stem Cell Physiology
17:10-18:00	Hall A Plenary Lecture *Keiichi Fukuda (Keio University)*		
18:15-20:30	Official dinner (학생식당)		

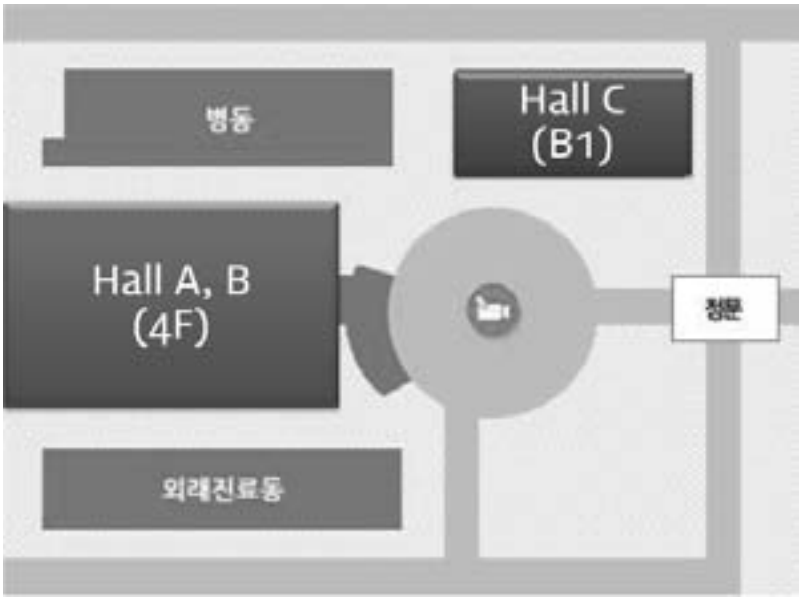
Friday, October 23

부산대학교병원 (양산): 모암홀 (A), 모암홀 옆 컨퍼런스 룸 (B),어린이병원 새싹홀 (C)

Time	Contents		
09:00-09:30	Registration (Lobby)		
09:30-10:00	Hall A Youdang Scholarship Award Lecture		
10:00-10:50	General Assembly (Hall A) and Group photo		
11:00-13:00	Hall A	Hall B	Hall C
	Symposium VI Physiology and Pathophysiology of ion homeostasis	**Symposium VII** The roles of TRP channels in cardiovascular systems	**Symposium VIII** Maladpative Pain Signaling
13:00-13:10	Closing remark (Hall A)		

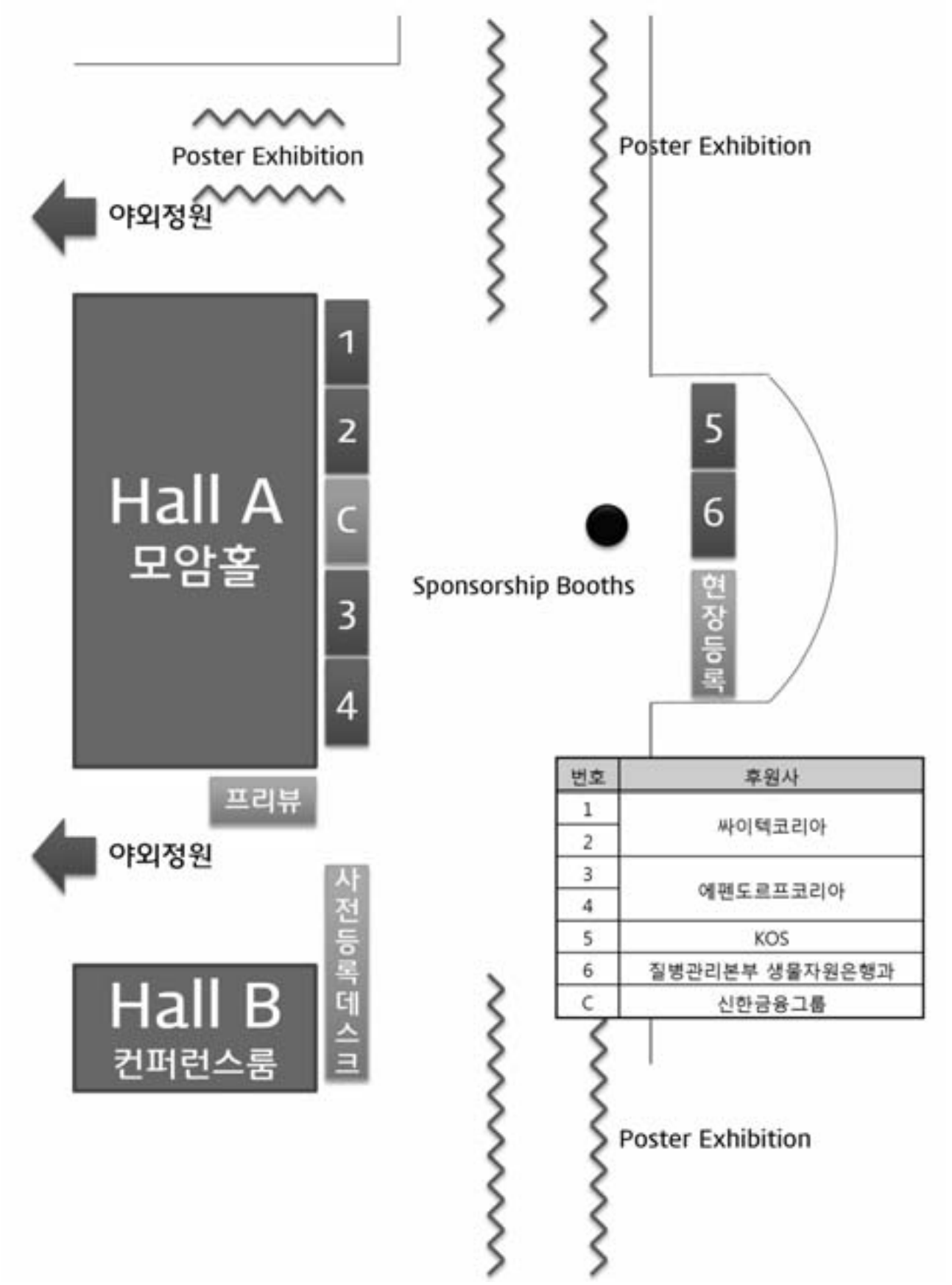
Venue Guide (학술대회장 안내)

양산 부산대학교 병원



본관 4층 – 등록데스크
– Hall A (모암 홀), Hall B (컨퍼런스 룸)
– 프리뷰룸, 포스터전시, 후원전시

어린이병원 지하1층 – Hall C (새싹 홀)


Poster Exhibition
야외정원
Hall A
모암홀
1
2
C
3
4
프리뷰
야외정원
Hall B
컨퍼런스룸
사전등록데스크
Poster Exhibition
5
6
Sponsorship Booths
현장등록
번호
후원사
1
2
싸이텍코리아
3
4
예편도르프코리아
5
KOS
6
질병관리본부 생물자원은행과
C
신한금융그룹
Poster Exhibition

Scientific Program (학술프로그램)

Pflügers Archiv Symposium - Cardiovascular physiology: Application and Translation

Time	Contents	
09:00-11:50	Registration	
11:50-12:00	Welcome Message and Introduction	Yung E Earm
	Session 1	**Chairs:** *Tong Mook Kang, Kyu Sang Park* **Pannel:** *JinKun, Yin Hua Zhang*
12:00-12:30	Mitochondrial DNA causes spreading necrosis in the heart	*James Downey (USA)*
12:30-13:00	Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial reactive oxygen species	*Thomas Krieg (UK)*
13:00-13:15	Coffee Break	
	Session 2	**Chairs:** *Jae Ho Kim, Byeong Hwa Jeon* **Pannel:** *Dawon Kang, Nari Kim*
13:15-13:45	Enlargement of myocardial infarct size by chronic kidney disease: a novel mechanism of disruption of Akt-GSK3beta/p70S6K signaling	*Tetsuji Miura (Japan)*
13:45-14:15	Dual roles of reactive oxygen species in myocardial ischemia/reperfusion injury and protection	*Huang-Tian Yang (China)*
14:15-14:30	Coffee Break	
	Session 3	**Chairs:** *Dae Kyu Song, Sun-Hee Woo* **Pannel:** *Sung Ryul Lee, Jae Hong Ko*
14:30-15:00	Physiological roles of unconventional eNOS expressed in the smooth muscle of skeletal and pulmonary arteries	*Sung Joon Kim (Korea)*
15:00-15:30	Zinc, zinc transporters and cardioprotection	*Zhelong Xu (China)*
15:30-15:45	Coffee Break	
	Session 4	**Chairs:** *Chae Hun Leem, Eun Hui Lee* **Pannel:** *Young Min Bae, Jae Boum Youm*
15:45-16:15	The role of TRPM4 in cardiac function and excitability	*Rudi Vennekens (Belgium)*
16:15-16:45	Bicarbonate permeation through anion channels	*Min Goo Lee (Korea)*
	Session 5	**Chairs:** *Moo Yoel Lee, Suhn Hee Kim* **Pannel:** *Ju Hyun Nam, Hyun Jin Kim*
16:45-17:15	Orai1 in ER/PM junctions	*Shmuel Muallem (USA)*
17:15-17:45	Endosomal and lysosomal chloride/proton exchange by CLC proteins: surprising roles in physiology and pathology	*Thomas Jentsch (Germany)*
17:45-18:00	Coffee Break	
	Session 6	**Chairs:** *Hyoweon Bang, Suk Hyo Suh* **Pannel:** *Seung-Kyu Cha, Hyoung Kyu Kim*
18:00-18:30	A short tribute to Pflügers Archiv (The European Journal of Physiology) and a sojourn to an endothelial anion channel	*Bernd Nilius (Belgium)*
18:30-18:40	Closing Remarks	
19:00	Pflügers Dinner	

Symposium

Contents	
Symposium I: Diverse scientific approach for resolve obesity epidemic	**Organizer :** *진영호*
Neuro-endocrine systems that targeted for anti-obesity research	*진영호 (경희대학교 의과대학, 생리학교실)*
Beige and Brown burns fat	*송영섭 (울산대학교 의과대학, 의생명과학)*
Bariatric to metabolic surgery; paradigm shift	*김용진 (순천향대학교 의과대학, 고도비만수술센터)*
Pharmacotherapy for resolving obesity	*김경곤 (가천의대, 가정의학과)*
Symposium II: Mechanosensation, nanoscience and optical modulation	**Organizer :** *배영민*
Recent advances in studying tactile sensation	*배영민 (건국대학교 생리학교실)*
Physical responses to pulsed laser stimulation in human skin	*박종락 (조선대학교)*
Cortical Responses to tactile sense induced by laser in humans	*김성필 (UNIST)*
When nanoscience meets optogenetics	*최철희 (KAIST)*
Symposium III: <KPS-PSJ Joint Symposium> Model-based analysis of physiological system	**Organizer :** *임채헌*
Physiome study on mitochondrial Ca2+ dynamics	*Satoshi Matsuoka (Univ. Fukui)*
Role of NKCC (Na-K-Cl cotransporter) and SOCE (store-operated calcium entry) in pacemaker activity in interstitial cells of Cajal	*염재범 (인제의대 생리학)*
A physiomic approach for the electrophysiological variation of the heart induced by the ischemia of coroanry artery	*심은보 (강원대 공대)*
Electromechanical delay in human ventricle under various load conditions: simulation study	*임기무 (금오공대)*
Model based interpretation of oral glucose tolerance test	*임채헌 (울산의대 생리학)*
Symposium IV: Exercise physiology	**Organizer :** *정덕조*
Oxygen availability and skeletal muscle mitochondrial function in health and disease	*Russell Richardson (University of Utah)*
Effects of exercise training on myokines expression and insulin sensitivity in diet-induced obese rats	*김기진(계명대학교)*
The effect of aerobics exercise on macrophage function in high fat induced obese mice	*이왕록(충남대학교)*
Gene exprression profiling in skeletal muscle with aerobic exercise	*박정준(부산대학교)*
Changes of muscle insulin-like growth factor-I and concentrations of inflammatory cytokines in rat skeletal muscle following denervation and diabetes-induced atrophy	*전병환(경성대학교)*
Symposium V: Stem Cell Physiology	**Organizer :** *김재호*
Therapeutic development of optimized mesenchymal stem cells and delivery technology for myocardial infarction	*안영근 (전남대학병원)*
Combining stem cells and biomaterials for regenerative medicine	*김병수 (서울대학교 공과대학)*
Mitochondrial function in stem cell differentiation	*한진 (인제의대 생리학)*
Hair growth promotion by adipose-derived stem cells: Fact or Fiction?	*성종혁 (연세대학교 약학대학)*

Symposium

Contents	
Symposium VI: Physiology and Pathophysiology of ion homeostasis	**Organizer :** *박규상*
Ambivalent role of phosphate in health and disease	*박규상 (원주의대)*
Roles of ion channels in cell death processes	*최용준 (아주의대)*
Role of zinc on hypoglycemia-induced neuron death	*서상원 (한림의대)*
Zinc homeostasis and osteoarthritis	*전장수 (광주과기원)*

Symposium VII : The roles of TRP channels in cardiovascular systems	**Organizer :** *서인석*
Gai-mediated TRPC4 activation by polycystin-1 contributes to endothelial dysfunction	*서인석 (서울의대)*
Simulation-based study of PIP2-meditated regulation of TRP channels	*Ryuji Inoue (Fukuoka University)*
Roles of TRPM4 in cardiac electrical activity and its perturbations	*Romain Guinamard (Université de Caen Basse-Normandie)*
Activation of Ca2+-dependent monovalent cation current by shear stress in atrial myocytes: possible role of TRPM4	*우선희 (충남대학교 약학대학)*

Symposium VIII: Maladaptive Pain Signaling	**Organizer :** *곽영섭/오석배*
Both sides of mGluR5 in maladaptive pain brain	*김상정 (서울의대 생리학교실)*
Targeting of Acid-Sensing Ion Channels (ASICs) to the plasma membrane	*서병창 (DGIST 뇌인지과학)*
Discovery of novel mGluR1 antagonists for the potential treatment of neuropathic pain	*배애님 (KIST 뇌의약연구단)*
The roles of GABA on neuropathic pain and reward following spinal cord injury	*곽영섭 (대구한의대 생리학교실)*

Pflügers Archiv Symposium - Cardiovascular physiology: Application and Translation

S 33 PAS-1 Mitochondrial DNA causes spreading necrosis in the heart
James Downey, Mikhail Alexeyev, Glenn Wilson, Xi-Ming Yang, and Michael Cohen
University of South Alabama, Mobile, Alabama, USA

S 33 PAS-2 Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial reactive oxygen species
Thomas Krieg
Department of Medicine, University of Cambridge, UK

S 33 PAS-3 Enlargement of myocardial infarct size by chronic kidney disease: a novel mechanism of disruption of Akt-GSK3beta/p70S6K signaling
Tetsuji Miura
Department of Cardiovascular, Renal and Metabolic Medicine, Sapporo Medical University School of Medicine, Japan

S 34 PAS-4 Dual roles of reactive oxygen species in myocardial ischemic injury and protection
Huang-Tian Yang
Key Laboratory of Stem Cell Biology and Laboratory of Molecular Cardiology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China

S 34 PAS-5 Physiological roles of unconventional eNOS expressed in the smooth muscle of skeletal and pulmonary arteries
Sung Joon Kim
Department of Physiology, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Korea

S 34 PAS-6 Zinc, zinc transporters and cardioprotection
Zhelong Xu
Department of Physiology and Pathophysiology, Tianjin Medical University, China

S 34 PAS-7 The role of TRPM4 in cardiac function and excitability
Rudi Vennekens
Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven, Belgium

S 35 PAS-8 Bicarbonate permeation through anion channels
Min Goo Lee
Department of Pharmacology and Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul 120-752, Korea

S 35 PAS-9 Orai1 in ER/PM junctions
Shmuel Muallem
National Institute of Health (NIH), NIDCR, USA, Bethesda Maryland, USA

S 35 PAS-10 Endosomal and lysosomal chloride/proton exchange by CLC proteins: surprising roles in physiology and pathology
Thomas J. Jentsch
Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) und Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany

S 36 PAS-11 A short tribute to Pflügers Archiv (The European Journal of Physiology) and a sojourn to an endothelial anion channel
Bernd Nilius
KU Leuven, Department of Cellular and Molecular Medicine, Laboratory Ion Channel Research, Campus Gasthuisberg, Herestraat 49, bus 802, Leuven, Belgium

Plenary Lecture

S 37 Clinical application of human iPS cells for cardiovascular Medicine
Keiichi Fukuda
Department of Cardiology, Keio University School of Medicine

Youdang Scholarship Award Lecture

S 38 Ca^{2+}-activated K^+ channel expression on cell membrane in physiological and pathophysiological conditions
Shinkyu Choi, Ji Aee Kim, Suk Hyo Suh
Department of Physiology, Medical School, Ewha Womans University, Seoul, Korea

Symposium

S 39 S-I-1 Neuro-endocrine systems that targeted for anti-obesity research
Young-Ho Jin
Department of Physiology, School of Medicine, Kyung Hee University, Seoul, 130-701, Korea

S 39 S-I-2 Beige and Brown burns fat
Youngsup Song, Aroom Hong
Department of Biomedical Sciences, University of Ulsan College of Medicine, Asan Medical Center, Seoul 138-736

S 39 S-I-3 Bariatric to metabolic surgery; paradigm shift
Yong Jin Kim
Department of Surgery, Soonchunhyang University, Seoul Hospital, Seoul, Korea

S 39 S-I-4 Pharmacotherapy for resolving obesity
Kyoung Kon Kim
Department of Family Medicine, Gachon University College of Medicine, Incheon, Korea

S 40 S-II-1 Recent advances in studying tactile sensation
Kyung Chul Shin, Hyunji Park, Sang Woong Park, In-Hwa lee, Jae Gon Kim, Young Min Bae
Department of Physiology Konkuk University School of Medicine

S 40 S-II-2 Physical responses to pulsed laser stimulation in human skin
Jong-Rak Park
Department of Photonic Engineering, Chosun University, 309 Pilmun-daero, Gwangju 61452, Korea

S 40 S-II-3 Cortical Responses to tactile sense induced by laser in humans
Sung-Phil Kim
Department of Human and Systems Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

S 40 S-II-4 When nanoscience meets optogenetics
Nambin Yim, Seung-Wook Ryu, Kyungsun Choi, Chulhee Choi
Department of Bio and Brain Engineering, KAIST, Daejeon, 305-701, South Korea

S 41 S-III-1 Physiome study on mitochondrial Ca^{2+} dynamics
Satoshi Matsuoka, Ayako Takeuchi
Department of Integrative and Systems Physiology, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan

S 41 S-III-2 Role of Na-K-Cl cotransporter and store-operated calcium entry in pacemaker activity of interstitial cells of Cajal
Jae Boum Youm[1,2], Haifeng Zheng[2], Mei Hong Zhu[2], Tae Sik Sung[2], Kenton M. Sanders[2], Sang Don Koh[2]
[1]Cardiovascular and Metabolic Disease Center (CMDC), Department of Physiology, College of Medicine, Inje University, Busan, South Korea, [2]Department of Physiology and Cell Biology, University of Nevada, School of Medicine, Reno, Nevada, USA

S 41 S-III-3 A physiomic approach for the electrophysiological variation of the heart induced by the ischemia of coronary artery
Ah-Jin Ryu, Gi-Tae Kim, Soon Sung Kwon, Eun Bo Shim
Department of Mechanical and Biomedical Engineering, Kangwon National University, Chuncheon 200-701, Korea

S 41 S-III-4 Electromechanical delay in human ventricle under various load conditions: simulation study
Aulia Heikhmakhtiar[1], Eun Bo Shim[2], Ki Moo Lim[1]
[1]Department of Medical IT Convergence Engineering, Kumoh National Institute of Technology, [2]Department of Mechanical & Biomedical Engineering, Kangwon National University

S 42 S-III-5 Model based interpretation of oral glucose tolerance test
Young Boum Lee, Jeong Hoon Lee, Ga Yul Kim, Ji Yeon Song, Pham Duc Doung, Chae Hun Leem
Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

S 43 S-IV-1 Oxygen availability and skeletal muscle mitochondrial function in health and disease
Russell S. Richardson
University of Utah

S 43 S-IV-2 Effects of exercise training on myokines expression and insulin sensitivity in diet-induced obese rats
Kijin Kim, Nayoung Ahn, Suryun Jung, Jayoung Byun, Kwangbae Park, Sungwook Kim, Yeunho Jung, Solee Park
Department of Physical Education, Keimyung University, 1095 Dalgubeuldaero, Dalseo-gu, Daegu, 704-701, Korea

S 43 S-IV-3 Moderate exercise training inhibits lipid metabolism and macrophage infiltration in high fat diet-induced obese mice
Wang Lok Lee, Young Ran Lee, Hee Geun Park, Jun Hyun Jeong
Department of Sport Science, College of Nautral Science, Chungnam National University

S 43 S-IV-4 Gene expression profiling in skeletal muscle with aerobic exercise
Jung-Jun Park
Division of Sport Science, Pusan National University

S 44 S-IV-5 Changes of muscle insulin-like growth factor-I and concentrations of inflammatory cytokines in rat skeletal muscle following denervation and diabetes-induced atrophy
Byeong-hwan Jeon
School of Sports and Health, Kyungsung University, Busan, 48434, Korea

S 44 S-V-1 Therapeutic development of optimized mesenchymal stem cells and delivery technology for myocardial infarction
Youngkeun Ahn
Department of Cardiology, Chonnam National University Hospital, Gwangju, South Korea, 501-757

S 44 S-V-2 Transforming stem cell therapy with nanobiomaterials
Byung-Soo Kim
School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea

S 45 S-V-3 Mitochondrial function in stem cell differentiation
Hye Jin Heo, Nari Kim, Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, 614-735, Korea

S 45 S-V-4 Hair growth promotion by adipose-derived stem cells: Fact or Fiction?
Jong-Hyuk Sung
College of Pharmacy, Yonsei University #162-1, Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea

S 46 S-VI-1 Ambivalent role of phosphate in health and disease
Tuyet Thi Nguyen, Xianglan Quan, Seung-Kyu Cha, Kyu-Sang Park
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Gangwon-Do 220-701, Korea

S 46 S-VI-2 Roles of ion channels in cell death processes
Soo Jeong Park, Sarah Yoon, Jeong Hee Kang, Sun Park, Ho-Joon SHin, Kyeongmin Kim, Yong-Joon Chwae
Department of Microbiology, Ajou University School of Medicine, Suwon, Korea

S 46 S-VI-3 Role of zinc on hypoglycemia-induced neuron death
Sang Won Suh
Department of Physiology, Hallym University, College of Medicine, Chuncheon, Gangwon-Do 200-702, Korea

S 46 S-VI-4 Zinc homeostasis and osteoarthritis
Jang-Soo Chun
School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

S 47 S-VII-1 Gαi-mediated TRPC4 activation by polycystin-1 contributes to the endothelial dysfunction in polycystic kidney diseases
Misun Kwak, Chansik Hong, Kotdaji Ha, Ju-Hong Jeon, Insuk So
Department of Physiology, Seoul National University College of Medicine

S 47 S-VII-2 Simulation-based study of PIP_2-meditated regulation of TRP channels based on voltage-sensing phosphatase and FRET measurements
Ryuji Inoue, Hu Yaopeng, Jun Ichikawa, Lin Kurahara, Tomohiro Numata
Department of Physiology, Fukuoka University School of Medicine, Fukuoka 814-0180, Japan

S 47 S-VII-3 Roles of TRPM4 in cardiac electrical activity and its perturbations
Romain Guinamard
Caen-Normandie University, France

S 47 S-VII-4 Activation of calcium-dependent monovalent cation current by shear stress in atrial myocytes: possible role of TRPM4
Min-Jeong Son, Joon-Chul Kim, Sung Woo Kim, Bojjibabu Chidipi, Insuk So[1], Krishna P. Subedi, Sun-Hee Woo
College of Pharmacy, Chungnam National Univeristy, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea, [1]Department of Physiology, College of Medicine, Seoul National University, Seoul 110-799, Korea

S 48 S-VIII-1 Both sides of mGluR5 in maladaptive pain brain
Geehoon Chung[1,3], Chae Young Kim[1,2], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2,3]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, [3]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences

S 48 S-VIII-2 Targeting of acid-sensing ion channels (ASICs) to the plasma membrane
Byung-Chang Suh
Department of Brain & Cognitive Sciences, DGIST, Daegu 711-873, Korea

S 49 S-VIII-3 Discovery of novel mGluR1 antagonists for the potential treatment of neuropathic pain
Ae Nim Pae
Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, South Korea

S 49 S-VIII-4 The of roles of GABA on neuropathic pain and reward following spinal cord injury in rats
Moon Yi Ko, Jun Yeon Lee, Su Phil Kim, Chae Ha Yang, Hee Young Kim, Young S. Gwak
Department of Physiology, Daegu Haany University, Daegu 42158

Poster Presentation (Poster Oral Presentation)

P01: Diet, Phytochemicals and Stress Physiology

S 50 P01-01 Glycosaminoglycans increase the phagocytic ability of macrophages via the activation of AMP-activated protein kinase and cytoskeletal reorganization
Ji Hyeon Ryu[1], Jina Sung[1], Chengliang Xie[2], Jaehee Han[1], Nam-Gil Kim[3], Yeung Joon Choi[4], Byeong Dai Choi[4], Sang Soo Kang[2], Dawon Kang[1]
Departments of [1]Physiology and [2]Anatomy, College of Medicine, Gyeongsang National University, Jinju 660-751, Republic of Korea [3]Department of Marine Biology and Aquaculture and Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Republic of Korea [4]Department of Seafood Science and Technology and Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Republic of Korea 50

S 50 P01-02 Inhibitory effects of cyanidin-3-glucoside on amyloid beta (25-35)-induced neuronal cell death in cultured rat hippocampal neurons
Ji Seon Yang[1], Kee Dong Yoon[2], Yang-Hyeok Jo[1], Shin Hee Yoon[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, [2]College of Pharmacy, The Catholic University of Korea, Bucheon-si 420-743, Republic of Korea

P02: Endocrine and Metabolic Physiology

S 50 P02-01 Electrophysiological profiling of cardiac myocytes underlying metabolic substrates-induced spontaneous contractions in normal and hypertensive rats
Jae Hwi Sung, Zai Hao Zhao, Sung Joon Kim, Yin Hua Zhang
Department of physiology, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S 51 P02-02 Endocytosis of K_{ATP} channels drives glucose-stimulated depolarization in pancreatic β-cell
Young-Eun Han, Young-Sun Ji, Jung Nyeo Chun, Sun-Hyun Park, Ajin Lim, Ju Hong Jeon, Sunghoe Chang, Suk-Ho Lee, Won-Kyung Ho
Department of Physiology, Seoul National University College of Medicine, Seoul, 110-799, Korea

S 51 P02-03 Effects of etomoxir on lipid metabolism and oxidative stress in peripheral tissues
Hye-Jun Jo, Hye-Na Cha, Jung-Yoon Heo, Suk-Jeong Kim, So-Young Park
Department of Physiology, College of Medicine Yeungnam University, Daegu, Korea, Rep.

S 51 P02-04 Angiotensin II impairs β2-adrenergic receptor stimulation in Human vascular progenitor cells via regulation of β2-adrenergic receptor expression
Seon Jin Lee, Sang Mo Kwon
Laboratory for vascular medicine & stem cell biology, Medical Research Institute, Department of physiology, Pusan National University, Pusan, Korea

S 51 P02-05 Oleanolic acid modulates body fluid and blood pressure homeostasis ; suppression the renin-angiotensin system
You Mee Ahn[1,2], Yun Jung Lee[1,2], Hye Yoom Kim[1,2], Rui Tan[1,2], So Heun Lee[1,2,3], Han Sol Lee[1,2,3], Kyung Woo Cho[1,2], Ho Sub Lee[1,2,3]*, Dae Gill Kang[1,2,3]*
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

P03: Exercise and Applied Physiology

S 52 P03-01(O-10) The effect of mitochondria-targeted antioxidant (MitoQ) on the age-related impairment in vasodilatory function in human skeletal muscle feed arteries
Oh Sung Kwon, Song Young Park, Russell Richardson
Department of Internal Medicine, Division of Geriatrics, University of Utah, Salt Lake City, Utah

S 52 P03-02(O-11) The impact of exercise training on vascular mitochondria and arterial function
Song-Young, Park, Russell S Richardson, Dale Abel, Matthew J Rossman, David Symons and Yi sub Kwak, Christian Riehle
[1]Geriatric Research, Education, and Clinical Center, George E. Whalen VA Medical Center, SLC, UT, [2]Department of Internal Medicine, Division of Geriatrics, University of Utah, SLC UT, [3]Department of Exercise and Sport Science, University of Utah, SLC, UT, [4]Division of Endocrinology, Metabolism, and Diabetes, University of Utah School of Medicine, SLC, UT, [5]Molecular Medicine Program, University of Utah, SLC, UT, 5Department of Physical Education Dong Eui University, [6]Fraternal Order of Eagles Diabetes Research Center Division of Endocrinology and Metabolism Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA 52

S 52 P03-03 The adiponectin response research of combined exercise in overweight child
Yi Sub Kwak
Department of Physical Education, Dong–Eui University, Busan 614-714, Korea

S 53 P03-04 Food-dependent exercise-induced anaphylaxis (FDEIAn) response research in human
Yi Sub Kwak
Department of Physical Education, Dong–Eui University, Busan 614-714, Korea

S 53 P03-05 Novel anthropometry-based calculation of the body heat capacity in the Korean population
Duong Duc Pham, Jeong Hoon Lee, Young Boum Lee, Eun Seok Park, Ga Yul Kim, Ji Yeon Song, Ji Eun Kim, Chae Hun Leem*
Department of Physiology, University of Ulsan College of Medicine, 88 OlympicRo 43-gil Songpa-gu, Seoul, Republic of Korea

S 53 P03-06 Effect of long-term exercise on circulating levels of Dickkpof-1 and frizzled-related protein-1 in breast cancer patients
Tae-Ho Kim[1], Jae Seung Chang[1], Hanul Kim[1], Park, jeeyeon[2], Nahyun Kim[3], In Deok Kong[1]
[1]Yonsei Univeristy Wonju College of Medicine, Wonju , Korea, [2]Keimyung University College of Nursing, Daegu, Korea, [3]Kyungsung University, Department of nursing science, Busan, Korea

S 54 P03-07 Effect of cardiorespiratory endurance training on power production in figure skaters
Seung Bo Park, Joung Kyue Han
Department of Sport Industry & Information, Graduate School of Chung-Ang University, Seoul 156-756, Republic of Korea

S 54 P03-08 The effect of AQP3 deficiency in fuel selection during a single bout of exhausting exercise
Ju Hyun Lim, Hae-Rahn Bae
Department of Physiology, College of Medicine, Dong-A University, Busan 602-714, Korea

P04: Ion Channels and Transporters

S 54 P04-01 Transient receptor potential canonical 4 (TRPC4) channel regulation by phosphotiesterase 5 inhibitor via the cyclic guanosine 3'5'-monophosphate
Jinhong Wie, Ju-Hong Jeon, Insuk So
Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

S 54 P04-02

Fluid flow facilitates inward rectifier K+ current by convective restoring of [K+] at cell membrane surface
Jae Gon Kim[1,2], Sang Woong Park[1], Hyang-Ae Lee[2], Doyoung Byun[3], Wan Soo Choi[4], Kyung Chul Shin[1], Ki-Suk Kim[2], kyung Kim[1], Sung Il Cho[1], Young Min Bae[1]*
[1]Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, Chungbuk 380-701, South Korea [2]Next-Generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, 141 Gajeong-Ro, Yuseong-Gu, Daejeon, South Korea [3]Department of Mechanical Engineering, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-Gu, Suwon, Gyeonggi 440-746, South Korea. [4]Department of Immunology, School of Medicine, Konkuk University, Chungju 380-701, South Korea

S 55 P04-03

Nicardipine inhibits hERG Channel
Eun Yong Chung[1], Hea-Young Cho[1], Ji-Hun Cha[2], Kyoung-Jin Kwon[2], Seol-Hee Jeon[2], Su Hyun Jo[3], Eun-Jung Kim[2], Hye Soo Kim[1], Hye Ju Chung[1]
[1]Korea Food and Drug Administration, [2]National Institute of Food and Drug Safety Evaluation, Seoul 122-704, [3]Department of Physiology and Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea

S 55 P04-04

Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease
Dong I. Lee[1], Guangshuo Zhu[1], Takashi Sasaki[2], Su-Hyun Jo[3], Nazha Hamdani[4], Ronald Holewinsky[5], Thomas Danner[1], Manling Zhang[1], Peter P. Rainer[1], Djahida Bedja[1], Jonathan Kirk[1], Wolfgang R. Dostmann[6], Hideki Uosaki[1], Chulan Kwon[1], Kenneth B. Margulies[7], Jennifer Van Eyk[5], Walter Paulus[4], Eiki Takimoto[1], David A. Kass[1]
[1]Division of Cardiology, Department of Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205 (USA), [2]Advanced Medical Research Laboratories, Research Division, Mitsubishi Tanabe Pharma Corporation, Yokohama, Kanagawa 227-0033, Japan, [3]Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea, [4]Department of Physiology and Cardiology, Institute for Cardiovascular Research, VU University Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands ,[5]Heart Institute and Advanced Clinical Biosystems Research Institute, Cedar Sinai Medical Center, 8700 Beverly Blvd, AHSP A9229 Los Angeles, CA 90048 (USA), [6]Department of Pharmacology, University of Vermont, Burlington, VT 05405 (USA), [7]Department of Medicine, Division of Cardiovascular Medicine, Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 (USA)

S 56 P04-05

Shear stress induces longitudinal Ca^{2+} wave via autocrine activation of P2Y1 purinergic signaling in atrial myocytes
Joon-Chul Kim, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, 34134, South Korea

S 56 P04-06

Shear stress enhances Ca^{2+} spark occurrence in rat ventricular myocytes via mitochondrial NOX-ROS signaling
Jun Wang, Joon-Chul Kim, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, 34134, South Korea

S 56 P04-07

Changes of K^+ channel currents in skeletal arterial smooth muscle by exercise training in sciatic nerve-injured rats
Ming Zhe Yin, Eun Young Seo, Sung Joon Kim
Department of Physiology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S 57 P04-08

Ion channel gene expression predicts survival in glioma patients
Donghee Lee, Young-Won Kim, Jeongyoon Choi, Misuk Yang, Hyemi Bae, Inja Lim, Hyoweon Bang, Jae-Hong Ko
Department of Physiology, College of Medicine, Chung-Ang University, Seoul 156-756, South Korea

S 57 P04-09

Tonic inhibition of TREK-2 K2P channels by intrinsic $PI(4,5)P_2$ is the physiological mode of regulation
Joohan Woo[1], Dong Hoon Shin[3], Yin-Hua Zhang[1], Joo Hyun Nam[2], Sung Joon Kim[2]
[1]Department of physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea [2]Department of physiology & Ion Channel Disease Research Center, College of Medicine, Dongguk University, Kyungju, 780-714, Korea [3]Division of Natural Medical Sciences, Chosun University, College of Health Science, Gwang-Ju, 501-759, Korea

S 57 P04-10

Inhibitory modulation of hERG K^+ channels by endogenous polyunsaturated fatty acid-derived electrophiles, 4-HNE and 4-ONE
Seong Woo Choi[1], Hyang-Ae Lee[1,2], Yin-Hua Zhang[1], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology

S 57 P04-11

TLR3-/4-Priming Differentially Promote Ca2+ Signaling and Cytokine Expression and Ca2+-Dependently
Kyoung Sun Park[1], Sun Hwa Kim[1], Kyoung Hwa Jung[1], Mi Kyung Kim[2], Yangmi Kim[3], Hyun Jin Kim[2], YoungGyu Chai[1]
[1]Department of Molecular and Life Sciences, Hanyang University, Ansan, Korea, [2]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea, [3]Department of Physiology, College of Medicine, Chungbuk National University, Cheongju, 361-763, Korea

S 58 P04-12 Close Spatio-Association of Transient Receptor Potential Canonical (TRPC4) channel with Gαi in TRPC4 activation process
JongYun Myeong, Misun Kwak, Jae-Pyo Jeon, Chansik Hong, Ju-hong Jeon and Insuk So
Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

S 58 P04-13 ATP sensitive potassium currents on Human Periodontal ligament cells
Tran Thi Huyen Phuong, Soo Joung Park, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

S 58 P04-14 Physiological temperature increase the calcium sensitivity and current activation of TMEM16F (ANO6)
Haiyue Lin[1], Joo Hyun Nam[2], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, 28 Yongon-dong, Chongro-gu, Seoul 110-799, Republic of Korea, [2]Department of Physiology, Dongguk University College of Medicine, 123 Dongdae-ro, Kyungju 780-714, Republic of Korea

S 59 P04-15 Electrophysiological Characterization of Novel KCNQ4 Mutant Channels
Hyun Been Choi[1], Mina Park[2], Min-Young Kim[3], Ah-Reum Kim[3], Byung Yoon Choi[3], Tong Mook Kang[1]
[1]Department of Physiology, SBRI, Sungkyunkwan University School of Medicine, Suwon, Korea, [2]Department of Otorhinolaryngology-Head and Neck Surgery and Healthcare Research Institute, Seoul National University Hospital, Healthcare System Gangnam Center, [3]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea

S 59 P04-16 Influence of Bisphenol-A on ion channel activities on Gonadotropin Releasing Hormone Neurons
Janardhan P. Bhattarai, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

S 59 P04-17 The Conserved Gating Elements in CIRB domain of TRPC4 Channel
Chansik Hong[1], Jongyun Myeong[1], Joo Hyun Nam[2], Young-Cheul Shin[1], Misun Kwak[1], Insuk So[1]
[1]Department of Physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea, [2]Department of Physiology, College of Medicine, Dongguk University, Gyeongju, 780-714, Korea

S 60 P04-18 KCNQ2/3 channel inhibition by ethanol is regulated by plasma membrane PI(4,5)P2 level
Kwon-Woo Kim, Dongil Keum, Hae-Jin Kweon, Byung-Chang Suh
Department of Brain and cognitive science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, South Korea

S 60 P04-19 Verapamil inhibits TRESK current in trigeminal ganglion neurons independently of the blockade of Ca2+ influx
Dawon Kang, Eun-Jin Kim, Ji Hyeon Ryu, Jaehee Han
Department of Physiology, College of Medicine and Institute of Health Sciences, Gyeongsang National University, Jinju 660-751, South Korea

S 60 P04-20 Stable interaction of Ca^{2+} channel β subunit with high voltage-activated Ca^{2+} channels α1 subunit revealed by translocatable CaV β subunit systems
Jun-Hee Yeon, Byung-Chang Suh
Department of Brain and Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology, Daegu, 711-873, South Korea

S 61 P04-21 Functional role of GABA as a gliotransmitter in epileptic hippocampus
Sudip Pandit, Hyang-Joo Lee, Hyun-Sill Cho, Yoon-Hyung Pai, Jin Bong Park
Department of Physiology, Brain Research Institute, School of Medicine, Chungnam National University, 6 Munhwa-Ro, Jung-gu, Daejeon, 301-131, Republic of Korea

S 61 P04-22 Gαi-mediated TRPC4 activation by Polycystin-1 contributes to cystic disease via STAT1 activation
Misun Kwak, Chansik Hong, Kotdaji Ha, Ju-Hong Jeon, Insuk So
Department of Physiology, Seoul National University College of Medicine

S 61 P04-23 Single channel recordings of the positive pressure-specific mechanosensitive piezo2 ion channels in human MCC-13 Merkel cell line
Kyung Chul Shin, Sang Woong Park, Hyunji Park, Young Min Bae
Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Chungju, Chungbuk, 380-701, South Korea

S 62 P04-24 Proton modulates common gate of ClC-1 chloride channel via helix O
Ju Yong Seong, Kotdaji Ha, Insuk So
Department of Physiology, College of medicine, Seoul National Univeristy

S 62 P04-25 The role of PIP_2 signaling in NALCN regulation
Jungeun Hong[1,3], Tae Jung Ahn[2], KyeongJin Kang[2,3], Hana Cho[1,3]
[1]Department of Physiology, [2]Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, [3]Samsung Biomedical Research Institute, Suwon, 440-746, Republic of Korea

S 62 P04-26 The Role of Kv Channels in Osteoblast Differentiation
Ji Eun Yang, Min Seok Song, Pan Dong Ryu, So Yeong Lee
Department of Veterinary Pharmacology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea

S 63 P04-27 Enhancements of contraction and L-type Ca^{2+} current by murrayafoline-A via protein kinase C in rat ventricular myocytes
Bojjibabu Chidipi, Min-Jeong Son, Nguyen Manh Cuong[1], Sun-Hee Woo
College of Pharmacy, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, South Korea, [1]Department of Bioactive Products, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Rd., Hanoi, Vietnam

S 63 P04-28 Enhancing skin barrier homeostasis via modulation of calcium ion channels by topical botanical products
Mi-Ok Lee[1], Joo Hyun Nam[1,2], Woo Kyung Kim[2,3]
[1]Department of physiology, College of Medicine, Dongguk University, Kyungju, 780-714, Korea, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, 27 Dongguk-ro, Goyang 410-773, Korea, [3]Department of Internal Medicine Graduate School of Medicine, Dongguk University, 27 Dongguk-ro, Goyang 410-773, Republic of Korea

S 63 P04-29 Epidermal growth factors activate TRPC4 and TRPC5, reducing desensitization of TRPC5 channel
Seungjoo Jeong, Jinhong Wie, Ju-Hong Jeon, Insuk So
Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

S 64 P04-30 Inhibition of the extrinsic aging-related ion channels TRPV1 and ORAI1 by constituents of the fruits of Foeniculum vulgare
Joo Hyun Nam[1], Dong-Ung Lee[2]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 780-714; and Channelopathy Research Center (CRC), Dongguk University College of Medicine, 27 Dongguk-ro, Ilsan Dong-gu, Goyang 410-773, Republic of Korea, [2]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea

S 64 P04-31 The rhizomes of Cyperus rotundus and its active component valencene inhibit skin photoaging related ion channels, TRPV1 and Orai1
Joo Hyun Nam[1], Dong-Ung Lee[2]
[1]Department of Physiology, Dongguk University College of Medicine, 123 Dongdae-ro, Kyungju 780-714, Republic of Korea, [2]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea

S 64 P04-32 Cryopreservation method of isolated adult cardiac myocytes of rat
Ga Yul Kim, Ji Yeon Song, Jeong Hoon Lee, Young Boum Lee, Pham Duc Doung, Chae Hun Leem
Department of Physiology, University of Ulsan College of Medicine, 88 OlympicRo 43-gil Songpa-gu, Seoul, Republic of Korea

S 64 P04-33 Unusual acid- and voltage-dependency of a prokaryotic CLC, ecCLC-2: A marginal ion channel or broken transporter?
Kun Woong Park, Jung Ha Kim, Hee Soon Choi, Hyun-Ho Lim
Lab. of Membrane Biochemistry and Biophysics, Dept. of Structure & Functional Neural Network, Korea Brain Research Institute (KBRI), Daegu, Korea 701-300

S 65 P04-34 Alcohol impair intracellular calcium oscillation in mouse pancreatic acinar cell
Mi Na Yoon, Min Jae Kim, Ye Jin Jo, Yeong Umg Baek, Dong Kwan Kim, Se hoon Kim, Hyung Seo Park
Department of Physiology, College of Medicine, Konyang University, Daejeon 35365, Korea

S 65 P04-35 Ketamine inhibits KCNQ2/3 channels and modulates excitability in hippocampal dentate gyrus granule cells
Seul Yi Lee, Xianlan Wen, Hana Cho
Department of Physiology and Samsung Biomedical Research Institute, School of Medicine, Sungkyunkwan University, Suwon, Korea

S 65 P04-36 Voltage gated sodium channel 1.7 as therapeutic target for treatment of neuropathic pain
Sung-Young Kim
New Drug Laboratory, Daewoong 72, Dugye-ro, Pogok-eup, Cheoin-gu, Yongin-si, Gyeonggi-do, 449-814, Korea

S 66 P04-37 Autocrine insulin stimulates plasma membrane trafficking of KATP channel via PI3K-VAMP2 pathway in MIN-6 cells
Shanhua Xu[1,2], Ji-Hee Kim[1], Kyu-Hee Hwang[1,2], Ranjan Das[1], Xianglan Quan[1], Tuyet Thi Nguyen[1], Soo-Jin Kim[1,2], Seong-Woo Jeong[1], In-Deok Kong[1], Seung-Kuy Cha[1], Kyu-Sang Park[1]
[1]Department of Physiology, [2]Department of Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, 220-701, Korea

S 66 P04-38 Effects of nitric oxide on voltage-dependent K^+ currents in human cardiac fibroblasts
Hyemi Bae, Donghee Lee, Misuk Yang, Youngwon Kim, Jeongyoon Choi, Jaehong Ko, Hyoweon Bang, Inja Lim
Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

S 66 P04-39 WNK1 promotes tumor progression via TRPC6 activation in clear cell renal cell carcinoma
Ji-Hee Kim[1,3], Kyu-Hee Hwang[1,3], Minseob Eom[2,3], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Kyu-Sang Park[1,3,4], Seung-Kuy Cha[1,3-5]
Departments of [1]Physiology, [2]Pathology, [3]Global Medical Science, [4]Institute of Lifestyle Medicine and [5]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 66 P04-40 Klotho inhibits tumor progression via targeting Orai1 channels in cancer cells
Ji-Hee Kim[1], Kyu-Hee Hwang[1], Seong-Woo Jeong[1,2], In Deok Kong[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2,3,¶]
Departments of [1]Physiology and Global Medical Science, [2]Institute of Lifestyle Medicine, and [3]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 67 P04-41 Inhibition of N-type Ca^{2+} currents in rat peripheral sympathetic neurons by Imidazoline I_2 receptors activation
Soo-Yeon Lee, Eun Jeong Kim, Ji-Hyun Joeng, Young-Hwan Kim, Duck-sun Ahn, Seungsoo Chung
Department of Physiology, Yonsei University College of medicine, Seoul, 120-752, Republic of Korea Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea

S 67 P04-42(O-2) TRPC6 induces hepatic stellate cell activation causing liver fibrosis
Kyu-Hee Hwang[1,2,3], Ji-Hee Kim[1,2,3], Soo-Jin Kim[1,2,3], Ranjan Das[1], Seong-Woo Jeong[1,2,3], In Deok Kong[1,2,3], Kyu-Sang Park[1,2,3], Seung-Kuy Cha[1,2,3,4]
Departments of [1]Physiology and [2]Global Medical Science, [3]Institute of Lifestyle Medicine, and [4]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 67 P04-43 The organellar Ca^{2+} channel TRPML3 regulates early autophagosome biogenesis by interaction with phosphoinositides
Mi Kyung Kim, So Woon Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, 440-746, Korea

S 68 P04-44 Trafficking-dependent N-glycan structure regulates cell surface expression of potassium channel Kv3.1b
Paul Christian Vicentea[1], Jin Young Kim[2], Ji Seon Shim[1], Jeong-Ju Ha[1], Dong-Hyeon Kim[3], Min-Young Song[1], Jin-Sung Choi[3], Kang-Sik Park[1]
[1]Department of Physiology, Kyung Hee University School of Medicine, Seoul 02447, Korea, [2]Division of Mass Spectrometry Research, Korea Basic Science Institute, Cheongju, Chungcheongbuk-do 28119, South Korea, [3]College of Pharmacy, Catholic University of Korea, Bucheon, Gyeonggi-Do 14662, Korea

S 68 P04-45(O-1) Tyrosine phosphorylation of Kv2.1 channel contributes to neuronal cell death in brain ischemia
Min-Young Song[1], Eun Ji Bae[1], Hye-Min Kang[2], Chan Park[2], Kang-Sik Park[1]
[1]Department of Physiology, Department of Anatomy, [2]Kyung Hee University School of Medicine, Seoul 02447, Korea

S 68 P04-46 The phosphorylation sites of potassium channel Kv2.1 determine cell background specific differences in function between cerebellum and cerebrum
Ji Yeon Hwang, Eun Ji Bae, Ji Seon Shim, Kang-Sik Park
Department of Physiology, School of Medicine, Kyung Hee University, Seoul 02447, Korea

P05: Molecular Physiology and Cell Signaling

S 69 P05-01 MicroRNA-200a/210 controls proliferation and Osteogenic differentiation of human adipose tissue stromal cells
Young Suk Kim[1], Hee Jeong Park[1], Keun Koo Shin[1], Seon Young Lee[1], Yong Chan Bae[2], Jin Sup Jung[1]
[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan (626-870), Korea, [2]Department of Plastic Surgery, School of Medicine, Pusan National University, Pusan (602-739), Korea 69

S 69 P05-02

MicroRNA-4284 controls proliferation and Adipogenic and Osteogenic differentiation of human adipose tissue stromal cells
Hee Jeong Park[1], Young Suk Kim[1], Keun Koo Shin[1], Sun Young Lee[1], Yong Chan Bae[2], Jin Sup Jung[1]
[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan (626-870), Korea, [2]Department of Plastic Surgery, School of Medicine, Pusan National University, Pusan (602-739), Korea

S 69 P05-03(O-12)

mTOR signaling in the insular cortex modulates neuropathic pain
Minjee Kwon[1,2,*], Jeongsoo Han[1,2,*], Myeounghoon Cha[1], Un Jeng Kim[1], Bae Hwan Lee[1,2,†]
[1]Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea, [2]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

S 70 P05-04

Nerve injury-induced neuroplasticity in the insular cortex contribute to pain hypersensitivity
Jeongsoo Han, Minjee Kwon, Motomasa Tanioka, Bae Hwan Lee
Department of Physiology, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

S 70 P05-05

Alteration of cardiac hypertrophic marker gene expression by PCB 126 and PCB 77
Mi-Hyeong Park , Su-Hyun Jo
Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea

S 70 P05-06

Impaired cholesterol homeostasis increases the secretion of beta-amyloid peptide in Familial Alzheimer's disease-associated presenilin mutant
Yoon Young Cho, Oh-Hoon Kwon, Sungkwon Chung
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

S 70 P05-07

Trichostatin A inhibits Angiotensin II-induced Hypertension in Vasocontraction and Blood Pressure via Inhibiting p66shc and Reactive Oxygen Species
Yu Ran Lee[1], Gun Kang[1], Hee Kyoung Joo[1], Myoung Soo Park[2], Cuk-Seong Kim[1], Sunga Choi[1], Byeong Hwa Jeon[1,2]
[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, 301-747, Republic of KOREA

S 71 P05-08

Regulation of basal autophagy and Aβ clearance by TRPM7
Hyun Geun Oh, Oh Hoon Kwon, Sungkwon Chung
Dept. of Physiol., Sungkyunkwan Univ. Sch. of Med., Suwon, Republic of Korea

S 71 P05-09

The 18-kDa translocator protein inhibits vascular cell adhesion molecule-1 expression via inhibition of mitochondrial reactive oxygen species
Hee Kyoung Joo[1], Yu Ran Lee[1], Myoung Soo Park[2], Su Hyeon Kim[1], Sunga Choi[1], Byeong Hwa Jeon[1]
[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, Daejeon, Republic of Korea, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Republic of Korea

S 71 P05-10

O-GlcNAcylation-induced GPAT Expression is Critical for Anti-apoptosis under Hypoxia
Hyun Jik Lee, Eun Ju Song, Ki Hoon Lee, Dah Ihm Kim , Jeong Yeon Kim, So Hee Ko, Gee Euhn Choi, Ji Young Oh, Ho Jae Han*
Department of Veterinary Physiology, BK21 PLUS Creative Research Center and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, 08826, Seoul, Korea

S 72 P05-11

Amyloid β-Induced abnormal Autophagolysosome formation leading defective mitochondrial accumulation causes neuronal cell death
Dah Ihm Kim, Jung Min Ryu, Ki Hoon Lee, Jeong Yeon Kim, Gee Euhn Choi, Ing Ing Chai, Ho Jae Han
Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea

S 72 P05-12

Aβ-Induced mTOR Activation is Important for Tau hyperphosphorylation through Regulation of Expression and Autophagy of CDK2 and CDK4
Ki Hoon Lee, Jung Min Ryu, Dah Ihm Kim, Jeong Yeon Kim, Gee Euhn Choi, Ho Jae Han
Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea

S 72 P05-13

Essential Role of Vibrio (V.) vulnificus VvpE in Promoting the Pyroptosis of Intestinal Epithelial Cells
Sei-Jung Lee[1], Hyeon Su Lim[1], Eun Ju Song[1], Jun Sung Kim[1], Kyung Ku Jang[2], Sang Ho Choi[2], Ho Jae Han[1,*]
[1]Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea, [2]National Research Laboratory of Molecular Microbiology and Toxicology, Department of Agricultural Biotechnology, and Center for Food Safety and Toxicology, Seoul National University, Seoul 08826, Korea

S 73 P05-14

EphB2-ephrinB2 signaling-induced Nanog expression is critical for maintaining the differentiation potential of umbilical cord blood derived mesenchymal stem cells
Young Hyun Jung, Sei-Jung Lee, Eun Ju Song, Hyeon Su Lim, Jun Sung Kim, Ho Jae Han*
Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 151-742, Korea

S 73 P05-15

Cdo regulates surface expression of the Kir2.1 K+ channel in myoblast differentiation
Jewoo Koh[1,4,*], Young-Eun Leem[2,4,*], Hyeon-Ju Jeong[2,4,*], Hyun-Ji Kim[1,4,*], Kyungjin Kang[3,4], Jong-Sun Kang[2,4,#], Hana Cho[1,4,#]
[1]Department of Physiology, [2]Department of Molecular Cell Biology, [3]Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, [4]Samsung Biomedical Research Institute, Suwon, 440-746, Republic of Korea

S 73 P05-16

Anti-adhesive activity of the ethanol extracts of Ulmus davidiana var japonica in cultured endothelial cells
Ki Mo Lee[1,*], Hee Kyoung Joo[1,*], Yu Ran Lee[1], Myoung Soo Park[2], Gun Kang[1], Sunga Choi[1], Kwon Ho Lee[3], Byeong Hwa Jeon[1]
[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Republic of KOREA, [3]Department of Physical Therapy, Joongbu University, 201 Daehak-Ro, Chubu-Myeon, Geumsan-Gun, Chungnam 312-702, Korea

S 73 P05-17

FBXO11 represses cellular response to hypoxia by destabilizing hypoxia-inducible factor-1α mRNA
Uk-Il Ju[1], Jong-Wan Park[1,2], Hyoung-Sook Park[1], Sang Jeong Kim[1,2,3], Yang-Sook Chun[1,2,3]
[1]Department of Biomedical Sciences, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea [3]Department of Physiology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea

S 74 P05-18

Fatty acid modulates nitric oxide synthase activity in hypertensive rat atrium
Yu Na WU, Ji Hyun Jang, Sung Joon Kim, Yin Hua Zhang
Department of Physiology & Biomedical Sciences, Seoul National University, College of Medicine, Seoul, Republic of Korea

S 74 P05-19

TGFBIp targeting peptide evaluation
Haeuk Jung[1,3,4], Hye-Nam Son[2], Soyoun Kim[2, 4], In-San Kim[5], Ha-Jeong Kim[1,3,4]
[1]Department of Physiology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea [2]Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea [3]BK21 Plus KNU Biomedical Convergence Program, School of medicine Kyungpook National University, Daegu 700-842, Korea [4]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea [5]Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Republic of Korea

S 74 P05-20(O-4)

The regulatory role of phosphodiesterase 4 inhibitor rolipram in lipopolysaccharides-induced signaling in submandibular glands
Dong Un Lee, Wanhee Suk, Jeong Hee Hong
Department of Physiology, College of Medicine, Gachon University, 191 Hambakmeoro, Yeonsu-gu, Incheon, 406-799, South Korea

S 75 P05-21

TRPC6 as a critical regulator in osteoclastogenesis
Jung Yun Kang, Yu-Mi Yang, Dong Min Shin
Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea

S 75 P05-22

Homer2/3 modulate RANKL-induced NFATc1, osteoclastogenesis and bone metabolism
Yu-Mi Yang, Aran Son, Jung Yun Kang, Dong Min Shin
Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea

S 75 P05-23

Osmo-mechanosensitive TRP channels regulate Ca^{2+}-mediated RANKL expression in mouse osteoblastic cells
Yu-Mi Yang[1], Jung Yun Kang[1], Aran Son[1], Hyo Jin Yang[2], Dong Min Shin[1]
[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, [2]Division of AIDS, Center for Immunopathology, Korea National Institute of Health, Chengju 28160, Korea

S 76 P05-24

Endothelin stimulates inflammatory bone loss in periodontitis
Sue Young Oh[1], So Yun Lee[1,2], Ga-Yeon Son[1], Inik Chang[1], Dong Min Shin[1,2]
Department of [1]Oral Biology, [2]BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, South Korea

S 76 P05-25(O-15)

Disarrangement of regulated exocytosis in TRPML1 knock-out mice
Soonhong Park[1], Min Seuk Kim[3], Shmuel Muallem[2], Dong Min Shin[1]
[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea, [2]Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Bethesda, MD 20892, USA, [3]Department of Physiology, Lab of Oral Biology, College of Dentistry, Wonkwang University, Iksan, Jeonbuk 54538, Korea

S 76 P05-26

Calcium ion regulates WNK/OSR1/NKCC1 pathway in HSG cell-line
Soonhong Park[1], Sang Kyun Ku[2], Hye Won Ji[1], Jong-Hoon Choi[2], Dong Min Shin[1]
[1]Department of Oral Biology, BK21 PLUS Project and [2]Department of Oral Medicine, Yonsei University College of Dentistry, Seoul 03722, Korea

S 77 P05-27

Hypotonic stress induces RANKL via transient receptor potential melastatin 3 (TRPM3) and vaniloid 4 (TRPV4) in human PDL cells
Ga-Yeon Son, Dong Min Shin
Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

S 77 P05-28

Induction of IL-6 and IL-8 by activation of thermosensitive TRP channels in human PDL cells
Ga-Yeon Son, Dong Min Shin
Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

S 77 P05-29

Bacterial PAMPs and allergens trigger increase in $[Ca^{2+}]_i$-induced cytokine expression in human PDL fibroblasts
Ga-Yeon Son, Dong Min Shin
Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

S 77 P05-30

Corn Silk Extract Prevents Carrageenan-Induced Inflammatory Edema by Suppressing Expression of P-Selectin Glycoprotein Ligand-1
Han Na Choi[1], Yong Hwan Kim[1], Soo Jin Kim[1], Yun A Kim[2], Byeong Hwa Jeon[1], Hyun Woo Kim[1], Dong Woon Kim[2], Sang Do Lee[1]
Department of [1]Physiology, Departmeent of [2]Anatomy, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

S 78 P05-31

Airborne allergens induce protease activated receptor-2-mediated production of inflammatory cytokines in human gingival epithelium
Ga-Yeon Son, Dong Min Shin
Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

S 78 P05-32

Macrophages programmed by apoptotic cells inhibit epithelial-mesenchymal transition in lung alveolar epithelial cells via PGE2, PGD2, and HGF
Young-So Yoon, Ye-Ji Lee, Jihee Lee
Department of Physiology, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans Uniersity, Seoul, 158-710, Korea

S 78 P05-33

Diverse effects of a 445 nm diode laser on isometric contraction of the rat aorta
Sang Woong Park, Kyung Chul Shin, Hyun Ji Park, Young Min Bae*
Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, Chungbuk 380-701, South Korea

S 78 P05-34

Suberoylanilide hydroxamic acid enhances apoptotic effect of TNF-α in human lung cancer cells via TNFR1 upregulation
Bo Ra You, Bo Ram Han, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim and Woo Hyun Park*
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, JeonJu, 561-180, Republic of Korea

S 79 P05-35

Hydroquinone intensifies the death of valproic acid-treated SK-LU-1 cells
Bo Ram Han, Bo Ra You, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, JeonJu, 561-180, Republic of Korea

S 79 P05-36

Differential Expression of Taste Receptors in Tongue Papillae
Ha-Jung Choi, Soo-Young Ki, Young-Kyung Cho, Ki-Myung Chung, Kyung-Nyun Kim
Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Sciences, Gangneung-Wonju National University, Gangneung, 210-702, Korea

S 79 P05-37

Expression of Bitter Taste Receptor Tas2r108 mRNA in Murine submandibular gland
Su-Young Ki, Ha-Jung Choi, Ki-Myung Chung, Young-Kyung Cho, Kyung-Nyun Kim
Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Sciences, Gangneung-Wonju National University, Gangneung, 210-702, Korea

S 80 P05-38

A monoclonal antibody against transmembrane proteins of human umbilical vein endothelial cells is a potential inhibitor of endothelium-dependent relaxation in rat aorta
Bong-Woo Park[1], Seung Hyo Jung[1], Donghyen Lee[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Hwan Myung Lee[2], Junghwan Kim[3], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, Konkuk University School of Medicine, Danwol-dong 322, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795; [3]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin 449-714, South Korea

S 80 P05-39(O-3)

TGF-β1-induced apoptosis via Nox4 is mediated by ERK1/2-mTORC1 activation in podocytes
Ranjan Das[1], Shanhua Xu[1,2], Xianglan Quan[1], Tuyet Thi Nguyen[1], Seung-Kuy Cha[1], Seong-Woo Jeong[1], In Deok Kong[1], Kyu-Sang Park[1]
[1]Department of Physiology, [2]Department of Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, Korea

S 80 P05-40

Scoparone inhibits PDGF-BB-induced vascular smooth muscle cells migration via inactivation of mitogen-activated protein kinases signaling pathway
Gyoung Beom Lee[1], Seung Hyo Jung[1], Kang Pa Lee[1], Donghyen Lee[1], Suji Baek[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan Myung Lee[2], Kyung Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

S 81 P05-41

Nafamostat mesilate induces protective effects against TNF-α-induced vascular endothelial cell dysfunction by inhibiting reactive oxygen species production
su jeong choi[1], Jung-Bum Park[1], Harsha Nagar[1], Shin Kwang Kang[2], Saet-byel Jung[3], Sungju Jee[4], Byeong Hwa Jeon[1], Cuk-Seong Kim[1]
[1]Department of Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea, [2]Department of Thoracic and Cardiovascular Surgery, [3]Department of Endocrinology, [4]Department of Rehabilitation Medicine

S 81 P05-42

HN1 Promotes Tumorigenicity through Activation of the SREBP-1 and -2 Lipogenic Signaling Pathway in Hepatocellular Carcinoma
Hua Jin, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

S 81 P05-43

TonEBP/NFAT5 suppresses adipogenesis via modulation of mitotic clonal expansion during early phase of differentiation in 3T3-L1 cells
Soo Jin Kim, Han Na Choi, Hyun-Woo Kim, Jin Bong Park, Byeong Hwa Jeon, Sang Do Lee
Department of Physiology, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

S 82 P05-44

Sirtuin 6 inhibits proliferation and invasion of hepatocellular carcinoma cells
Hua Jin, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

S 82 P05-45

Ursolic Acid inhibits the growth of human esophageal squamous cell carcinoma cells by inducing autophagy
Navin Ray, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

S 82 P05-46

CTHRC1 Stimulates Growth and Metastasis in Esophageal Adenocarcinoma Cells by Activation of the β-catenin/c-Myc Signaling Pathway
Jie Gao[1,2], Kwang Bok Lee[2], Soo Mi Kim[1]
[1]Department of Physiology, [2]Department of Orthopetid Surgery, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

S 82 P05-47

Effect of Macrophage on Induction of Gefitinib Resistance in EGFR Mutated Non Small Cell Lung Cancer Cells
Subodh Sharma[1], Soo Jin Kim[1], Taehee Kim[1], Young Hwan Kim[2], Ji Yeong Mun[1], Han Na Choi[1], Min Woong Kang[2], Sang Do Lee[1]
[1]Department of Physiology, [2]Department of thoracic surgery, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

S 83 P05-48

Gas6/Mer signaling induces transactivation of LXRα-target gene arginase 2 and vascular endothelial growth factor via STAT1 transcription factor in macrophages
Eunjin Lim, Si Yoon Kim, Youn-Hee Choi, Jihee Lee
Injury Defense Research Center, School of Medicine, Ewha Womans University, Seoul 158-056, Korea

S 83 P05-49

Nafamostat mesilate attenuates transient focal ischemia/reperfusion-induced brain injury via the inhibition of endoplasmic reticulum stres
Sun Kwan Kwon, Moonsang Ahn, Hee-Jung Song, Shin Kwang Kang, Saet-byel Sun Kwan Kwon, Moonsang Ahn, Hee-Jung Song, Shin Kwang Kang, Saet-byel Jung, Nagar Harsha, Sungju Jee, Jae Young Moon, Kwang-sun Suh, Sang Do Lee, Byeong Hwa Jeon, Dong Woon Kim, Cuk seong Kim
Department of Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea

S 83 P05-50

Statin pretreatment inhibits LPS-induced EMT via the downregulation of TLR4 and NF-κB in hBECs
Seon Mee Park[1], Yangmi Kim[2]
Department of [1]Gastroenterology, Department of [2]Physiology, College of Medicine, Chungbuk National University, Korea

S 84 P05-51

Regulation of Autophagy by Rapamycin has inhibition cardio-toxicity role in Doxorubicin-Induced Cardiac Progenitor/Stem cells Dysfunction
Ji Hye Park[1,2,3,#], Sang Mo Kwon[1,2,*]
[1]Laboratory of Vascular Medicine and Stem Cell Biology, Department of Physiology, Pusan National University School of Medicine, South Korea, [2]Convergence Stem Cell Research Center, Department of Physiology, Pusan National University School of Medicine, South Korea. [3]Pusan National University Medical Science, Education Center (BK21 Program), Pusan National University School of Medicine, Yangsan 626-870, Korea

S 84 P05-52(O-8)

Serum protein Fetuin-B is involved in immune cells and vascular smooth muscle cells-linked atherosclerotic plaque stability
Donghyen Lee[1], Seung Hyo Jung[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Suji Baek[1], Bong-Woo Park[1], Junghwan Kim[2], Hwan-Myung Lee[3], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin 449-714, Korea, [3]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

S 84 P05-53

DJ-1 contributes to sphingophosphorylcholine-induced differentiation of human mesenchymal stem cells into smooth muscle cells
Suji Baek[1], Kang Pa Lee[1], Seung Hyo Jung[1], Gyoung Beom Lee[1], Donghyen Lee[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan-Myung Lee[2], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

S 85 P05-54

532 nm laser irradiation suppresses restenotic lesion-related responses in PDGF-BB-stimulated vascular smooth muscle cells
Seung Hyo Jung[1], Suji Baek[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan-Myung Lee[2], Young Min Bae[1], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

S 85 P05-55

Angiotensin II induces migration via APE/Ref-1-mediated transactivation of sphingosine-1-phosphate receptor in vascular smooth muscle cells
Kang Pa Lee[1], Dong-Youb Lee[1], Dong Hyen Lee[1], SeungHyo Jung[1], Suji Baek[1], Yuri Park[1], Hwan Myung Lee[2], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, Seoul 143-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

S 85 P05-56

Inhibition of Pi transport across plasma and mitochondrial membrane prevents high phosphate-induced vascular calcification
Tuyet Thi Nguyen[1], Shanhua Xu[1,2], Ranjan Das[1], Xianglan Quan[1], Ji-Hee Kim[1], Kyu-Hee Hwang[1,2], Seung-Kuy Cha[1], Seong-Woo Jeong[1], In Deok Kong[1], Kyu-Sang Park[1]
Department of [1]Physiology and [2]Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, Korea

S 86 P05-57 Cul3-KLHL22 E3 ubiquitin ligase and TWIST target anterior gradient-2 and regulate tumor progression
Seok Yun Jung, Sang-Mo Kwon
Laboratory for Vascular Medicine & Stem Cell Biology, Department of Physiology, School of Medicine, Pusan National University, 49, Busandaehak-ro, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do, Korea

S 86 P05-58 Engineered M13 Phage as a Novel Therapeutics to enhance Endothelial Progenitor Cell-based Neovascularization
Sung Wook Kim, Jun Hee Lee, Sang Mo Kwon
Laboratory for Vascular Medicine&Stem cell biology, Medical Research Institute, Department of Physiology, Pusan National University, Pusan, Korea

S 86 P05-59 Tat-biliverdin reductase A protects insulin-producing INS-1 cells from islet amyloid polypeptide (IAPP)-induced apoptosis by alleviating oxidative and endoplasmic reticulum stresses
Su Jin Lee, Hyung Kyung Kang, Won Sik Eum[1], Soo Young Choi[1], Hyeok Yil Kwon
Department of Physiology, College of Medicine, [1]Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Chunchon 200-702, Korea

P06: Muscle, Cardiovascular, and G-I System

S 87 P06-01 Absence of of hypoxic augmentation of vasoconstriction in the femoral artery from eNOS deficient mice
Hae Jin Kim, Hae Young Yoo, Yin Hua Zhang, Sung Joon Kim
Department of Physiology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, KOREA

S 87 P06-02 Augmented vascular reactivity and hypoxic pulmonary vasoconstriction in monocrotaline-induced pulmonary arterial hypertension rats
Hae Jin Kim[1,2], Yin Hua Zhang[1], Sung Joon Kim[1,2], Hae Young Yoo[3]
[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea, [3]Chung-Ang University College of Nursing, Seoul 156-756, Korea

S 87 P06-03 Smooth Muscle Cell Genome Browser: Enabling the Identification of Novel Serum Response Factor Target Genes
Moon Young Lee[1,2], Chanjae Park[1], Robyn M. Berent[1], Paul J. Park[1], Robert Fuchs[1], Hannah Syn[1], Albert Chin[1], Jared Townsend[1], Craig C. Benson[3], Doug Redelman[1], Tsaiwei Shen[4], Jong Kun Park[5], Joseph M. Miano[3], Kenton M. Sanders[1], Seungil Ro[1]
[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, United States of America, [2]Department of Physiology, Wonkwang Digestive Disease Research Institute and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, Jeollabuk-do, Korea, [3]Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York, United States of America, [4]LC Sciences, 2575 West Bellfort Street Suite 270, Houston, Texas, United States of America, [5]Division of Biological Science, Wonkwang University, Iksan, Jeollabuk-do, South Korea

S 88 P06-04 Serum Response Factor Is Essential for Prenatal Gastrointestinal Smooth Muscle Development and Maintenance of Differentiated Phenotype
Chanjae Park[1], Moon Young Lee[1,2], Paul J Park[1], Se Eun Ha[1], Robyn Berent[1], Robert Fuchs[1], Joseph M Miano[3], Laren S Becker[4], Kenton M Sanders[1], Seungil Ro[1]
[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, USA, [2]Department of Physiology, Wonkwang Digestive Disease Research Institute and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, Jeollabuk-do, Korea, [3]Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA, and [4]Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, California, USA

S 88 P06-05 Loss of Cdo leads to alteration in N-cadherin and connexin with intercellular coupling defects and cardiomyopathy
Hyun-Ji Kim[1,3,#], Myong-Ho Jeong[2,3,#], Kyu-Sil Choi[3], Young-Hwan Song[4], Gordon F. Tomaselli[5], Jong-Sun Kang[2,3,*], Hana Cho[1,3,*]
[1]Department of Physiology, [2]Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, 440-746, [3]Samsung Biomedical Research Institute, Samsung medical center, Seoul 135-710, [4]Devision of Cardiology, Seoul National University Bundang Hospital, 463-707, Republic of Korea, [5]Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

S 88 P06-06 Sildenafil is effective to enhance the proliferation of skeletal myoblasts
Mei Huang, Keon Jin Lee, Mi Kyoung Ahn, Eun Hui Lee
Department of Physiology, College of Medicine, The Catholic University of Korea, Banpo-daero 222, Seocho-gu, Seoul 137-701, Republic of Korea

S 88 P06-07

Interaction between mitsugumin 29 and TRPC3 participates in regulating calcium transients in skeletal
Jin Seok Woo[1], Ji-Hye Hwang[1], Mei Huang[1], Mi Kyoung Ahn[1], Mi Ri Oh[1], Chung-Hyun Cho[2], Jianjie Ma[3], Eun Hui Lee[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Banpo-daero 222, Seocho-gu, Seoul 137-701, Republic of Korea , [2]Department of Pharmacology, College of Medicine, Seoul National University, Seoul 110-799, Republic of Korea, [3]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210, USA

S 89 P06-08

Inhibition of nNOS facilitates myofilament disarray and cardiac hypertrophy in Ang II-induced hypertensive rat
Ji Hyun Jang, Zai Hao Zhao, Sung Joon Kim, Yin Hua Zhang
Department of Physiology, Seoul National University, College of Medicine, Seoul, Republic of Korea

S 89 P06-09

Signaling pathway and physiological role of WNK1 in mouse skeletal muscle
Hanul Kim[1,2,3], Ji-Hee Kim[1,2,3], Kyu-Hee Hwang[1,2,3], Kyu-Sang Park[1,2,3], Seong-Woo Jeong[1,2,3], Seung-Kuy Cha[1,2,3], In Deok Kong[1,2,3]
Departments of [1]Physiology and [2]Global Medical Science, and [3]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 89 P06-10

Inhibition of Endoplasmic Reticulum Stress Normalizes Augmented Myogenic Responses in Coronary Arteries of the Spontaneously Hypertensive Rats
Soo-Kyoung Choi, Mihwa Lim, Duck-Sun Ahn, Young-Ho Lee
Department of [1]Physiology, College of Medicine, [2]BK 21 Plus Project for Medical Sciences, Yonsei University, C.P.O Box 8044, Seoul,120-752, Korea

S 90 P06-11(O-6)

Does eNOS-palmitoylation involve in palmitic acid-enhanced cardiac inotropy in rat cardiac myocyte?
Chun Li Jin, Ji Hyun Jang, Yu Na Wu, Zhai Hao Zhao, Sung Joon Kim, Yin Hua Zhang*
Department of Physiology, Seoul National University College of Medicine, Seoul 110-799, Korea

S 90 P06-12

Beta1-adrenergic receptor antagonist and nitric oxide stimulator, nebivolol, prevents spontaneous contraction induced by metabolic substrates in rat cardiomyocytes
Zai Hao Zhao, Ji Hyun Jang, Jae Hwi Sung, Yin Hua Zhang*
Department of Physiology & Biomedical Sciences, Seoul National University, College of Medicine, Seoul, 110-799 Korea

S 90 P06-13

Angiotensin IV protects cardiac reperfusion against via AT4R by inhibiting apoptosis and inflammation
Byung Mun Park[1], Seung Ah Cha[1], Sun Hwa Lee[2], Byung Hyun Park[3], Yuan Kuichang[1], Suhn Hee Kim[1]
Department of [1]Physiology, [2]Internal Medicine, and [3]Biochemistry, Chonbuk National University Medical School, Jeonju, Korea

S 91 P06-14

Cereblon gene dysfunction improves cardiac performance and mitochondrial energy metabolism in mice
Sujin Noh, Hyoung Kyu Kim, Tae Hee Ko, Seung Hun Jeong, In-Sung Song, Sung Ryul Lee, Hye Jin Heo, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Department of Health Sciences and Technology, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

S 91 P06-15

DQAsome, a Mitochondria targeting carrrier, shows cardiac toxicity via suppresing cardiac Ca2+ signaling
Hyoung Kyu Kim, Seung Hun Jeong, Tae Hee Ko, Sujin Noh, In-Sung Song, Sung Ryul Lee, Hye Jin Heo, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Joon Sig Choi, Jin Han
Cardiovascualr and Metabolic Disease Center, Colleage of Medicine, Inje University, Busan, Korea

S 91 P06-16

The difference between vascular smooth muscle contraction and relaxation in four different aortic regions and their aortic parameters in rats
Bolor-Erdene Sarankhuu*, Nari Kim*
National Research Laboratory for Mitochondrial Signaling Laboratory, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan 613-735, Korea

S 92 P06-17

Role of Formyl Peptide Receptors on Mobilization of Peripheral Blood Stem Cells in Myocardial Ischemia Injury
Soon Chul Heo[1], Yang Woo Kwon[1], Geun Ok Jeong[1], Jung Won Yoon[1], Tae Wook Lee[1], Il Ho Jang[1], Jae Kyung Park[1], Jae Ho Kim[1,2]
[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-870, Gyeongsangnam-do, Republic of Korea, [2]Research Institute of Convergence Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 626-770, Gyeongsangnam-do, Republic of Korea

S 92 P06-18 Antihypertensive effects of fermented garlic extract through NO-cGMP-PKG pathway in SHR
Byung Mun Park[1], Seung Ah Cha[1], Yuan Kuichang[1], De Gil Kang[1], Suhn Hee Kim[1]
[1]Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea, [2]Department of Physiology, Wonkwang University Oriental Medicine, Iksan, Korea

S 92 P06-19 Echinochrome A inhibits vascular smooth muscle cell phenotype changing
Kyowon Seo, Seunghun Jeong, Jin Han, Nari Kim
Cardiovascular and Metabolic Disease Center (CMDC), National Research Laboratory for Mitochondrial Signaling, Inje University, Busan 614-735, Korea

S 93 P06-20 The effect of microRNA-34c on angiogenesis capacity of high glucose-insulted mesenchymal stem cells
Yong Sook Kim[1], Youngkeun Ahn[2]
[1]Biomedical Research Institute, Chonnam National University Hospital, Gwangju, South Korea [2]Department of Cardiology, Chonnam National University Hospital, Gwangju, South Korea

P07: Neurophysiology

S 93 P07-01 Epigallocatechin-3-Gallate Rescues LPS-impaired Adult Hippocampal Neurogenesis through Suppressing the TLR4-NF-κB Signaling Pathway in Mice
Kyung-Joo Seong[1,2], Hyun-Gwan Lee[1,2], Hyun-Mi KO[3], Min Suk Kook[4], Ji-Yeon Jung[1,2], Won-Jae Kim[1,2,**]
[1]Dental Science Research Institute, Medical Research Center for Biomineralization Disorders, [2]Department of Oral Physiology, [3]Department of Oral and Maxillofacial Surgery, School of Dentistry, Chonnam National University, Gwangju 500-757, Republic of Korea, [4]Department of Microbiology, collage of Medicine, Seonam Universtity, Namwon 55724, Republic of Korea

S 93 P07-02 The effect of BD1047 in CCL2 mediated microglia activation in zymosan induced hyperalgesia in rats
Young Bae Kwon
Department of pharmacology, Medical School, Chonbuk National University, Jeonju, Korea

S 94 P07-03 Repetitive motor cortex stimulation for the chronic neuropathic pain
Myeounghoon Cha, Bae Hwan Lee
Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea

S 94 P07-04 Maresin 1 inhibits TRPV1 in temporomandibular joint (TMJ)-related trigeminal nociceptive neurons and TMJ inflammaion-induced synaptic plasticity in the trigeminal nucleus
Sang Taek Im, Jee Eun Lee, Chul-Kyo Park
Department of Physiology, College of Medicine, Gachon University, Incheon 406-799, Republic of Korea

S 94 P07-05 Neuroprotective Effects of Okadaic Acid Following Oxidative Injury in Organotypic Hippocampal Slice Culture
Un Jeng Kim[1,*], Kyung Hee Lee[2], Bae Hwan Lee[1,3,†]
[1]Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea, [2]Department of Dental Hygiene, Division of Health Science, Dongseo University, Busan 47011, Korea, [3]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

S 94 P07-06 NDL-PCBs inhibit store-operated Ca2+ entry
Se-Young Choi[1,2], Keimin Lee[1], Yurim Park[3], Seung-Hyun Lee[1], Mi-Hyeong Park[4], Sungkwon Chung[3], Kyong-Tai Kim[2], Su-Hyun Jo[4]
[1]Department of Physiology, Dental Research Institute, Seoul National University School of Dentistry, Seoul 110-749, Korea, [2]Department of Life Sciences, Division of Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang 790-784, Korea, [3]Department of Physiology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea, [4]Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea

S 95 P07-07 The effect of ultrasound stimulation on neurogenesis
Dough Kim[1,2], Ha-Jeong Kim[1,2,3], Hak Jong Lee[4], Hyung Soo Han[1,2,*]
[1]Department of Physiology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea, [2]BK21 Plus KNU Biomedical Convergence Program, Department of Biomedical Science, Kyungpook National University, Korea, [3]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea, [4]Department of Radiology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam 463-707, Korea

S 95 P07-08(O-7)

Necrotic cells Influence Migration and Proliferation of Glioblastoma cells through NF-κB/IL-8 Signaling
So-Hee Ahn, Hyunju Park, Jiwoo Lim, Yieun Jung, Jihee Lee Kang, Youn-Hee Choi
Department of [1]Physiology, Ewha Womans University School of Medicine, Seoul 911-1, Korea, [2]Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

S 95 P07-09

Utilizing Ultrasound to Transiently Increase Blood-Brain Barrier Permeability, Modulate of the Tight Junction Proteins, and Alter Cytoskeletal Structure
Mi Jung Bae[1,2], Young Mi Lee[1], Su Yeon Ryu[1,2,4], Yeoun Hee Kim5, Hyung Soo Han[1], Hak Jong Lee[3]
Department of [1]Physiology, [2]BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, 101 Dongin 2 Ga, Jung Gu, Daegu 700-422, Korea Department of [3]Radiology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam 463-707, Korea, [4]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea 5Cheil eye research institute, Cheil eye hospital, 1 Ayang-ro, Dong-gu, Daegu 701-820, Korea

S 96 P07-10

PAMAM dendrimer-conjugated TA attenuates mechanical allodynia by inhibiting spinal cord microglia activation
Hwisung Kim[1], Hyoungsub Lim[1], Hyunjung Min[1], Sunghyoun Choi[2], Jong-sang Park[2], Sung Joong Lee[1,*]
[1]Department of Neuroscience and Physiology, Dental Research Institute, BK21-Plus, School of Dentistry, Seoul National University, Seoul 03080, Republic of Korea, [2]School of Chemistry and Molecular Engineering, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, 08826, Republic of Korea

S 96 P07-11

Activation of satellite glia after peripheral nerve injury induces spinal cord microglia activation and neuropathic pain via ganglioside-TLR2 signaling
Hyoung Sub Lim[1,2], Hyun Kyoung Lee[1], Kyung Chul Noh[1], Byung Hyun You[1], Jae Hoon Oh[3], Hyuck Jun Mok[4], Byung Gon Kim[5], Jong-Sang Park[3], Kwang Pyo Kim[4], Sung Joong Lee[1,2,*]
[1]Department of Neuroscience and Physiology, Dental Research Institute, BK21-Plus, School of Dentistry, Seoul National University, Seoul 110-799, Republic of Korea, [2]Interdisciplinary Program in Neuroscience, College of Natural Science, Seoul National University, Seoul 151-747, Republic of Korea, [3]Department of Chemistry, Seoul National University, Seoul 151-747, Republic of Korea, [4]Department of Applied Chemistry, College of Applied Sciences, Kyung Hee University, Yongin 446-701, Republic of Korea, [5]Departments of Brain Science and Neurology, Ajou University School of Medicine, Suwon 443-721, Republic of Korea

S 96 P07-12

Spinal leptin enhances NMDA receptor-mediated tactile hypersensitivity via the reactive oxygen species-phosphatidylinositol 3-kinase (ROS-PI3K) pathway in neuropathic rats
Se Jung Jung[1], Euichan Lee[1,2], Jae Beom Jun[1,2], Min Kyung Ko[1], Joong Woo Leem[1,2]
Department of [1]Physiology, [2]Brain Research Institute, Yonsei University College of Medicine, Seoul 120-752, Korea

S 97 P07-13

Spinal D-serine induces increase in GluN1 phosphorylation and nociception via nNOS activation in mice: involvement of sigma-1 receptors
Sheu-Ran Choi[1], Ji-Young Moon[2], Soon-Gu Kwon[1], Hoon-Seong Choi[1], Mi-Ji Lee[1], Ho-Jae Han[1], Jang-Hern Lee[1]
[1]Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea, [2]KM Fundamental Research Division, Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea

S 97 P07-14

Astrocyte gap junction contribute to development of mirror-image mechanical allodynia in peripheral inflammatory rat: Suppressive effect of spinal interleukin-1β on connexin 43 expression
Hoon-Seong Choi, Sheu-Ran Choi, Soon-Gu Kwon, Mi-Ji Lee, Ho-Jae Han, Jang-Hern Lee*
Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

S 98 P07-15

Effects of TCDD exposure on the gonadotropin releasing hormone neurons in mice
Pravin Bhattarai[1], Janardhan Prasad Bhattarai[1], Dong Hyu Cho[2], Seong Kyu Han[1]
[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, [2]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine, Chonbuk National University

S 98 P07-16

Action of calcitriol on NMDA and kainate receptor-mediated actions in juvenile GnRH neurons
Pravin Bhattarai[1], Janardhan P. Bhattarai[1], Min Sun Kim[2], Dong Hyu Cho[3], Seong Kyu Han[1]
[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, [2]Department of Pediatrics & Research Institute of Clinical Medicine, School of Medicine, [3]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine, Chonbuk National University

S 98 P07-17

Effect of BPA over pre- and post- natal development of Gonadotropin Releasing Hormone Neurons
Janardhan P. Bhattarai, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

S 98 P07-18

Loss of Tumor Suppressor PML Promotes Cell Cycle Progression and Proliferation By Enhancing STAT-3 Activity
Jiwoo Lim, Hyunju Park, So-Hee Ahn, Youn-Hee Choi
Department of Physiology, Ewha Womans University School of Medicine, Seoul 911-1, Korea Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

S 99 P07-19

Necrotic cells Influence Glioblastoma progression through regulating MCP-1 and MIP-3α expression
Yieun Jung, So-Hee Ahn, Hyunju Park, Jihee Lee Kang, Youn-Hee Choi
Department of Physiology, Ewha Womans University School of Medicine, Seoul 911-1, Korea, Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

S 99 P07-20(O-14)

Generation and regulation of pacemaker activity by TRPC3 channels in nigral dopamine neurons
Ki Bum Um, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

S 99 P07-21

Noradrenergic Regulation of Cerebellar Output during Arousal
Seung-Eon Roh[1,2], Seung-Ha Kim[1], Chang-Eop Kim[1], Yong-Gyu Kim[1,2], Sun Kwang Kim[3], Sang Jeong Kim[1,2]
[1]Department of Physiology and [2]Department of Biomedical Science, College of Medicine, Seoul National University, [3]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, South Korea

S 100 P07-22

Treatment With Diluted Bee Venom Reduces Both Spinal Inflammatory Responses And Central Neuropathic Pain Behaviors After Spinal Cord Injury In Rats
Ji-Young Moon, Suk-Yun Kang, Seong Jin Cho, O Sang Kwon, Sun Hee Yeon, Kwang-Ho Choi, Jang-Hern Lee[1], Yeonhee Ryu
KM Fundamental Research Division, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea, [1]Deptment of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

S 100 P07-23

Immunosuppressive effect of estrogen ameliorates pruritic atopic dermatitis in the pubertal female rats
Jeahee Lee[1], Hye Young Kim[1], Taeho Han[1], Seung Keun Back[2,*] and Heung Sik Na[1,*]
[1]Neuroscience Research Institute and Department of Physiology, Korea University College of Medicine, 126-1 Anam-dong 5 Ga, Seongbuk-Gu, Seoul, 136-705, Korea, [2]Department of Pharmaceutics & Biotechnology, College of Medical Engineering, Konyang University, Chungnam 320-711, Korea

S 100 P07-24

Spontaneous firing system of substantia nigra dopamine neurons: proximal dendrites as an accelerator and the soma as a counteract balancer
Jinyoung Jang, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Suwon, Korea

S 100 P07-25(O-5)

Novel function of histone demethylase of JHDM in spatial learning and memory
Hye-Jin Kim, Seon-Young Kim, Myoung-Hwan Kim, Sang Jeong Kim, Yang-Sook Chun
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 101 P07-26

Hypotaurine action mediated by α-homomeric & αβ-hetromeric glycine receptors in medullary dorsal horn neurons
Sun Mi Oh, Seong Kyu Han, Soo Joung Park
Department of Oral Physiology & Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, Korea

S 101 P07-27

Role of Neuregulin-2 in synaptogenesis in newborn granule cells
Kyu-Hee Lee, Hyun-Su Lee, Che Ho Yang, Won-kyung Ho, Suk-Ho Lee
Department of Physiology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul, Korea

S 101 P07-28

Portal hypertension is associated with the impairment of arterial baroreflex and hypoexcitability of aortic baroreceptor neurons in cirrhotic rats
Choong-Ku Lee, Jae-Won Lee, Seong-Woo Jeong
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju 220-701, Korea

S 102 P07-29

Intraplantar injection of DHEAS or PREGS enhance P2X mediated mechanical allodynia via sigma-1 receptors in rats
Soon-Gu Kwon, Sheu-Ran Choi, Hoon-Seong Choi, MiJi Lee, Jang-Hern Lee
Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

S 102 P07-30

Investigation of leak channels important for pacemaking in the nigral dopamine neurons
Su Yun Hahn, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

S 102 P07-31

LTP of dendritic spines in nigral dopamine neurons : possible link of reward and burst
Min Jung Kim, Miae Jang, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

S 103 P07-32

Electroacupuncture alleviates mechanical allodynia via spinal opioidergic and alpha2-adrenergic mechanisms in oxaliplatin- or vincristine-induced neuropathy mice model
Jung-Wan Choi[1], Suk-Yun Kang[1], Kwon O Sang[1], Yeon Sun Hee[1], Yeon-Hee Ryu[1], Hyun-Woo Kim[2]
[1]Korea Institute of Oriental Medicine, Daejeon, 305-811, South Korea [2]Department of Physiology and Institute of Brain Research, Chungnam National University School of Medicine, Daejeon, 301-747, South Korea

S 103 P07-33(O-13)

Agonist-independent activity of mGluR1 underlies homeostatic control of intrinsic excitability via IH in cerebellar Purkinje cells
Hyun Geun Shim[1,2], Sung-Soo Jang[1,3], Dong Cheol Jang[1,4], Joo Min Park[5], Sang Jeong Kim[1,2,3]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Science, Seoul National University College of Medicine, [3]Neuroscience Research Institute, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul, 110-799, Korea, [4]Department of Brain and Cognitive Sciences, College of Science, Seoul National University, Kwanak-gu, Seoul, 151-742, Korea, [5]Center for Cognition and Sociality, Institute for Basic Science, Daejeon, 305-811, Korea

S 103 P07-34

Distinct responses of vagal and splanchnic nerves innervating liver to 5-HT receptor agonists in Guinea pigs
Yong Seok Yang*, Jae Jun Han, kyung Min Choi, Hong Soon Lim, Min-Goo Lee
Department of Physiology, Korea University College of Medicine, Seoul 136-705

S 104 P07-35

Primary afferents temporally encode the noxious stimulus for pain signaling
K.W. CHO[1,*], J.Z. LIM[2], S.-P. KIM[3], D.P. JANG[1], S.J. JUNG[2]
[1]Department of Biomedical Engineering, Hanyang University, Seoul, Korea, [2]Department of Biomedical Sciences, Hanyang University, Seoul, Korea, [3]School of Design and Human Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

S 104 P07-36

Three distinct cerebellum-dependent eye movement learning in pcp2-cre mice
Dong Cheol Jang[1,2], Sang Jeong Kim[1,2]
[1]Department of Brain and Cognitive Science, College of Science , [2]Department of Physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea

S 104 P07-37

Dysregulation of metabotropic glutamate receptor 5 in periaqueductal gray perpetuate chronic neuropathic pain
Geehoon Chung[1,2], Hyun Geun Shim[2,3], Chae Young Kim[2,3], Sang Jeong Kim[1,2,3]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences, [3]Department of Biomedical Sciences, Seoul National University College of Medicine

S 105 P07-38

Upregulation of prelimbic metabotropic glutamate receptor 5 in chronic neuropathic pain state
Chae Young Kim[1,2], Geehoon Chung[1,3], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2,3]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, [3]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences

S 105 P07-39

Changes in Field Potentials Following Transcranial Direct Current Stimulation on the Motor Cortex of Rats in Vivo
Ho Koo[1], Yong-Il Shin[2], Yu Fan[3], Sang Hu Han[1], Min Sun Kim[1]
[1]Department of Physiology, Wonkwang University School of Medicine, Iksan, Korea, [2]Department of Rehabilitation Medicine, Pusan National University School of Medicine, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Pusan, Korea, [3]Department of Meridian & Acupoint, College of Korean Medicine, Wonkwang University, Iksan, Korea

S 105 P07-40

Characterizing the function of Negr1, a newly-identified obesity-related gene, in the nervous system
Kyungchul Noh, Hyunkyoung Lee, Soo-Jeong Kim, Tae-Yong Choi, Se-Young Choi, Sung Joong Lee
Department of Neuroscience and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, 03080, Republic of Korea

S 105 P07-41

The of roles of GABA on the motivational driving force for the pain attenuation following spinal cord injury in rats
Moon Yi Ko[1], Jun Yeon Lee[1], Su Phil Kim[2], Hee Young Kim[2], Chae Ha Yang[2], Young S. Gwak[2]
[1]Department of Aroma Application Industry, Daegu Hanny University, Kyungsan si, Kyungsanbukdo, 38610, Korea, [2]Department of Physiology, Daegu Haany University, Daegu 42158, Korea

S 106 P07-42

Carvacrol inhibits mGluR1-evoked slow currents in cerebellum
Da Eun Jeon, Sang Jeong Kim
Department of Physiology, College of Medicine, Seoul National University

S 106 P07-43 Anti-inflammatory role of cytoplasmic Ref-1 in cultured astrocytes
Hyang-Joo Lee, Hyun-Sill Cho, Sudip Pandit, Yoon-Hyung Pai, Byeong Hwa Jeon, Jin Bong Park
Department of Physiology, Brain Research Institute, School of Medicine, Chungnam National University, 6 Munhwa-Ro, Jung-gu, Daejeon, 301-131, Republic of Korea

S 106 P07-44 Clonidine, an alpha-2 adrenoceptor agonist relieves mechanical allodynia in oxaliplatin-induced neuropathic mice; potentiation by spinal p38 MAPK inhibition without motor dysfunction and hypotension
Ji-Hee Yeo[1], Seo-Yeon Yoon[2], Sol-Ji Kim[1], Jang-Hern Lee[3],
Alvin J. Beitz[4], Dae-Hyun Roh[1,*]
[1]Department of Oral Physiology and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea, [2]Pain Cognitive Function Research Center, Department of Brain and Cognitive Sciences College of Natural Sciences, Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea, [3]Department of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, [4]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

S 107 P07-45 Role of capsaicin-sensitive primary afferents in the development of hypersensitivity in a new mouse model for nitroglycerin-induced chronic migraine
Sol-Ji Kim[1], Seo-Yeon Yoon[2], Ji-Hee Yeo[1], Dae-Hyun Roh[1,*]
[1]Department of Oral Physiology and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea, [2]Pain Cognitive Function Research Center, Department of Brain and Cognitive Sciences College of Natural Sciences, Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea

P08: Physiome and Systems Biology

S 107 P08-01 Altered rhythmic behaviors in Alzheimer's disease model flies by dim light exposure at night
Manivannan Subramanian[1], Mari Kim[2], Eunil Lee[2], Joong-Jean Park[1]
[1]Department of Physiology, [2]Department of Preventive Medicine, Korea University College of Medicine, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

P09: Renal, Respiratory and Reproductive Physiology

S 107 P09-01 Signals governing the trafficking of PKD1L1 and PKD2L1 to primary cilia
Kotdaji Ha[1], Insuk So[1]
Department of Physiology, Seoul National University, College of Medicine, Seoul, Republic of Korea[1]

S 108 P09-02 Klotho ameliorates proteinuria through protecting podocyte injury
Ji-Hee Kim[1], Kyu-Hee Hwang[1], Seong-Woo Jeong[12], In Deok Kong[12], Kyu-Sang Park[12], Seung-Kuy Cha[1-3¶]
Departments of [1]Physiology and Global Medical Science, [2]Institute of Lifestyle Medicine and [3]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 108 P09-03 Klotho inhibits tumor progression by IGF-1 receptor activation in human clear cell renal cell carcinoma
Ji-Hee Kim[1,3], Kyu-Hee Hwang[1,3], Minseob Eom[2,3], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Kyu-Sang Park[1,3,4], Seung-Kuy Cha[1,3-5,¶]
Departments of [1]Physiology, [2]Pathology, [3]Global Medical Science, [4]Institute of Lifestyle Medicine and [5]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 108 P09-04 Orai1 expression is closely related with favorable prognostic factors in clear cell renal cell carcinoma
Kyu-Hee Hwang[1,3,4], Ji-Hee Kim[1,3,4], Sayamaa Lkhagvadorj[2,3], Minseob Eom[2,3], Kyu-Sang Park[1,3,4], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Seung-Kuy Cha[1,3,4]
Departments of [1]Physiology, [2]Pathology and [3]Global Medical Science, and [4]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

P10: Other Area

S 109 P10-01 Improved application of the electrophoretic tissue clearing technology, CLARITY, to intact solid organs
Hyunsu Lee, Incheol Seo, Shin Kim, Jae-Hyung Park
Department of Physiology, Keimyung University School of Medicine, 1095 Dalgubeoldaero, Dalseo-Gu, Daegu, 704-701, Korea

S 109 P10-02 The Effect of Bio-active Materials Coated Fabric on Rat Skeletal Muscular Mitochondria
Donghee Lee, Young-Won Kim, Misuk Yang, Hyemi Bae, Inja Lim, Hyoweon Bang, Jae-Hong Ko
Department of Physiology, College of Medicine Chung-Ang University, Seoul 156-756

S 109 P10-03

1Identification of Primo-Vascular System in Abdominal Subcutaneous Tissue Layer of Rats
Chae Jeong Lim, So Yeong Lee, Pan Dong Ryu
Laboratory of Veterinary Pharmacology, College of VeterinaryMedicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea University, Seoul 151-742, Republic of Korea

S 110 P10-04

Toll-like Receptor 2 is Dispensable for an Immediate-early Microglial Reaction to Two-photon Laser-induced Cortical Injury In vivo
Heera Yoon[1], Yong Ho Jang[2], Sang Jeong Kim[3,*], Sung Joong Lee[2,*], Sun Kwang Kim[1,*]
[1]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul 130-701, [2]Department of Oral Physiology and Neuroscience, School of Dentistry, Seoul National University, Seoul 110-749, [3]Department of Physiology, School of Medicine, Seoul National University, Seoul 110-799, Korea

S 110 P10-05

MLN4924 can promote U373MG cell migration via src dependent phosphorylation of caveolin-1
Sung Yeon Park, Yang-Sook Chun
Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S 110 P10-06

Study investigated the effects of Oligonol supplementation on sudomotor activity during heat load in human subjects
Jeong Beom Lee, Sun Jong Kang, Sang Eun Im, Jae Young Heo, Hyun Soo Kim, Sang Mook Kim, Hyun Kyu Kang, Jung Ho Kim, Sung Woon Kim
Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea

S 110 P10-07

PSA-NCAM-Negative Neural Crest Cells Emerging During Neural Induction of Pluripotent Stem Cells Cause Mesodermal Tumors and Unwanted Grafts
Dongjin R. Lee[1], Jeong-Eun Yoo[1], Jae Souk Lee[1], Sanghyun Park[1], Junwon Lee[1], Chul-Yong Park[1], Eunhyun Ji[1], Han-Soo Kim[2], Dong-Youn Hwang[3], Dae-Sung Kim[4,*], Dong-Wook Kim[1,*]
[1]Department of Physiology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, [2]Cell Therapy Center and Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul 120-752, Korea, [3]Department of Biomedical Science, College of Life Science, CHA University, Seongnam, Gyeonggi-do 463-840, Korea, [4]Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea

S 111 P10-08

The role of TRPM7 in the progression of human renal cell carcinoma (RCC)
Soon Hee Kim[1], Su Yeon Ryu[1,2,3], Jae Sik Park[1], Eun Kyoung Yang[1]
Department of [1]Physiology, Kyungpook National University, School of Medicine, Daegu 700-422, [2]BK21 Plus KNU Biomedical Convergence Program, School of medicine Kyungpook National University, Daegu 700-842, [3]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea

S 111 P10-09

A novel function of JHDM in Hepatic steatosis
Jung-Yup Song[1], Kyung-Hwa Lee[1], Yang-Sook Chun[1,2,3]
[1]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea, [2]Departments of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Korea, [3]Departments of Physiology, Seoul National University College of Medicine, Seoul 110-799, Korea

S 111 P10-10

KSP inhibitor SB743921 induces death of multiple myeloma cells via inhibition of the NF-kB signaling pathway
Bayalagmaa Nyamaa, In Sung Song, Yu Jeong Jeong, Hyoung Kyu Kim, Naru Kim, Jin Han
Department of Physiology, College of Medicine, Inje University, Busan, Korea

S 112 P10-11

The Effect of Low-Intensity Ultrasound in Resolution of Synovitis
Jee-In Chung, A Young Kim, Sumit Barua, Soo Yeon Lee, Eun Joo Baik
Department of Physiology, Department of Biomedical Science, Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

S 112 P10-12

Role of CXCR2 in Acetylated Pro-Gly-Pro(Ac-PGP)-induced Vascular Regeneration in Murine Hind limb Ischemia Model
Yang Woo Kwon, Soon Chul Heo, Jung Won Yoon, Tae Wook Lee, Ba Reun Kim, Geun Ok Jeong, Jae Ho Kim
Medical Research Center for Ischemic Tissue Regeneration & Medical Research Institute, Department of Physiology, School of Medicine, Pusan National University, Yang san 626-870, Republic of Korea

S 112 P10-13

Wnt signaling pathway augments Endothelial Progenitor Cells commitment and its angiogenic potential through SDF1-CXCR4 axis
Yeon Ju Kim, Sang Mo Kwon
Laboratory for vascular medicine&Stem cell Biology, Medical Research Institute, Department of Physiology, Pusan National University, Korea

S 112 P10-14(O-9)

The Sulfated Polysaccharide Fucoidan Rescues Senescence of Endothelial Colony Forming Cells for Ischemic Repair
Jun Hee Lee[1], Takayuki Asahara[2], Sang-Mo Kwon[1]
[1]Laboratory for Vascular Medicine and Stem Cell Biology, Medical Research Institute, Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-870, Korea, [2]Department Regenerative Medicine Science, Tokai University School of Medicine, Isehara, Kanagawa, 259-1193, Japan

S 113 P10-15

Novel angiogenic peptide stimulates mouse hindlimb ischemia repair
TaeWook Lee, YangWoo Kwon, SoonChul Heo, Ilho Jang, JaeHo Kim
Physiology, Pusan national university medical school, Yangsan 626-870, South Korea

S 113 P10-16

Caffeine links dopamine, serotonin and prolactin release during thermal stress in human
Tae Wook Kim[1,2,*], Jeong Beom Lee[2], Sun Jong Kang[2], Sang Eun Im[2], Jae Young Heo[2], Hyun Soo Kim[2], Sang Mook Kim[2], Hyun Kyu Kang[2], Jung Ho Kim[2], Sung Woon Kim[2]
[1]Department of Health Care, Graduate School, Soonchunhyang University, 646 Eupnae-ri, Shinchang-myeon, Asan 336-745, [2]Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea113

S 113 P10-17

Improved sweat gland function during active heating in physically trained human
Jeong Beom Lee[1,*], Tae Wook Kim[1,2], Sun Jong Kang[1], Sang Eun Im[1], Jae Young Heo[1], Hyun Soo Kim[1], Sang Mook Kim[1], Hyun Kyu Kang[1], Jung Ho Kim[1], Sung Woon Kim[1], Sang Woo Jang[2]
[1]Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea, [2]Department of Health Care, Graduate School, Soonchunhyang University, 646 Eupnae-ri, Shinchang-myeon, Asan 336-745

S 114 P10-18

Assessment of eye irritation potential of hair dye chemicals using human conjunctival keratinocytes
Ju Hyun Lim[1], Jeong Bum Bae[2], Hae-Rahn Bae[1]
[1]Department of Physiology, [2]Department of Ophthalmology, College of Medicine, Dong-A University, Busan 602-714, Korea

S 114 P10-19

Effect of Samultang on HO-1 Mediated Vascular protection in HUVECs
Eun Sik Choi[1,2], Yun Jung Lee[1,2], Jung Joo Yun[1,2], Min Chol Kho[1,2], Ji Hun Park[1,2], Xian Jun Jin[1,2,3], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

S 114 P10-20

Inhibitory Mechanism of Samchuleum on Renal Fibrosis
Jung Joo Yoon[1,2], Yun Jung Lee[1,2], Byung Hyuk Han[1,2,3], Seung Namgung[1,2,3], Min Chol Kho[1,2], Ji Hun Park[1,2], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, 2Hanbang Body-fluid Research Center, 3Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

S 115 P10-21

Inhibitory Effect of Hwagryunhaedoktang on TNF-α-induced vascular inflammation in human umbilical vein endothelial cells
Byung Hyuk Han[1,2,3], Yun Jung Lee[1,2], Eun Sik Choi[1,2], Seung Namgung[1,2,3], Xian Jun Jin[1,2,3], Ho Sub Lee[1,2,3,*], Dae Gill Kang[1,2,3,*]
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

S 115 P10-22

Study on the mechanism of vascular relaxation by mantidis ootheca
Hye Yoom Kim[1,2], Yun Jung Lee[1,2], You Mee Ahn[1,2], Rui Tan[1,2], So Heun Lee[1,2,3], Han Sol Lee[1,2,3], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

PAS-1

Mitochondrial DNA causes spreading necrosis in the heart

James Downey, Mikhail Alexeyev, Glenn Wilson, Xi-Ming Yang, and Michael Cohen

University of South Alabama, Mobile, Alabama, USA

The past decade has seen a marked improvement in outcomes in patients with acute myocardial infarction (AMI) treated by reperfusion with percutaneous intervention (PCI). Much of that improvement appears to derive from loading with platelet $P2Y_{12}$ ADP receptor inhibitors prior to PCI to block platelet aggregation. In animals clopidogrel, cangrelor, and ticagrelor have powerful anti-infarct effects themselves. Importantly, no additional protection occurs when any of the above platelet inhibitors is combined with ischemic pre- or postconditioning, presumably because the platelet inhibitor has already "conditioned" the heart. That is reflected in the disappointing results of all clinical trials of ischemic postconditioning that have been conducted since loading with platelet inhibitors gained widespread use. Unfortunately, although post-infarction morbidity and mortality has been cut in half by anti-platelet drugs, it is far from eliminated. While further protection is needed, conditioning based therapies will not provide it. Any effective treatment must target a process of cell killing against which conditioning is unable to protect. We recently found evidence that much of the cell death that occurs in myocardial infarction is due to inflammatory pyroptosis triggered by mitochondrial (mt) DNA released from dying myocardial cells. We propose that this mtDNA causes a self propagating wave of spreading necrosis in the infarcted heart. Attenuation of oxidative damage of mtDNA with a mitochondrially-directed DNA repair enzyme administered at reperfusion reduces infarct size in *in situ* rat hearts. Importantly this repair enzyme provides additive protection when combined with cangrelor. Another approach is DNase-1 given iv at reperfusion to destroy any extracellular mtDNA. DNase limits infarction in both *in situ* and neutrophil-free, isolated hearts. DNase treatment also has an additive effect when combined with cangrelor. Giving purified rat liver mtDNA to the isolated heart increases infarct size showing that it is very toxic; incubating mtDNA with DNase prior to injection detoxifies it. Fragments of mtDNA are known activators of TLR9. Accordingly, TLR9-activating oligodeoxynucleotides also increase infarct size in this model suggesting that TLR9 is probably involved in the toxicity. We conclude that strategies that prevent mtDNA-induced inflammation are very likely to effectively reduce infarct size in today's AMI patients.

PAS-2

Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial reactive oxygen species

Thomas Krieg

Department of Medicine, University of Cambridge, UK

Ischaemia-reperfusion (IR) injury occurs when blood supply to an organ is disrupted and then restored, and underlies many disorders, notably heart attack and stroke. While reperfusion of ischaemic tissue is essential for survival, it also initiates oxidative damage, cell death, and aberrant immune responses through generation of mitochondrial reactive oxygen species (ROS)[1-5]. Although mitochondrial ROS production in IR is established, it has generally been considered a non-specific response to reperfusion[1,3]. Here, we developed a comparative *in vivo* metabolomic analysis and unexpectedly identified widely conserved metabolic pathways responsible for mitochondrial ROS production during IR. We showed that selective accumulation of the citric acid cycle (CAC) intermediate succinate is a universal metabolic signature of ischaemia in a range of tissues and is responsible for mitochondrial ROS production during reperfusion. Ischaemic succinate accumulation arises from reversal of succinate dehydrogenase (SDH), which in turn is driven by fumarate overflow from purine nucleotide breakdown and partial reversal of the malate/aspartate shuttle. Upon reperfusion, the accumulated succinate is rapidly re-oxidised by SDH, driving extensive ROS generation by reverse electron transport (RET) at mitochondrial complex I. Decreasing ischaemic succinate accumulation by pharmacological inhibition is sufficient to ameliorate *in vivo* IR injury in murine models of heart attack and stroke. Thus, we have identified a conserved metabolic response of tissues to ischaemia and reperfusion that unifies many hitherto unconnected aspects of IR injury. Furthermore, these findings reveal a novel pathway for metabolic control of ROS production *in vivo*, while demonstrating that inhibition of ischaemic succinate accumulation and its oxidation upon subsequent reperfusion is a potential therapeutic target to decrease IR injury in a range of pathologies.

PAS-3

Enlargement of myocardial infarct size by chronic kidney disease: a novel mechanism of disruption of Akt-GSK3beta/p70S6K signaling

Tetsuji Miura

Department of Cardiovascular, Renal and Metabolic Medicine, Sapporo Medical University School of Medicine, Japan

Chronic kidney disease (CKD), defined as chronic reduction of glomerular filtration rate and/or proteinuria, is a major risk factor of cardiovascular events and mortality after myocardial infarction. Enlargement of infarct size by CKD has been demonstrated in animal models, but its mechanism remains unclear. Our previous studies showed that different co-morbidities, including hypertension and diabetes, disrupt intracellular signaling pathways at distinct steps upstream of GSK3beta/p70S6K phosphorylation (Miki et al. Circulation 2000, Miki et al. Diabetes 2009, Yano et al. Hypertension 2011, Hotta et al. Circ Res 2010). Here we systematically analyzed the effect of CKD on cytoprotecive signaling by use of a rat model of CKD, two-stage 5/6 nephrectomy. Infarct size after 20-min ischemia/2-h reperfusion was larger by 30% in CKD than in the control. CKD increased the level of Thr308 phosphorylation in Akt at baseline by 375%, though its levels upon reperfusion were similar in CKD and the control. In contrast, phosphorylation of Akt at Ser473 upon reperfusion was significantly suppressed by CKD, though its baseline phosphorylation level was unaffected. Inhibition of Akt-Ser473 phosphorylation upon reperfusion by Ku-0063794, an mTOR inhibitor, significantly enlarged infarct size in control rats. Protein levels of PDK1 and mTORC2, which phosphorylate Thr308 and Ser473 in Akt, respectively, were not changed by CKD. However, of PP2A regulatory subunits, B55alpha, a subunit targeting Thr308 in Akt, was selectively reduced by 24% in CKD. By overexpressing HA-tagged wild-type Akt and phospho-Thr308-mimetic mutant Akt (T308D) in HEK293 cells, we found that constitutive phosphorylation of Akt-Thr308 negatively regulates the response of Akt-Ser473 phosphorylation to its upstream signaling. These results indicate that a novel mechanism of Akt-GSK-3beta/S6 signaling disruption, i.e., intramolecular inhibition of Ser473 phosphorylation in Akt upon reperfusion by reduction of B55alpha-mediated Thr308 dephosphorylation, contributes to infarct size enlargement by CKD.

PAS-4

Dual roles of reactive oxygen species in myocardial ischemic injury and protection

Huang-Tian Yang

Key Laboratory of Stem Cell Biology and Laboratory of Molecular Cardiology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Shanghai, 200025, China

Myocardial injury following ischemia/reperfusion (I/R) is a common clinical scenario in patients suffering from ischemic heart disease. An excessive production of reactive oxygen species (ROS) during the early phase of reperfusion following myocardial ischemia has been proposed to contribute to the reperfusion injury. Paradoxically, the ROS has also been recognized as a trigger of pro-survival signaling pathways mediating cardioprotection at a low level. However, the precise mechanisms for the dual role of ROS have not yet been fully clarified. To address this question, using intermittent hypobaric hypoxia (IHH) as a cardioprotective model, combining with H_2O_2 pre- and post-conditioning, we studied the level and roles of ROS during early reperfusion following ischemia in I/R injury and protection. Our results reveal that the elevated ROS generated during early reperfusion are injurious but insufficient to reach the threshold to efficiently trigger protective signaling pathways. The moderate level of ROS, higher than that elevated by I/R, during early reperfusion is critical for triggering cardioprotection against I/R injury via alleviating intracellular Ca^{2+} overload and preserving mitochondrial function through the efficient activation of Akt, PKCε and JAK2/STAT3 pathways. We then identified the downstream target of ROS-JAK2/STAT3 signals that interacts with the sarcoendoplasmic reticulum Ca^{2+}-ATPase 2 (SERCA2) to improve the activation of SERCA2 and subsequently regulate intracellular Ca^{2+} homeostasis. These findings provide a new angle to interpret the controversial roles of ROS in myocardial I/R and demonstrate that the differential effects of ROS during myocardial I/R are derived from a quantity-dependent wrestling between its detrimental and signaling roles (grants: the National Natural Sciences Foundation of China (81170119), the Major State Basic Research Development Program of China (2012CB518203), and the National Science and Technology Major Project of China (2012ZX09501001).
Key Words: reactive oxygen species, intermittent hypobaric hypoxia, ischemia/reperfusion injury

PAS-5

Physiological roles of unconventional eNOS expressed in the smooth muscle of skeletal and pulmonary arteries

Sung Joon Kim

Department of Physiology, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Korea

The role of eNOS is well known as the source of vasorelaxing NO released from endothelium, and vascular smooth muscle is conventionally regarded as a passive recipient of the endothelial NO. Recent reports provide evidences that arterial smooth muscle cells (ASMCs) also express eNOS. Since the activation of eNOS is triggered by increased $[Ca^{2+}]_{ji,}$ such muscular eNOS expression might mitigate the Ca^{2+}-dependent contractile signals. We have found that deep femoral arterial smooth muscle cells (DFASMCs) and pulmonary arterial smooth muscle cells (PASMCs) express eNOS. In deep femoral arteries (DFAs) of rats, under alpha-adrenergic stimulation or membrane depolarization, hypoxia inhibited the concomitantly activated eNOS and largely augmented the contraction. The hypoxic vasoconstriction (HVC) of DFA was mimicked by the addition of L-NAME to the PhE-pretreated DFA irrespective of endothelium. Interestingly, the HVC of FA was not observed in the eNOS (-/-) mice. In rat pulmonary arteries (PAs), however, L-NAME did not simulate the hypoxic pulmonary vasoconstriction (HPV). Instead, increased mechanical stretch (S(+)) combined with thromboxane A_2 (TXA2/S(+)) stimulate eNOS in pulmoarny arterial smooth muscle cells (PASMCs), preventing their excessive contraction of PA. H_2O_2 or angiotensin II treatment could substitute the S(+) condition for the L-NAME-induced contraction in the endothelium-deprived PAs. The relaxing influence from myogenic eNOS might participate the physiological low resistance of pulmonary circulation.
Key Words: nitric oxide, eNOS, smooth muscle, hypoxia, skeletal artery, pulmonary artery, stretch

PAS-6

Zinc, zinc transporters and cardioprotection

Zhelong Xu

Department of Physiology and Pathophysiology, Tianjin Medical University, China

As an important trace element, zinc is required for the normal cellular structure and function, and impairment of zinc homeostasis is associated with a variety of health problems including cardiovascular disease. Zinc homeostasis is regulated through zinc transporters, zinc binding molecules, and zinc sensors. Zinc also plays a critical role in cellular signaling. Studies have documented that zinc homeostasis is impaired by ischemia/reperfusion in the heart and zinc dyshomeostasis may play a role in the pathogenesis of myocardial ischemia/reperfusion injury. Both exogenous and endogenously released zinc may play an important role in cardioprotection against ischemia/reperfusion injury. The goal of this review is to summarize the current understanding of the roles of zinc homeostasis and zinc signaling in myocardial ischemia/reperfusion injury.

PAS-7

The role of TRPM4 in cardiac function and excitability

Rudi Vennekens

Laboratory of Ion Channel Research, Department of Cellular and Molecular Medicine, KU Leuven, Belgium

TRPM4 is a Ca2+-activated non-selective cation channel that belongs to the family of the Transient Receptor Potential (TRP) ion channels. Importantly, TRPM4 is impermeable for Ca2+ and is involved in different Ca2+-dependent cell functions, such as exocytosis, contraction and cell death. Trpm4 is known to be expressed in atrial and ventricular cardiomyocytes. The interest in the functional role of TRPM4 in the heart has risen further by the discovery of Trpm4 mutations that are linked to cardiac conduction disorders, including Progressive Familial Heart Block type I (PFHBI) and Brugada Syndrome. Both gain-of-function and loss-of-function mutation were described in patients with cardiac conduction diseases. Recently, our group showed that TRPM4 plays a role during the late repolarization phase of the action potential

in murine ventricular cardiomyocytes and that deletion of the Trpm4 gene leads to shorter ventricular action potentials. To characterize if deletion of Trpm4 has an effect on the conduction properties of the heart, an in depth electrophysiological study was performed in living mice. An octapolar catheter was inserted into the right atrium and ventricle of the heart to measure intracardial electrograms. The atrial-His (AH) and His-ventricular (HV) intervals were calculated and no differences were found between WT and Trpm4-/- mice. Additionally, more detailed conduction parameters of the heart were determined by use of programmed electrical stimulation (PES) protocols. Sinus node recovery time (SNRT) was not different between WT and Trpm4-/- mice. Effective refractory period of atrium (AERP), AV node (AVNERP) and ventricle (VERP) were in the same range in WT and Trpm4-/- mice. Wenckebach periodicity, the parameter for AV nodal conduction, was also not different between WT and Trpm4-/- mice. These results suggest that deletion of Trpm4 has no effects on the conduction properties of the murine heart.

PAS-8

Bicarbonate permeation through anion channels

Min Goo Lee

Department of Pharmacology and Brain Korea 21 Project for Medical Sciences, Yonsei University College of Medicine, Seoul 120-752, Korea

Anion channels are an essential component of the cells for keeping them alive and mediating their diverse functions. Although many anions can permeate anion channels, chloride and bicarbonate are the two most abundant anions that can be the charge carrier of anion channels in animal cells. Increasing evidence indicates that bicarbonate permeation though anion channel is involved in many basic biologic processes ranging from epithelial fluid secretion to neuronal excitation. However, the principle of ion selection and permeation by the anion channels, in particular that of bicarbonate, is largely unknown. By employing an integrated study of combined molecular, physiological, structural, and mathematical approaches, we provide evidence that electric permittivity and channel pore diameter are cardinal features, which determine the ion selectivity of anion channels. Importantly, many cellular stimuli dynamically modulate anion channel ion selectivity by changing pore size. Pore size change affects the bicarbonate permeability of anion channels by altering energy barriers of size-exclusion and ion dehydration of bicarbonate permeation. These findings provide key insights into the mechanism of how the ion permeation and selectivity of anion channels are determined.

PAS-9

Orai1 in ER/PM junctions

Shmuel Muallem

National Institute of Health (NIH), NIDCR, USA, Bethesda Maryland, USA

When activated Orai1 and STIM1 cluster at ER/PM junctions both in model systems and *in vivo*. Until recently, the proteins that tether the ER and plasma membrane were not known and how targeting the STIM1-Orai1 complex to the ER/PM junctions affects channel activity. In this presentation the role of tether proteins in recruitment of the Orai1-STIM1 complex to specific ER/PM junctions and its regulation by SARAF that mediated the $\mathbf{Ca^{2+}}$-dependent inactivation of Orai1 will be discussed. In polarize secretory cells, like acinar cells Orai1 and STIM1 are assembled at apical pole ER/PM junctions. The significance of Orai1 function in pancreatic acinar cells junctions, as a model for other secretory cells, will be discussed.

PAS-10

Endosomal and lysosomal chloride/proton exchange by CLC proteins: surprising roles in physiology and pathology

Thomas J. Jentsch

Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP) und Max-Delbrück-Centrum für Molekulare Medizin (MDC), Berlin, Germany

The CLC family of anion transporters comprises both plasma membrane Cl^- channels and vesicular $2Cl^-/H^+$-exchangers that are differentially expressed along the endosomal-lysosomal pathway. Their physiological and medical importance became apparent from human genetic disease and mouse models. ClC-5 is expressed on endosomes, mainly in epithelia. Its mutation in human Dent's disease leads to proteinuria and kidney stones. Our KO mouse model revealed that ClC-5 is important for proximal tubular endocytosis. It disruption leads to hypercalciuria and kidney stones because of defective endocytosis and processing of calciotropic hormones. ClC-7, together with its beta-subunit Ostm1, resides on lysosomes. Disruption of either subunit leads to lysosomal storage, neurodegeneration and osteopetrosis in mice and men. ClC-4 mutations lead to human mental retardation, while disruption of ClC-3 and ClC-6 in mice entail neurodegeneration. The role of these transporters in endosomal/lysosomal function was previously attributed exclusively to impaired vesicular acidification, as these transporters may provide a shunt for the vesicular proton ATPase. While a role of ClC-5 in endosomal acidification has been ascertained, the lysosomal pH of ClC-7 KO mouse is, however, unchanged owing to a parallel cation conductance. We were puzzled by the fact that the vesicular CLCs are $2Cl^-/H^+$-exchangers rather than Cl^- channels. Both are suited, in principle, as shunts for proton pumping. We asked whether chloride/proton exchange is essential for their function and converted ClC-3, ClC-5 and ClC-7 into pure chloride conductances in KI mice. This is possible by single point mutations. Surprisingly, these mice revealed that these mice have almost identical phenotypes as the respective KO mice, suggesting an important role for H^+-exchange dependent vesicular Cl^- accumulation or changes in vesicular voltage. Another ClC-7 mouse model, in which we disrupted its ion transport totally without affecting the expression of the protein, furthermore indicated that the loss of protein-protein interactions explains some aspects of the ClC-7 KO mouse.

PAS-11

A short tribute to Pflügers Archiv (The European Journal of Physiology) and a sojourn to an endothelial anion channel

Bernd Nilius

KU Leuven, Department of Cellular and Molecular Medicine, Laboratory Ion Channel Research, Campus Gasthuisberg, Herestraat 49, bus 802, Leuven, Belgium

This lecture will comprise two parts: ***First***, a short description of the oldest Physiology journal in the world will be given highlighting the main achievements which were published since 1868. This will be a short historical, philosophical sojourn. ***Second***, I will shortly refer to one of the last orphan ion channels, the "Volume Regulated Anion channel (VRAC) ". This channel is a main player in cellular volume regulation and plays an important functional role in the vascular endothelium, especially related to "Regulatory Volume Decrease" (RVD) and "Apoptotic volume decrease" (AVD). VRAC is activated by hypotonic cell challenges, by GTPγS, and as a more principle mechanisms by decreased intracellular ionic strength. Several activation cascades will be discussed. Endothelial VRAC is slowly activated, shows variable inactivation at positive potentials, has a single channel of ~50 pS in outward direction versus ~10pS in inward direction, thus, rectification reflects no change in open probability but in conductance. Its permeation profile: I- > Cl-, taurine, glutamate, aspartate, and but also ATP is permeable. Important physiological effects of VRAC for endothelial function will be described, e.g. the involvement in the driving force for Ca^{2+} entry into endothelial cells and its role in regulation of endothelial cell proliferation and also angiogenesis.

The discovery of VRAC, stimulated many laboratories worldwide to analyze this sensor in many other cells types and to unravel the activation mechanisms of this channel. Unfortunately, it turned out to be extremely difficult to identify the molecular identity of this channel. Until the end of 2014, more than 10 possible candidates for VRAC have been put forward (for a comprehensive review see 1,2). Thus, during the last 15 years, shrinking and expanding list of candidates comprised *P64 intracellular Cl channels CLIC1*, the *Band 3* exchanger of chloride for bicarbonate from the AE family, *FXYD domain-containing ion transport regulators*, *phospholemman*, *mat-8* (a family of small membrane proteins that share a 35-amino acid signature sequence domain and is a ion), the chloride channels *ClC2* and *ClC3*, the *P-glycoprotein multi-drug resistance transporter MDR1* (a member of the superfamily of ATP-binding cassette transporters), the small cytosolic protein I_{Cln} (possibly a protein involved in spliceosomal snRNP, small nuclear ribonucleic particles, biogenesis), *VDAC* (Voltage-dependent anion channels, a class of porin ion channel located on the outer mitochondrial membrane) and recently *TMEM16F* (*ANO6*, probably Ca^{2+} activated Cl^- channels, scramblases of even cation channels).

Now finally, the true nature of VRAC has been identified independently by two laboratories via screening of large whole-genome human siRNA libraries. The *Leucine-Rich Repeats Containing 8A* (LRRC8A) protein, belonging to family of proteins (LRRC8A-E) distantly related to pannexins, is likely the pore-forming subunit of VRAC (se 3,4). I will finally describe some properties of the LRRC8 proteins, highlight some features of the LRRC8A knockout mouse, and discuss the impact of the discovery of LRRC8 as VRAC on future research. The *Lrrc8* gene has been first discovered in a girl with a-gamma-globulinemia and has been identified as a component of the pre-B-cell receptor (pre-BCR) (see also 5). Surprisingly, in the LRRC8A ko, no B cell defect but severe problems with T-cell development, thymocyte depletion, survival and function have been decribed. There are also some striking puzzles left: over expression does not increase the current, pore identification is still weak, the knock outs have in addition to a T cell phenotype, an increased early in utero mortality, increased postnatal lethality, growth retardation, curly hair, hind limb weakness, hydronephrosis, sterility, epidermal hyperkeratosis, thin skeletal muscle bundles, vacuolar renal tubular cells (cysts), and no ovarian corpora lutea (see 5). The link to the vascular function has still to be evaluated. More striking and exciting new properties are to expect and we are obviously in the beginning of an in depth understanding of the important ubiquitous anion channel VRAC .

References

1. Nilius, B. & Droogmans, G. 2003. Amazing chloride channels: an overview. Acta Physiol Scand. 177: 119-147
2. The identification of a volume-regulated anion channel: an amazing Odyssey. Pedersen SF, Klausen TK, Nilius B. Acta Physiol (Oxf). 213: 868-81, 2015.
3. Qiu, Z., Dubin, A. E., Mathur, J., Tu, B., Reddy, K., Miraglia, L. J., Reinhardt, J., Orth, A. P. & Patapoutian, A. 2014. SWELL1, a plasma membrane protein, is an essential component of volume-regulated anion channel. *Cell* 157: 447-458
4. Identification of LRRC8 heteromers as an essential component of the volume-regulated anion channel VRAC. Voss FK, Ullrich F, Münch J, Lazarow K, Lutter D, Mah N, Andrade-Navarro MA, von Kries JP, Stauber T, Jentsch TJ, 2014. Science. 344: 634-8, 2014
5. Kumar, L., Chou, J., Yee, C. S., Borzutzky, A., Vollmann, E. H., von Andrian, U. H., Park, S. Y., Hollander, G., Manis, J. P., Poliani, P. L. & Geha, R. S. 2014. Leucine-rich repeat containing 8A (LRRC8A) is essential for T lymphocyte development and function. J.Exp.Med. 211: 929-942, 2014.Plenary Lecture Keiichi Fukuda (Keio University)

PL

Clinical application of human iPS cells for cardiovascular Medicine

Keiichi Fukuda

Department of Cardiology, Keio University School of Medicine

Although heart transplantation can drastically improve the survival, shortage of the donor heart is a serious problem. The regenerative medicine of the failing heart had been long awaited. To address this question, we had developed novel methods to induce human iPS cells from circulating human T lymphocytes using Sendai virus containing Yamanaka 4 factors. We had screened the factor that were expressed in future heart forming area of the early mouse embryo, found several growth factors and cytokines that can induce cardiomyocytes differentiation and proliferation, and applied them to human iPS cells. We performed transcriptome of the metabolic enzymes and fluxome analysis using 13glucose and 13lactic acid on ES/iPS cells and cardiomyocytes, and found that their metabolic pathways were completely different. Based on these findings, we purified cardiomyocytes using glucose-free lactate-supplemented medium. Purity of the cardiomyocytes was >99%, and they did not make teratoma formation. The transplanted cardiomyocytes using our technique can survive in the heart with more than 90%, and can show physiological growth after transplantation. Transplantation of ES/iPS-derived cardiomyocytes into the infarcted myocardium could improve cardiac function in rat and porcine model. We expect the combination of these techniques can achieve future heart regeneration.

CURRICULUM VITAE

Name: Keiichi Fukuda, M.D., Ph.D, FACC

Academic Carreer

1983	Graduated Keio University School of Medicine, Tokyo, Japan
1983 - 1987	Post Graduate School, Keio University, Tokyo, Japan
1987	PhD in Clinical Cardiaology
1983 – 1985	Resident in Internal Medicine, Keio University School of Medicine
1985 – 1991	Resident in Cardiology, Keio University School of Medicine
1991 – 1992	Growth Factor Division, National Cancer Center Research Institute
1992 – 1994	Dept of Molecular Medicine, Beth Israel Hospital, Harvard Medical School
1994 – 1995	Cardiovascular Reserch Center, University of Michigan
1995 – 1999	Lecturer, Deapartment of Cardiology, Keio University School
1999 – 2004	Assistant Professor, Institute for Advanced Cardiac Therpeutics, Keio University
2005 – 2010	Professor, Dept of Regenerative Medicine, Keio University
2010-Present	Professor, Dapartment of Cardiology, Keio University
2007 – Present	Vice Dean, Keio niversity School of Medicine

Honors and Awards

1. Keio University School of Medicine Sanshikai Kitajima Prize (2000)
2. Tokyo Metoropolitan Medical Association Medical Prize (2001)
3. Japan Medical Association Science Promotion Prize (2002)
4. Keio University Sakaguchi Memorial Medical Prize (2002)
5. Japanese Circulation Society/Japan Heart Foundation Sato Memorial Prize (2005)
6. Erwin von Bälz Prize, (2010)
7. Mochida Memorial Medical Science Prize (2011)
8. Imura Memorial Clinical Research Award (2012)
9. President lecture Award of International Society for Heart Research (2014)

Youdang Scholarship Award Lecture

Ca^{2+}-activated K^+ channel expression on cell membrane in physiological and pathophysiological conditions

Shinkyu Choi, Ji Aee Kim, Suk Hyo Suh

Department of Physiology, Medical School, Ewha Womans University, Seoul, Korea

Ion channels play key roles in the control of cellular functions, and thus cellular functions are greatly affected by altered expression of ion channels on cell membrane. Ca^{2+}-activated K^+ channels (K_{Ca}1.1, K_{Ca}2.3, and K_{Ca}3.1), which are expressed in smooth muscle cells (SMCs) and endothelial cells (ECs), regulate cellular functions by modulating Ca^{2+} influx through Ca^{2+} entry channels or endothelium-dependent responses, such as NO release and endothelium-dependent hyperpolarization. Altered expression of these Ca^{2+}-activated K^+ channels on cell membrane was found in physiological (normal pregnancy, and aging) and pathophysiological (aging, and vascular diseases such as pregnancy-induced hypertension and Fabry diseases) conditions.

K_{Ca}1.1, or K_{Ca}2.3 and K_{Ca}3.1 was upregulated in gastric SMCs or ECs from aged mice, respectively, compared to young mice. Ca^{2+}-activated K^+ currents were markedly increased without affecting the channel activity. K_{Ca}1.1 upregulation in gastric SMCs impaired intracellular Ca^{2+} mobilization and decreased p-MLC levels, causing contractile dysfunction of aged gastric smooth muscle. On the other hand, endothelial K_{Ca}2.3, and K_{Ca}3.1 upregulation markedly increased K_{Ca}3.1 activation-induced, NO- and prostacyclin-resistant endothelium-dependent relaxation, and thereby compensating diminished endothelium-dependent relaxation to NO in aged mice. In addition, K_{Ca}2.3, and K_{Ca}3.1 were upregulated in ECs from normal pregnancy, which might cause pregnancy-associated vasodilation and angiogenesis. On the other hand, K_{Ca}2.3, and K_{Ca}3.1 were downregulated in ECs from vascular diseases, such as pregnancy-induced hypertension and Fabry disease, and the downregulation of these K^+ channels contributes to endothelial dysfunction in vascular diseases. Finally, we'll discuss about the mechanisms to control the expression of these K^+ channels on cellular membranes.

Key Words: Ca^{2+}-activated K^+ channel, Smooth muscle cells, Endothelial cells, Cellular dysfunction, K^+ channel expression on cell membrane

CURRICULUM VITAE

Name: Suh, Suk Hyo

Academic History

1985	Seoul National University, College of Medicine, B.S., M.D.
1993	Seoul National University, Ph.D. (Physiology)

Professional Experience

1995-2006	Assistant Professor, Department of Physiology, College of Medicine, Ewha Women's University
1998-2000	Guest Professor, Catholic Unversity in Leuven (Belgium)
2006-Present	Professor, Department of Physiology, College of Medicine, Ewha Women's University

Publication List (Representiveitves)

1. Choi S, Kim JA, Kim TH, Li H, Shin K, Lee Y, Oh S, Pewzner-Jung Y, Futerman AH, Suh SH. Altering sphingolipid composition with aging induces contractile dysfunction of gastric smooth muscle via KCa1.1 upregulation. Aging Cell. 2015; in press.
2. Choi S, Kim JA, Na H, Cho S, Park S, Jung S, Suh SH Globotriaosylceramide induces lysosomal degradation of endothelial KCa3.1 in Fabry disease. Arterioscler Thromb Vasc Biol. 2014;34:81-89
3. Choi S, Kim JA, Na H, Kim J, Park S, Han KH, Kim YJ, Suh SH. NADPH oxidase 2-derived superoxide downregulates endothelial Kca3.1 in preeclampsia. Free Radic Biol Med. 2013;57:10-21
4. Choi S, Kim MY, Joo KY, Park S, Kim JA, Jung J, Oh S, Suh SH. Modafinil inhibits KCa3.1 currents and muscle contraction via a cAMP-dependent mechanism. Pharmacol Res 2012;66:51-59 1043-6618
5. Park S, Kim JA, Joo KY, Choi S, Choi EN, Shin JA, Han KH, Jung SC, Suh SH. Globotriaosylceramide leads to KCa3.1 channel dysfunction: A new insight into endothelial dysfunction in fabry disease. Cardiovasc Res. 2011;89:290-299 6.064 0008-6363

S-I-1

Neuro-endocrine systems that targeted for anti-obesity research

Young-Ho Jin

Department of physiology, School of Medicine, Kyung Hee University, Seoul, 130-701, Korea

Epidemic increases of the obesity in the industrialized countries not only deteriorate personal health but also become heavy burden for national health care system by increasing health care costs. To address this problem, great effort has been made to investigate body weight regulation mechanism and found that hormones originate from adipose tissue, intestine, pancreas, and hypothalamus regulate energy balance via activating neurons in hypothalamus, vagus nerve and nucleus tractus solitarii (NTS). Among them, two distinct type neurons each of them co-express agouti-related peptide and neuropeptide Y (AgRP/NPY neuron) or proopiomelanocortin and cocaine- and amphetamine-regulated transcript (POMC/CART neuron) was become primary research subject. Many hormones that activate POMC/CART neuron reduce appetite and increased energy expenditure. Contrary, activation of AgRP/NPY neuron has opposite effect. POMC/CART expressing neuron is present both of the arcuate nucleus (ARC) neurons of the hypothalamus and NTS but until recently most of the obesity research focused on neuron in ARC. In addition that nutrients and sensory signals including mechanical stimuli from other brain regions are integrated with ARC-mediated hormonal signal for regulate energy balance. Despite latest advances, still there is no effective medicine which can break or slow down epidemic increase of obesity. In this presentation, I will make short summary of the recent progress in obesity research and will discuss about why such progress couldn't lead to new drug development. In addition, I will introduce some alternative attempts to reduce appetite by stimulating satiety transmitting cranial vagus nerves.

Key Words: Obesity, metabolism, appetite, vagus nerve, nucleus tractus solitarii

S-I-2

Beige and Brown burns fat

Youngsup Song, Aroom Hong

Department of Biomedical Sciences, University of Ulsan College of Medicine, Asan Medical Center, Seoul 138-736

Obesity is a serious health risk factor highly associated with type 2 diabetes, heart diseases, and certain cancers. Currently one thirds of US population and 18% of adult population in OECD countries are obese. Simple reason for being obese is imbalance between energy intake and expenditure. Adipose tissue is a multi-functional organ, providing mechanical protection of other organs, insulating, and regulating physiology as an endocrine organ. Two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT) have been identified. In the aspect of energy balance, the main function of WAT is storing excessive of energy in the form of triglycerides and distributing it to other organs when needed. In contrast, BAT by oxidizing fuels and dissipating energy in the form of heat regulating body temperatures and energy expenditures. Here, we discuss how energy balance between WAT and BAT is regulated, especially by catecholamine and its downstream mediators CREB and Crtc3 and BAT as a potential therapeutic target for the treatment of obesity.

Key Words: Brown adipose tissue, Beige adipose tissue, catecholamine, energy expenditures, Crtc3

S-I-3

Bariatric to metabolic surgery; paradigm shift

Yong Jin Kim

Department of Surgery, Soonchunhyang University, Seoul Hospital, Seoul, Korea

The most common surgical options for extreme obesity include Roux-en-Y gastric bypass, sleeve gastrectomy, and adjustable gastric banding. Substantial weight loss (roughly 25% initial body weight for Roux-en-Y gastric bypass) has been reported up to 20-year follow up. Further, gastric bypass corrects obesity-induced biologic changes or adaptations which might explain why bariatric surgery is the only available treatment to show long-term effectiveness. Although there is a powerful evidence, the reason why surgery could not be generalized in the management of obesity is because, clinicians is not aware of the struggling of a patient in weight loss. Second, referring physicians and candidates have a vague fear in surgery. And financial problem could be the last reason. In this session, there are four things that I want to point out. Reason why surgery is carried out in the obesity treatment, basic principle of the bariatric surgery, my own personal experiences for surgical outcomes and lastly current worldwide trend toward bariatric to metabolic or diabetic surgery.

Key Words: Morbid Obesity, Bariatric Surgery, Metabolic syndrome

S-I-4

Pharmacotherapy for resolving obesity

Kyoung Kon Kim

Department of Family Medicine, Gachon University College of Medicine, Incheon, Korea

In October, 2015, in Korea, the approved anti-obesity drugs that can be used for long-term are orlistat and lorcaserine, and in US, liraglutide, phentermine/topiramate and naltrexone/bupropion were approved recently. Orlistat is a reversible intestinal lipase inhibitor and administered orally. Lorcaserine is an oral anti-obesity drug that is a highly selective 5-HT2c agonist. Liraglutide is a synthetic GLP-1 analogue that should be injected subcutaneously once a day. Phentermine/topiramate and naltrexone/bupropion are combined drugs that were developed to enforce the efficacy and reduce the adverse events. In general, the long-term anti-obesity drugs induce 5-10% weight reduction, and have good safety profiles. There are differences in the weight reducing efficacies among the drugs and their dosages, and the adverse events profiles are also different according to the administered drugs. Therefore, both of efficacy and safety must be considered together in selecting the drug, titrating the dosage, and monitoring patients. Common adverse events of the drugs can be controlled by properly adjusting the dosage. Fatty stool is a common and famous adverse event for orlistat. The common adverse events of lorcaserine are headache and URI symptoms. Nausea, vomiting and other gastrointestinal related symptoms are common adverse events of liraglutide. Paresthesia, dry mouth and irritability are the adverse events profile of phentermine/topiramate. The severe nausea and contipation commonly occurred for naltrexone/bupropion. The uncommon serious side effects or the concern are liver injury for orlistat; valvulopathy for lorcaserine; thyroid C-cell pathology and pancreatitis for liraglutide; renal stone and glaucoma for phentermine/topiramate.

Key Words: anti-obesity drugs, efficacy, safety

S-II-1

Recent advances in studying tactile sensation

Kyung Chul Shin, Hyunji Park, Sang Woong Park, In-Hwa lee, Jae Gon Kim, Young Min Bae

Department of Physiology Konkuk University School of Medicine

Compared with other types of senses, understanding of tactile sensing is relatively undeveloped because molecular identities of the ion channels that mainly contribute to the generation of receptor potentials at mechanosensing cells are not fully uncovered yet. Transient receptor potential, degenerin/epithelial Na channels, and piezo have been known as good candidates for the ion channels of mechanoreceptors. Activation of these channels generates inward currents and receptor potential in a physiological setting. Especially, critical roles of piezo2 ion channel in Merkel cells and their Aβ afferent fibers have been suggested in a couple of recent studies. In this talk, I'll briefly review on this promising piezo2 touch-sensing channel. In addition, I would like to suggest a possibility of non-contact tactile sensation based on the following observations: To validate the feasibility of utilizing laser for non-contact tactile stimulation, we provide evidence at cell and ion channel levels. We found that 532 nm laser-stimulation activated the piezo-like mechanosensitive ion channels in mechanosensing neuro2A and Merkel cells, which implies the plausibility of non-contact tactile sensation using laser. These results support the hypothesis that laser stimulation may induce tactile sensation by activating mechanosensitive ion channels of somatosensory cells in human skin.
Key Words: Tactile sensation, Piezo, Merkel cell, Laser, Non-contact

S-II-2

Physical responses to pulsed laser stimulation in human skin

Jong-Rak Park

Department of Photonic Engineering, Chosun University, 309 Pilmun-daero, Gwangju 61452, Korea

Recently, it has been demonstrated by physical, perceptual, and simulation studies that laser-induced thermoelastic effects in human skin can evoke tactile sensations. The temporal evolution of the thermoelastic effects induced by pulsed laser absorption in human skin can be divided into four regimes: heating, transient, quasi steady-state, and thermal diffusion regimes. When the inertial or stress confinement condition is met in the heating regime, very high thermoelastic stresses build up. In the transient regime, the skin reconfigures itself as it responses to the forces caused by the thermoelastic stresses. In the quasi steady-state regime, the skin reaches mechanical equilibrium state, where the net force becomes zero but the thermoelastic stresses still exist because of the nonuniform temperature distribution. In the last, or thermal diffusion, regime, the thermoelastic stresses decay to zero as the temperature distribution becomes uniform by the thermal diffusion process. In this presentation, physical responses to pulsed laser stimulation in human skin are investigated in terms of the laser-induced thermoelastic effects. Optical, thermal, and mechanical responses to pulsed laser absorption in human skin are examined in detail.
Key Words: Laser–tissue interactions, thermoelastic effects, photomechanical effects

S-II-3

Cortical Responses to tactile sense induced by laser in humans

Sung-Phil Kim

Department of Human and Systems Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

The present study investigates the human cortical responses to tactile stimuli generated by laser. Laser has been primarily used in neurophysiological studies to evoke nociceptive feelings in humans. However, our recent study has shown that laser could evoke non-nociceptive tactile sensations in humans. We examine human cortical activity related to such non-painful feelings by laser and compare different cortical activities when participants experienced painful feelings or non-painful feelings. The human EEG is particularly measured and analyzed to study cortical responses. The sensorimotor rhythms in the alpha and beta frequency bands are main features examined here. We also utilize the decoding analysis to compare cortical patterns in response to different tactile stimuli. Taken together, we demonstrate that cortical responses to non-nociceptive laser stimuli are more similar to those to mechanical stimuli than those to painful stimuli or thermal stimuli.
Key Words: laser, EEG, tactile, sensorimotor

S-II-4

When nanoscience meets optogenetics

Nambin Yim, Seung-Wook Ryu, Kyungsun Choi, Chulhee Choi

Department of Bio and Brain Engineering, KAIST, Daejeon, 305-701, South Korea

Nanoparticle-mediated delivery of functional macromolecules is a promising method for treating a variety of human diseases. Among nanoparticles, cell-derived exosomes have recently been highlighted as new therapeutic strategies for the in vivo delivery of nucleotides and chemical drugs. Here, we describe a new tool for intracellular delivery of target proteins, named 'exosomes for protein loading via optically reversible protein–protein interaction" (EXPLOR). By integrating a reversible protein–protein interaction module controlled by blue light with the endogenous process of exosome biogenesis, we were able to successfully load cargo proteins into newly generated exosomes. Treatment with protein-loaded EXPLORs was shown to significantly increase intracellular levels of cargo proteins in recipient cells in both a time- and dose-dependent manner. These results clearly indicate the potential of EXPLORs as a mechanism for the efficient intracellular transfer of protein-based drugs into recipient cells and tissues both in vitro and in vivo.
Key Words: optogenetics, drug delivery, protein transduction

S-III-1

Physiome study on mitochondrial Ca^{2+} dynamics

Satoshi Matsuoka, Ayako Takeuchi

Department of Integrative and Systems Physiology, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuokashimoaizuki, Eiheiji-cho, Yoshida-gun, Fukui 910-1193, Japan

Mitochondria are pivotal organelle as ATP producing factories as well as Ca^{2+} stores. However, the mechanisms and physiological significances of mitochondrial Ca^{2+} dynamics are not well understood. To get more insight into the mechanisms and significances, we performed physiome studies by combining wet physiological experiments and *in silico* mathematical analyses, especially focusing on mitochondrial Ca^{2+} extruding Na^{+}/Ca^{2+} exchanger (NCLX). In B lymphocytes, analyses of a mathematical model predicted that NCLX activity affects endoplasmic reticulum (ER) Ca^{2+} content and antigen receptor-mediated cytoplasmic Ca^{2+} rise. The model predictions were validated by NCLX knockout and knockdown in DT40 and A20 B lymphocytes and by pharmacological inhibition of NCLX. In HL-1 cardiomyocytes, NCLX knockdown resulted in reduction of sarcoplasmic reticulum (SR) Ca^{2+} content and in prolongation of cycle length of spontaneous Ca^{2+} oscillation and action potential generation. Our mathematical model well reproduced and explained the experimental results. We propose that NCLX functions as a Ca^{2+} provider to ER/SR and is important for antigen receptor-induced Ca^{2+} response of B lymphocytes and automaticity of cardiomyocytes.

Related publications

1. Takeuchi A, Kim B, Matsuoka S. The destiny of Ca^{2+} released by mitochondria. *J Physiol Sci* 2015;65:11-24.
2. Takeuchi A, Kim B, Matsuoka S. The mitochondrial Na^{+}-Ca^{2+} exchanger, NCLX, regulates automaticity of HL-1 cardiomyocytes. *Sci Rep* 2013;3:2766.
3. Kim B, Takeuchi A, Koga O, Hikida M, Matsuoka S. Mitochondria Na^{+}-Ca^{2+} exchange in cardiomyocytes and lymphocytes. *Adv Exp Med Biol* 2013;961:193-201.
4. Kim B, Takeuchi A, Koga O, Hikida M, Matsuoka S. Pivotal role of mitochondrial Na^{+}-Ca^{2+} exchange in antigen receptor mediated Ca^{2+} signalling in DT40 and A20 B lymphocytes. *J Physiol* 2012;590:459-474.
5. Kim B, Matsuoka S. Cytoplasmic Na^{+}-dependent modulation of mitochondrial Ca^{2+} via electrogenic mitochondrial Na^{+}-Ca^{2+} exchange. *J Physiol* 2008;586:1683-1697.

Key Words: physiome, mitochondria, calcium, B lymphocyte, cardiomyocyte

S-III-2

Role of Na-K-Cl cotransporter and store-operated calcium entry in pacemaker activity of interstitial cells of Cajal

Jae Boum Youm[1,2], Haifeng Zheng[2], Mei Hong Zhu[2], Tae Sik Sung[2], Kenton M. Sanders[2], Sang Don Koh[2]

[1]Cardiovascular and Metabolic Disease Center (CMDC), Department of Physiology, College of Medicine, Inje University, Busan, South Korea, [2]Department of Physiology and Cell Biology, University of Nevada, School of Medicine, Reno, Nevada, USA

Pacemaker depolarization in interstitial cells of Cajal (ICCs) is believed to be induced by oscillatory increases in cytosolic Ca2+ and subsequent activation of Ano1 channel. However, there are findings that the block of SOCE (store-operated calcium entry) or Na-K-Cl cotransporter (NKCC) can terminate pacemaker activity of ICCs indicating that they might be involved in the initiation or maintenance of pacemaker depolarization. We hypothesized that the SOCE contributes to pacemaker depolarization by mediating Ca2+ depletion in endoplasmic reticulum (ER) with activation of Ano1. As for the NKCC, we hypothesized that NKCC contributes to plateau phase by mediating Cl- loss by Ano1 activation during pacemaker depolarization with the reverse-mode operation of Na/Ca exchanger. We updated our recent mathematical model of ICCs in mouse small intestine by incorporation of SOCE and NKCC. The updated model faithfully reproduces the experimentally obtained recordings of SOCE and Ano1 current. Block of either NKCC or SOCE in our mathematical model of ICCs terminates pacemaker activity. However, the contribution of NKCC to pacemaker activity in a beat-to-beat manner was not envisioned in our mathematical model. Instead, NKCC seems to play a significant role in maintaining Cl-equilibrium which is critical for Ano1 activation. Incorporation of SOCE allows the model to drive pacemaker activity without diastolic depolarization. Animal experiments are essential to validate the role of NKCC in pacemaker mechanism of ICCs.

Key Words: ICCs, NKCC, Ano1, SOCE, pacemaker activity

S-III-3

A physiomic approach for the electrophysiological variation of the heart induced by the ischemia of coronary artery

Ah-Jin Ryu, Gi-Tae Kim, Soon Sung Kwon, Eun Bo Shim

Department of Mechanical and Biomedical Engineering, Kangwon National University, Chuncheon 200-701, Korea

Heart ischemia due to coronary aretry stenosis can induce electrophysiological change in heart muscle, showing abnormal EKG signals. The sequential physiological events from stenosis of a coronary artery to variation in the heart's electric field have not been delineated. To investigate this phenomenon mathematically, we implemented an integrative model of the heart covering a wide range of levels (from cells to organs, the torso, and coronary hemodynamics). An electrophysiological model of an ischemic cell was incorporated into tissue and organ models of the human ventricle. This was coupled with a stenosed coronary model to simulate the effect of reduced blood flow through a stenosed coronary artery on cardiac electrophysiology and virtual EKG signals according to the location and duration of coronary stenosis. Coronary blood flow was solved by computational fluid dynamics coupled with a lumped parameter model. Using this model, we first predicted the regional blood perfusion pressure and determined whether the myocardial cells in the region were in a state of ischemia. If so, KATP channels were activated to reduce the action potential duration (APD), which eventually affected the heart's electric activity. Then the electrical pattern across the surface of the torso was converted into EKG signals.

Key Words: coronary artery stenosis, ischemic cell, ATP-sensitive potassium channel, numerical simulation, patient- specific model

S-III-4

Electromechanical delay in human ventricle under various load conditions: simulation study

Aulia Heikhmakhtiar[1], Eun Bo Shim[2], Ki Moo Lim[1]

[1]Department of Medical IT Convergence Engineering, Kumoh National

Institute of Technology, [2]Department of Mechanical & Biomedical Engineering, Kangwon National University

One of the subset of the heart failure (HF) is dyssynchrony of the heart depolarization and the myofiber shortening phase which leads to sever HF condition. Time interval between the heart depolarization (electrical activation) and onset myo-fiber shortening (mechanical activation) in one cycle of heart rhythm is known as electromechanical delay (EMD). Experimental study of Russell et al. in dog and human heart also showed that mechanical load prolonged the EMD. These studies leads to a presumable solution that if the mechanical load of the ventricle is decreased, the EMD will also be decreased. However, no one ever proved that. To measure the mechanical load effect on three-dimensional (3D) EMD distribution cannot be obtained experimentally so far due to the limitation of measurement devices. The purpose of the study is to quantify the effect of mechanical afterload on EMD by using 3D cardiac physiome model. To construct an integrated model of a cardiovascular system, we combined the 3D image-based electromechanical model of failing canine ventricles with a lumped model of the circulatory system. In order to apply various mechanical afterload condition, we changed the aortic flow resistance with scale factors of 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0. The local electrical activation times, which is obtained from Durrer et al., were mapped to the ventricular computational mesh. The local mechanical activation time was defined as the 10% of the strain lowest value of the ventricle models following Constantino et al. Therefore, EMD can be derived by subtracting EAT from MAT in space. Finally, EAT, MAT and EMD were compared among in silico experimental groups. We obtained cardiac responses such as cardiac tension, strain, ATP consumption, mechanical activation time (MAT), and EMD, etc. The MAT and EMD increased in the degradation of Ca2+ concentration level, which means that the MAT and EMD are emphasized under more severe HF condition induced by Ca2+ remodeling. And we found that the MAT and EMD increased depending on mechanical afterload. Ventricles with five times higher aortic flow resistance induced almost 20% more prolonged EMD than normal case. Although this study have proved that mechanical afterload increases EMD with computational method, it can be used for treatment of patients who suffer from prolonged EMD in novel way.

Key Words: Cardiac physiome model, Electromechanical delay, Mechanical activation time, Mechanical afterload

S-III-5

Model based interpretation of oral glucose tolerance test

Young Boum Lee, Jeong Hoon Lee, Ga Yul Kim, Ji Yeon Song, Pham Duc Doung, Chae Hun Leem

Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

Oral glucose tolerance tests (OGTTs) are used commonly to diagnose diabetes mellitus (DM). The changes on blood glucose and insulin by OGTTs contain information of the intestinal absorption, hepatic control of glucose and insulin, pancreatic insulin secretion and peripheral tissue glucose and insulin control. Therefore, an appropriate dynamic model could reveal those information from OGTT data. We developed an OGTT model containing five compartments for insulin dynamics and two compartments for glucose dynamics based on previous reports. Anthropometric data of individuals were used to assume the cardiac output. Simplex and Levenberg-Marquardt algorithms were then used to fit the data obtained from 42 normal subjects (24 males and 20 females) and eight subjects with DM. We found clear gender differences in the intestinal glucose absorption kinetics, glucose sensitivity in the pancreas, maximal insulin production capacity and endogenous glucose production. There were also differences between normal and DM subjects. For example, pancreatic and liver dysfunctions were evident in DM cases. The differences between normal and DM subjects in glucose and insulin dynamics in the pancreas, liver and peripheral tissues, such as insulin resistance, insulin secretion and the relative roles of glucose disposal in each organ, were demonstrated clearly and quantitatively in a time-dependent manner. This study revealed the quantitative dynamic interaction between glucose and insulin using OGTT data and revealed organ function during the OGTT. Using this approach, we identified the dysfunctional organs for glucose and insulin regulation. Data produced using this model will allow a personalized and targeted approach for health issues related to glucose and insulin. (Supported by the grant No. NRF-2015M3A9B6028310, NRF-2014M3A9D7034366 & 2015K000247 from MSIP/COMPA)

Key Words: Oral glucose tolerance test, glucose, insulin, diabetes mellitus, model

S-IV-1

Oxygen availability and skeletal muscle mitochondrial function in health and disease

Russell S. Richardson

University of Utah

Oxygen, as the final electron acceptor in the electron transport system, the major pathway for producing the human biological energy currency, adenosine triphosphate (ATP), is vital for life. However, until relatively recently, our understanding of the influence of oxygen availability has been hindered by the inability to assess intramuscular oxygenation and estimate intramuscular oxygen partial pressure (PiO_2). Therefore, the evaluation of basal intracellular oxygenation of human skeletal muscle utilizing proton nuclear magnetic resonance spectroscopy (^{1}H NMRS) of myoglobin (Mb) is an important development that can facilitate the assessment of the oxygen cascade from air to myocyte and provide valuable insight into the impact of oxygen transport and availability on mitochondrial function in health and disease. In combination with other techniques such as direct Fick measurements (arterial and venous blood gases and blood flow) across a muscle bed and phosphorous (^{31}P) MRS of the muscle itself, these Mb assessments have provided significant insight into the role and impact of oxygen availability at rest, at the onset of exercise, maximal exercise, and the impact of exercise training. Finally, some of these *in vivo* findings, related to PiO_2, have recently been corroborated by the novel combination of both *in vivo* and *in vitro* mitochondrial function assessments that provide a unique perspective on the role of oxygen availability, mitochondria, and physical function.

S-IV-2

Effects of exercise training on myokines expression and insulin sensitivity in diet-induced obese rats

Kijin Kim, Nayoung Ahn, Suryun Jung, Jayoung Byun, Kwangbae Park, Sungwook Kim, Yeunho Jung, Solee Park

Department of Physical Education, Keimyung University, 1095 Dalgubeuldaero, Dalseo-gu, Daegu, 704-701, Korea

The purpose of this study was to investigate effects of resistance training on myokines expression and insulin sensitivity in young and middle-aged rats. 50wks and 10wks of male Wistar rats were randomly assigned for exercise and sedentary groups after 1-week of adaptation period. The resistance exercise training was carried out using ladder climbing with weight attached to the tail (3day/week, 8weeks). The high-fat induced rats were randomly assigned for 4 groups (FD, FP, FEx, FPEx). The 8-week of resistance exercise and high-protein diet significantly reduced body weight and abdominal fat weight. Also an insulin resistance of skeletal muscle significantly improved. In the basal level, TNF-α, IL-1β and NFκB protein were expressed 2-3 fold higher in middle-aged group than in young group. After 8 weeks of training, there was no change in the level of NFκB and IL-1β in both middle-aged and young groups. The level of TNF-α was highly decreased in ME group, but this decreased level was still higher than any of young group. Therefore resistance exercise training with use ladder climbing were effective tool to decrease inflammation in skeletal muscle, but training responses were gradually decreased with aging. And an 8-week resistance training and high-protein diet improve insulin resistance as the improvement of body composition and mitochondrial biogenesis in sarcopenic obese rats.

Key Words: sarcopenia, myokine, resistance exercise, insulin sensitivity, aging

S-IV-3

Moderate exercise training inhibits lipid metabolism and macrophage infiltration in high fat diet-induced obese mice

Wang Lok Lee, Young Ran Lee, Hee Geun Park, Jun Hyun Jeong

Department of Sport Science, College of Nautral Science, Chungnam National University

PURPOSE: This study was investigated to determine the effect of moderate exercise training on adipose tissue remodeling and macrophage infiltration in high fat diet induced obese mice. **METHODS:** To accomplish the purpose of this study, C57BL/6 male mice were fed high fat diet (45% fat diet) during experimental period. The animals were divided into 2 groups ; HD (high fat diet control, n=10), and HE (high fat diet with moderate exercise training, n=10). Exercise training was performed for 12 weeks on a treadmill running for 40-60 min/day at 10-22 m/min, 0% grade, 5 days/week. **RESULTS:** As a result, body weight and epididymal fat pad weight were significantly decreased in HE compared with HD ($p < .05$). Also, the size of adipose was significantly reduced in HE compared with HD ($p < .05$). Macrophage infiltration markers (CD11c, F480, CD86) mRNA expression were significantly decreased in HE compared to HD ($p < .05$). Moreover, adipogenesis marker (aP2) mRNA expression was significantly decreased in HE compared to HD ($p < .05$). **CONCLUSION:** These findings suggest that moderate exercise training has beneficial effects to inhibit adipose tissue remodeling and macrophage infiltration in high fat diet-induced obese mice.

Key Words: high-fat diet, moderate exercise, adipocyte, macrophage infiltration, lipid metabolism

S-IV-4

Gene expression profiling in skeletal muscle with aerobic exercise

Jung-Jun Park

Division of Sport Science, Pusan National University

It is well known that regularly performed aerobic exercise induces major adaptations in skeletal muscle. These include increases in the mitochondrial content and respiratory capacity of the muscle fibers. The major metabolic consequences of these adaptations are a slower utilization of muscle glycogen and blood glucose, a greater reliance on fat oxidation, and less lactate production during exercise of a given intensity. These results in the large increase in the ability to perform prolonged strenuous exercise that occurs in response to aerobic exercise training. However, molecular mechanism underlying these adaptations still remains unclear. One of the potential reasons for this difficulty may include limited time to investigate each candidate gene related these adaptations. Therefore, we studied gene expression profile of skeletal muscle on middle-aged overweight men (n=5) and women (n=5) with fasting hyperinsulinemia (> 10 IU/ml) following aerobic exercise training. Aerobic exercise training program was composed of high intensity (65-80% of VO2 max) and high dose (4-5 days/week) for 9 months. Affymetrix U133A chips were used for gene expression profiling. Analysis was done by using d-Chip, RMA, GeneSpring software with a fold change cutoff of 1.5 and statistical difference at $p < .05$. Two-round amplification was performed to produce enough target cRNA for expression profiling. Not surprisingly, we found that there was a trend towards the increased expression of those genes most strongly

associated with the more oxidative/type I fiber/heart-like phenotype, and the down-regulation of genes associated with the glycolytic, type II fiber phenotype. Most interesting founding was that many of these genes have functionally related promoter elements. These data suggest the possibility that an "aerobic transcriptional phenotype" may be present in exercise trained skeletal muscle prior to its detection by classic immuno/histochemical fiber-typing techniques, which means these physical adaptations are responsible for the improved glucose uptake, insulin sensitivity and lipid profiles.
Key Words: Aerobic exercise, Skeletal muscle, Gene expression profile

S-IV-5

Changes of muscle insulin-like growth factor-I and concentrations of inflammatory cytokines in rat skeletal muscle following denervation and diabetes-induced atrophy

Byeong-hwan Jeon

School of Sports and Health, Kyungsung University, Busan, 48434, Korea

Muscle atrophy is the result of several diseases and conditions. In systemic disease, many factors contribute to muscle atrophy. Insulin-like growth factor-I (IGF-I) is a local and systemic hormone that contributes to muscle growth. The aim of this study was to investigate the role of local IGF-I in muscle atrophy during systemic disease. Local muscle tissue damage was observed and compared in both streptozotocin (STZ)-induced diabetic and denervated rats. In these animal models, we measured the expression of IGF-1 in muscle by real-time PCR and serum concentrations of inflammatory cytokines by ELISA. In addition, muscle weight and blood glucose levels were observed for six weeks. The results showed that muscle mass decreased during the first week in denervated rats. In diabetic rats, muscle mass showed no significant reductions but was significantly lower than that in the control group. Inflammatory factors including TNF-α and IL-6 were significantly higher in diabetic rats than in the control group. The level of tumor necrosis factor-α (TNF-α) was increased at four weeks; however, IL-6 levels did not change in denervated rats. The expression of IGF-1 mRNA did not change significantly in the two experimental groups. In conclusion, IGF-1 did not have an effect on muscle atrophy. Muscle atrophy may be attributed to inflammatory factors via activation of protein degradation.
Key Words: Denervation, Diabetic, Growth Hormone, Inflammatory Markers, Muscle Atrophy

S-V-1

Therapeutic development of optimized mesenchymal stem cells and delivery technology for myocardial infarction

Youngkeun Ahn

Department of Cardiology, Chonnam National University Hospital, Gwangju, South Korea, 501-757

Despite substantial advances in therapeutic modalities, cardiovascular disease remains the leading cause of death worldwide. Cell-based therapy represents a new generation in the evolution of biomedical therapeutics. The mesenchymal stem cells (MSC) have been emerged as representative adult stem cells due to their safety and efficacy and have rapidly been applied in a broad field of disorders. MSC have the capability to transdifferentiate, modulate immune system, and stimulate endogenous regenerative potential in injured heart. In Korea, FCB Pharmicell developed the first MSC product (Hearticellgram-AMI) to be approved for clinical use in acute myocardial infarction. It was approved for sale in July 2011, although concerns have been raised over the unsatisfactory efficacy. Accumulating data from preclinical and clinical studies demonstrate the safety of MSC in a range of cardiovascular diseases, but also raise an issue about a wide gap between the results and expected outcomes. Our research findings suggest that the regenerative activity of MSC is evidently associated with donor's pathophysiological factors such as diabetic stress. The potential disadvantage of autologous cell therapy is that patients with risk factors may fail to get functional recovery. We identified the mediators in MSC insulted with diabetic stress such as Krüppel-like factor 2 (KLF2), angiopoietin-like 4 (Angptl4), microRNA-132 (miR-132), and miR-34c. Additionally, we are searching for safe priming agent to reprogram the stem cell fate. In this lecture, I will discuss the current status of MSC therapy for cardiovascular disease, and demonstrate the underlying mechanism of MSC exposed by diseased niche to optimize their effectiveness in the clinical setting.
Key Words: mesenchymal stem cell, myocardial infarction, therapy, optimization

S-V-2

Transforming stem cell therapy with nanobiomaterials

Byung-Soo Kim

School of Chemical and Biological Engineering, Seoul National University, Seoul, Korea

Nanobiomaterials can direct stem-cell fate both in vitro and in vivo by displaying stem-cell-regulatory signals in a precise fashion. This presentation will show how new technologies of biomaterials can be used to regulate stem cell differentiation. Graphene and its derivatives can promote adsorption of cell-adhesion signals and soluble signals, which can be applied to enhancement of stem cell differentiation into cardiac [1-3] and chondrogenic lineages [4]. Although these materials can promote the differentiation of stem cells, the difficulties associated with engineering graphene into 3D macrostructured scaffolds have hampered the application of graphene in tissue engineering and regenerative medicine. From a practical perspective, carbonized polyacrylonitrile (cPAN), a highly ordered carbon isomorph that resembles the graphitic structure of graphene, could be a promising alternative, as cPAN can be easily processed into 3D scaffolds. We demonstrate the fabrication of microporous 3D scaffolds of cPAN

and excellent osteoinductivity of cPAN, suggesting utility of 3D cPAN scaffolds as synthetic bone graft materials [5]. Iron oxide nanoparticles (IONPs) can be used to develop gap junctional crosstalk among cardiomyocytes and stem cells in co-culture and induce cardiomyogenic differentiation of stem cells, generating therapeutic cells that exceed the reparative potentials of conventional stem cells [6]. Mesenchymal stem cells have tumor tropism and can be used to deliver anti-tumor drugs to tumor [7]. References [1] Park J, Kim YS, Ryu S, Kang WS, Park S, Han J, Jeong HC, Hong BH, Ahn Y, Kim BS. Advanced Functional Materials 25(17): 2590-2600 (2015) (IF=11.800) [2] Park J, Kim B, Han J, Oh J, Park S, Ryu S, Jung S, Shin JY, Le BS, Hong BH, Choi D, Kim BS. ACS Nano 9(5):4987-4999 (2015) (IF=12.881) [3] Ryu S, Yoo J, Jang Y, Han J, Yu SJ, Park J, Jung SY, Ahn KH, Im SG, Char K, Kim BS. ACS Nano (in press) (IF=12.881) [4] Yoon HH, Bhang SH, Kim T, Yu T, Hyeon T, Kim BS. Advanced Functional Materials 24: 6455–6464 (2014) (IF=11.800) [5] Ryu S, Lee C, Park J, Lee JS, Kang S, Seo YD, Jang J, Kim BS. Angewandte Chemie-International Edition 53(35):9213-9217 (2014) (IF=11.800) [6] Han J, Kim B, Shin JY, Ryu S, Noh M, Woo J, Park JS, Lee Y, Lee N, Hyeon T, Choi D, Kim BS. ACS Nano 9(3):2805-2819 (2015) (IF=12.881) [7] Kang S, Bhang SH, Hwang S, Yoon J, Song J, Jang H, Kim S, Kim BS. ACS Nano (in press) (IF=12.881)
Key Words: stem cell therapy, nanobiomaterials, differentiation

S-V-3

Mitochondrial function in stem cell differentiation

Hye Jin Heo, Nari Kim, Jin Han

National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, 614-735, Korea

Cardiomyocytes that differentiate from pluripotent stem cells (PSCs) provide a crucial cellular resource for cardiac regeneration. The mechanisms of mitochondrial metabolic and redox regulation for efficient cardiomyocyte differentiation are, however, still poorly understood. Here, we show that inhibition of the mitochondrial permeability transition pore (mPTP) by Cyclosporin A (CsA) promotes cardiomyocyte differentiation from PSCs. We induced cardiomyocyte differentiation from mouse and human PSCs and examined the effect of CsA on the differentiation process. The cardiomyogenic effect of CsA mainly resulted from mPTP inhibition rather than from calcineurin inhibition. The mPTP inhibitor NIM811, which does not have an inhibitory effect on calcineurin, promoted cardiomyocyte differentiation as much as CsA did, but calcineurin inhibitor FK506 only slightly increased cardiomyocyte differentiation. CsA treated cells showed an increase in mitochondrial calcium, mitochondrial membrane potential, oxygen consumption rate, ATP level, and expression of genes related to mitochondrial function. Furthermore, inhibition of mitochondrial oxidative metabolism reduced the cardiomyogenic effect of CsA while antioxidant treatment augmented the cardiomyogenic effect of CsA. Our data show that mPTP inhibition by CsA alters mitochondrial oxidative metabolism and redox signaling, which leads to differentiation of functional cardiomyocytes from PSCs.
Key Words: pluripotent stem cells, mitochondria, cardiomyocytes, Cyclosporin A

S-V-4

Hair growth promotion by adipose-derived stem cells: Fact or Fiction?

Jong-Hyuk Sung

College of Pharmacy, Yonsei University #162-1, Songdo-dong, Yeonsu-gu, Incheon, 406-840, Korea

Adipose-derived stem cells (ASCs) have been used in tissue repair and regeneration. Recently, it was reported that ASC transplantation promotes hair growth in animal experiments, and a conditioned medium of ASCs (ASC-CM) induced the proliferation of hair-compositing cells in vitro. However, ASCs and their conditioned medium show a limitation to their effectiveness in clinical uses. ASC preconditioning is one strategy that can be used to enhance the efficacy of ASCs and ASC-CM. Therefore, I will highlight the functional role of ASCs in hair cycle progression and also the advantages and disadvantages of their application in hair regeneration. In addition, I will introduce novel ASC preconditioning methods to enhance hair regeneration using ASC stimulators, such as vitamin C, platelet-derived growth factor, hypoxia, and ultraviolet B.
Key Words: Adipose-derived stem cells (ASCs), conditioned medium, hair regeneration, ASC preconditioning

S-VI-1

Ambivalent role of phosphate in health and disease

Tuyet Thi Nguyen, Xianglan Quan, Seung-Kyu Cha, Kyu-Sang Park

Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Gangwon-Do 220-701, Korea

Inorganic phosphate (P_i) plays an essential role in cell signaling and energy metabolism. Particularly, P_i in mitochondria not only provides a substrate for ATP synthesis but also activates respiratory chain. Mitochondrial P_i uptake is driven by nutrient-generated pH gradient, which results in further development of mitochondrial electrical gradient. This hyperpolarization accelerates electrogenic ATP^{4-}/ADP^{3-} translocation, and exported ATP in cytosol acts as a main stimulus for insulin exocytosis. However, mitochondrial P_i overload elicits reactive oxygen species (ROS) generation and precipitates opening of PT pore. High P_i increases the abundance of plasmalemmal P_i transporters and induces cytosolic alkalinization, which further augments mitochondrial P_i uptake and superoxide production. Mitochondrial ROS caused by excessive P_i leads to mitochondrial dysfunction and ER stress and, as a consequence, deteriorates insulin content and secretion. In vascular smooth muscle cells, oxidative stress due to mitochondrial P_i uptake triggers osteogenic gene upregulation and calcific changes. Most of pathologic alterations by exposure of high P_i can be prevented by either mitochondrial antioxidants or suppressions of plasmalemmal and mitochondrial P_i transporters. There are conflicting roles of cellular P_i, which might be a novel therapeutic target to improve mitochondrial energy metabolism or to prevent pathogenic consequences such as metabolic and cardiovascular morbidities.
Key Words: Inorganic phosphate, Mitochondria, Oxidative stress, Phosphate transporters

S-VI-2

Roles of ion channels in cell death processes

Soo Jeong Park, Sarah Yoon, Jeong Hee Kang, Sun Park, Ho-Joon SHin, Kyeongmin Kim, Yong-Joon Chwae

Department of Microbiology, Ajou University School of Medicine, Suwon, Korea

Apoptosis, which is anti-inflammatory, and necrosis, which is pro-inflammatory, represent the extremes of the cell death spectrum. In the context of cellular volume regulation of two processes, apoptosis and necrosis can be characterized by apoptotic volume decrease (AVD) and necrotic volume increase (NVI), respectively. AVD concurrent with cell shrinkage is induced by activation of K^+ and Cl^- channels at early apoptosis before caspase activation, whereas NVI is caused by activation of Na^+ channels. Here, we introduce a novel combined mode of cellular demise–caspase-dependent regulated necrosis. Most importantly, it is mainly characterized with release of marked amount of oligo- or poly-nucleosomes and their attached damage-associated molecular patterns (DAMPs) and initiated by caspase activation. Caspase-activated DNase has dual roles in nucleosomal release as it can degrade extracellularly released chromatin into poly- or oligo-nucleosomes although it prohibits release of nucleosomes. Osmotically triggered water movement following Cl^- influx and subsequent Na^+ influx appears to be the major driving force for nucleosomal and DAMPs release although the specific Cl^- channels are not yet certain.
Key Words: Cancer cell death, Nucleosome, DAMPs, Caspase, Calpain, Cl^- Channel

S-VI-3

Role of zinc on hypoglycemia-induced neuron death

Sang Won Suh

Department of Physiology, Hallym University, College of Medicine, Chuncheon, Gangwon-Do 200-702, Korea

Oxidative stress and zinc release are both known to contribute to neuronal death after hypoglycemia; however, the cause-effect relationships between these events are not established. Here we found, using a rat or a mice model of profound hypoglycemia, that the neuronal zinc release and translocation that occur immediately after hypoglycemia are prevented by the nitric oxide synthase inhibitor 7-nitroindazole but not by overexpression of superoxide dismutase-1 (SOD-1). However, overexpression of SOD-1 prevented activation of poly(ADP-ribose) polymerase-1 (PARP-1) and neuronal death, suggesting that zinc release is upstream of superoxide production. Accordingly, zinc-induced superoxide production was blocked in neuronal cultures by the NADPH oxidase inhibitor apocynin and by genetic deficiency in the p47(phox) subunit of NADPH oxidase. A key role for the vesicular zinc pool in this process was suggested by reduced superoxide formation and neuronal death in mice deficient in zinc transporter 3. Together, these findings suggest a series of events in which nitric oxide production triggers vesicular zinc release, which in turn activates NADPH oxidase and PARP-1. This sequence may also occur in other central nervous system disorders in which zinc, nitric oxide, and oxidative stress have been linked.
Key Words: Zinc, Oxidative stress, Hypoglycemia, Neuronal Death, NADPH oxidase

S-VI-4

Zinc homeostasis and osteoarthritis

Jang-Soo Chun

School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

Osteoarthritis (OA), primarily characterized by cartilage degeneration, is caused by an imbalance between anabolic and catabolic factors. Here, we investigated the role of zinc (Zn2+) homeostasis, Zn2+ transporters, and Zn2+-dependent transcription factors in OA pathogenesis. Among Zn2+ transporters, the Zn2+ importer ZIP8 was specifically upregulated in OA cartilage of humans and mice, resulting in increased levels of intracellular Zn2+ in chondrocytes. ZIP8-mediated Zn2+ influx upregulated the expression of matrix-degrading enzymes (MMP3, MMP9, MMP12, MMP13, and ADAMTS5) in chondrocytes. Ectopic expression of ZIP8 in mouse cartilage tissue caused OA cartilage destruction, whereas Zip8 knockout suppressed surgically induced OA pathogenesis, with concomitant modulation of Zn2+ influx and matrix-degrading enzymes. Furthermore, MTF1 was identified as an essential transcription factor in mediating Zn2+/ZIP8-induced catabolic factor expression, and genetic modulation of Mtf1 in mice altered OA pathogenesis. We propose that the zinc-ZIP8-MTF1 axis is an essential catabolic regulator of OA pathogenesis.
Key Words: Zinc, ZIP8, MTF1, Cartilage, Osteoarthritis

S-VII-1

Gαi-mediated TRPC4 activation by polycystin-1 contributes to the endothelial dysfunction in polycystic kidney diseases

Misun Kwak, Chansik Hong, Kotdaji Ha, Ju-Hong Jeon, Insuk So

Department of Physiology, Seoul National University College of Medicine

Polycystin-1 (PC1) is a candidate protein for polycystic kidney diseases (PKD) and regulates a number of cellular processes, for example, heterotrimeric G protein and transcription factor. We have previously reported that TRPC4/C5 channel can be activated by Gαi-coupled receptors. We assumed that PC1 might act as a Gαi-coupled receptor, so there might be interaction between PC1 and TRPC4/5 via Gαi proteins based on the phenotypes, that is, aneurysm in PKD and endothelial dysfunction in TRPC4 -/- mice. Here, we identified that PC1 dominantly interacts with Gαi3 using co-immunoprecipitation. Thus we recorded the activity of TRPC4/C5 heterologously co-expressed with PC1 in HEK293 cells. PC1 activated TRPC4β channel (4 ± 1 → 41 ± 14 pA/pF) by modulating G-protein signaling without change in TRPC4 translocation. Intracellular 0.2 mM GTPγS-induced TRPC4 activation was not significantly different in the presence or absence of PC1. C-terminal fragment (CTF) of PC1 did not affect TRPC4/C5 activity due to loss of N-terminus containing G-protein coupled receptor proteolytic site (GPS). Dominant negative Gαi3 (G202T) mutant inhibited PC1-activated TRPC4 current. TRPC5 also was activated by full-length PC1 (54 ± 8 → 114 ± 16 pA/pF). Using Fura-2 indicator, we observed intracellular Ca2+ increase by PC1 through TRPC4 channel. We, next, investigated whether TRPC4 induces activation of STAT (signal transduction and transcription) proteins, leading to cell proliferation or death. We observed that STAT1 and STAT3, but not STAT6 activation by PC1 is independent on Src kinase cascades. Interestingly, TRPC4 promoted STAT1 activation. When PC1 co-expressed with TRPC4, STAT1 activation was further increased compared to each sole expression, causing cystic cell death. To determine the role of PC1 with TRPC4 activation in endothelial cell migration, we performed a loss of function screening assay and a wound-healing assay in HUVECs (human umbilical vein endothelial cells). The downregulation of PC1 and TRPC4 activity by the PC1 knockdown or TRPC4 antagonist inhibited the migration of HUVECs. Our findings indicated an important function between PC1 and TRPC4/C5 in modulation of intracellular Ca2+ signaling and provided a new potential therapeutic approach targeting TRPC4/C5 channel in polycystic kidney disease, especially intracranial aneurysms.

S-VII-2

Simulation-based study of PIP_2-meditated regulation of TRP channels based on voltage-sensing phosphatase and FRET measurements

Ryuji Inoue, Hu Yaopeng, Jun Ichikawa, Lin Kurahara, Tomohiro Numata

Department of Physiology, Fukuoka University School of Medicine, Fukuoka 814-0180, Japan

Transient receptor potential (TRP) protein constitutes a large super-family of cation channels, which activate in response to a variety of physicochemical stresses and have been implicated in an unprecedentedly huge repertoire of body functions and dysfunctions. However, despite much efforts, quantitative studies defining the exact functions of TRP channels are entirely missing.
To make a break-through in this situation, we recently introduced the Förster Resonance Transfer (FRET) technique to chase a dynamic change in the endogenous phosphatidylinositol 4,5-bisphosphate (PIP_2) level and investigated its impact on TRPC3/C6/C7 and TRPM4 channels. To control the endogenous PIP_2 level in a graded manner, we employed the voltage-sensing phosphatase (VSP), the activation of which can readily be controlled by changing the intensity and duration of depolarization. With this optimal combination, we could quantify the temporal relationships between the endogenous PIP_2 level and the activities of these channels whereby to be able to construct dynamic numerical models. The results of simulation based on these models indicate that seemingly small differences in PIP_2 sensitivity among different homologues or among wild type and single amino acid mutants can nonlinearly be amplified to cause greatly different ultimate responses. In this talk, I would discuss the significance of these findings in their possible connections to hypertensive and arrhythmic disorders.

S-VII-3

Roles of TRPM4 in cardiac electrical activity and its perturbations

Romain Guinamard

Caen-Normandie University, France

TRPM4 forms a non-selective cation channel activated by internal Ca^{2+}. It is widely expressed among tissues, with a high level in heart. Its functional expression was demonstrated in cardiomyocytes of several mammalian species including human. The identification of TRPM4 inhibitors (flufenamic acid, 9-phenanthrol) allowed revealing TRPM4 current properties in cardiac cells and unmasking its role in action potential. This was also supported by the generation of *Trpm4* null mice allowing studying the impact of gene disruption. At the electrical level, TRPM4 participates in diastolic depolarization and beating rate in the sino-atrial node. It prolongs the duration of the action potential in cardiomyocytes and Purkinje fibers cells. In rat, TRPM4 inhibition prevents cardiac ischemia-reperfusion injuries and decreases the occurrence of arrhythmias. In addition to these *in-vitro* studies, *TRPM4* mutations have been identified in patients with inherited cardiac diseases including conduction blocks and Brugada syndrome. Even if a global understanding of its roles is not yet available, these data indicate that TRPM4 is an actor in cardiac electrophysiology. Note that in addition to these direct implications on cardiac electrophysiology, TRPM4 was also reported to modulate ventricular contractility and cardiac development or hypertrophy.

S-VII-4

Activation of calcium-dependent monovalent cation current by shear stress in atrial myocytes: possible role of TRPM4

Min-Jeong Son, Joon-Chul Kim, Sung Woo Kim, Bojjibabu Chidipi, Insuk So[1], Krishna P. Subedi, Sun-Hee Woo

College of Pharmacy, Chungnam National Univeristy, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea, [1]Department of Physiology, College of Medicine, Seoul National University, Seoul 110-799, Korea

Atrial myocytes are subjected to shear stress during the cardiac cycle under physiological or pathological conditions. The ionic currents

regulated by shear stress remain poorly understood. We report the characteristics, molecular identity and activation mechanism of the shear stress-sensitive current (Ishear) in rat atrial myocytes. Shear stress of about 16 dyn/cm^2 was applied to single myocytes using a pressurized microflow system, and the current was measured by whole-cell patch clamp. In symmetrical CsCl solutions with minimal concentrations of internal EGTA, Ishear showed an outwardly rectifying current–voltage relationship (reversal at approximately -2 mV) and was well sustained. The current was conducted primarily (approximately 80%) by monovalent cations, but not Ca^{2+}. It was suppressed by intracellular dialysis with 15 mM EGTA, selective inhibitors of transient receptor potential melastatin subfamily 4 (TRPM4), intracellular introduction of TRPM4 antibodies, or knock-down of TRPM4 expression, suggesting that TRPM4 carries most of this current. A notable reduction in Ishear occurred upon inhibition of Ca^{2+} release through the ryanodine receptors or inositol 1,4,5-trisphosphate receptors (IP_3Rs) and upon depletion of sarcoplasmic reticulum Ca^{2+}. In type 2 IP_3R (IP_3R2) knock-out atrial myocytes, Ishear was 10–20% of that in wild-type myocytes. Inhibition of protein kinase C, another protein activated by phospholipase C signaling, eliminated the sustained Ishear with no effect on initial Ishear. Immunocytochemistry revealed that TRPM4 and IP_3R2 were expressed at peripheral junctional sites with considerable co-localization. Our data suggest that shear stress activates TRPM4 current by triggering Ca^{2+} release from the IP_3R2 in peripheral domains of atrial myocytes.

Key Words: shear stress, atrial myocytes, TRPM4, type 2 IP3R

S-VIII-1

Both sides of mGluR5 in maladaptive pain brain

Geehoon Chung[1,3], Chae Young Kim[1,2], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, [3]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences

Neuropathic pain patients often suffer from long-lasting severe pain symptoms still after the nerve injury-induced peripheral change is diminished. This chronic pain is considered as maladaptive byproduct of nociceptive circuits of the nervous system. In contrast to detailed understanding of alterations related to increased pain transmission of primary afferent and spinal cord, much less is known about the alteration of brain functions related to pain perception and modulation. Here we hypothesized that maladaptive change of pain circuit in the brain contributes to pathogenesis of chronic neuropathic pain. That is, with the peripheral dysregulation settled, altered brain itself should reproduce pain and maintain it even in the absence of peripheral sensitization. We focused on the role of metabotropic glutamate receptor 5 (mGluR5) in the brain in that mGluR5 is widely expressed in the nervous system and contributes to the plastic change of the neurons in the diverse variety of physiological and pathological states. Given the diverse role of mGluR5 in neuronal plasticity, it is assumable that mGluR5 also plays a role in maladaptive change of pain processing in the brain during chronic neuropathic pain state. We identified the functional role of mGluR5 in pain processing and the altered mGluR5 activity in pain brain using behavior analysis combined with microPET imaging and slice patch-clamp techniques. Our study revealed bidirectional actions of mGluR5 in pain processing, demonstrating its functional roles in pain modulation and pain perception, respectively.

Key Words: Neuropathic pain, Metabotropic glutamate receptor 5, Brain imaging, Electrophysiology

S-VIII-2

Targeting of acid-sensing ion channels (ASICs) to the plasma membrane

Byung-Chang Suh

Department of Brain & Cognitive Sciences, DGIST, Daegu 711-873, Korea

Acid-sensing ion channels (ASICs) are proton-activated cation channels which play important roles as typical proton sensors during pathophysiological conditions and normal synaptic activities. Among the ASIC subunits, ASIC2a and ASIC2b are alternative splicing products from the same gene, ACCN1. It has been known that ASIC2b is insensitive to proton, unlike ASIC2a. Here we report their differential subcellular distribution in heterologous expression system, and elucidate underlying mechanisms for their trafficking to the cell surface. By constructing chimeras, we identified the first transmembrane (TM1) domain and the proximal post-TM1 domain (17 amino acids) of ASIC2a as critical forward trafficking signals of ASIC2 proteins. Additionally, we demonstrated that the proximal post-TM1 domain of ASIC2a is also involved in proton-sensitivity of ASIC2. Finally, we discovered that ASIC2b can be delivered to the plasma membrane from the ER by heteromeric assembly with ASIC2a. Our study has uncovered hidden trafficking mechanisms of ASIC2.

Key Words: Acid-sensing ion channels, proton sensor, membrane trafficking, regulation

S-VIII-3

Discovery of novel mGluR1 antagonists for the potential treatment of neuropathic pain

Ae Nim Pae

Center for Neuro-Medicine, Brain Science Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, South Korea

Glutamate is the major excitatory neurotransmitter and known to activate the metabotropic and ionotropic glutamate receptors in the brain. Among these glutamate receptors, metabotropic glutamate receptor 1 (mGluR1) has been implicated in various brain disorders including anxiety, schizophrenia and chronic pain. Several studies demonstrated that the blockade of mGluR1 signaling reduced pain responses in animal models, suggesting that mGluR1 is a promising target for the treatment of neuropathic pain. In an effort to identify potent and selective mGluR1 negative allosteric inhibitors for treatment of neuropathic pain, structure and ligand-based molecular modeling studies were undertaken. Hierachical combining ligand-based vHTS protocols of pharmacophore and naïve Bayesian classification models with the structure-based protocol showed high synergistic effects in enrichment factors. We have developed mGluR1 antagonists with an aryl isoxazole and tetrahydrothieneo pyridine scaffold. Several compounds are orally active in vivo. We believe that these compounds can serve as a useful tool for the investigation of the role of mGluR1 and a promising lead for the potential treatment of neuropathic pain.
Key Words: metabotropic glutamate receptor 1

S-VIII-4

The of roles of GABA on neuropathic pain and reward following spinal cord injury in rats

Moon Yi Ko, Jun Yeon Lee, Su Phil Kim, Chae Ha Yang, Hee Young Kim, Young S. Gwak

Department of Physiology, Daegu Haany University, Daegu 42158

GABA is the major inhibitory neurotransmitter in the central nervous system includes sensory and psychiatric pathophysiology. To investigate the roles of GABA at both sensory and psychiatric abnormality, the present study tested GABA-mediated neuropathic pain and reward mechanism following spinal cord injury in rats. SCI was produced by T10 clip compression injury (35g, 1 mins) in ages with 180-225 g male SD rats. To test the roles of GABA, paw withdrawal response, ultrasound vocalization, in vivo extracellular single cell recording, HPLC-microdialysis and immunohistochemistry were performed. Prior to injury, the baseline paw withdrawal threshold and ultrasound vocalization was recorded, respectively. Post injury days 40, SCI groups showed significantly decreased paw withdrawal threshold at both hindpaws and increased ultrasound vocalization compared to before injury, respectively (*$p<0.05$). In vivo extracellular electrophysiology at the ventral tegmental area (VTA), GABA neuron activity (characterized by less than 1 ms full action potential duration and > 5 Hz frequency) showed increased firing rates (13.6 ± 1.7 spikes/sec) compared to sham control groups (7.3 ± 1.1 spikes/sec). In addition the ultrasound vocalization also showed increased changes in SCI groups. In immunohistochemistry, glutamic acid decarboxylase (GAD) 67 showed increased expression compared to sham control groups (*$p<0.05$) at the VTA. In HPLC-microdialysis at the VTA, the concentration of glutamate (608.3±0 nM) and GABA (98.8±50.5 nM) in SCI groups showed increases compared to sham controls (353±0 nM and 4±1.3 nM), respectively. Taken together, the present study suggests that chronic neuropathic pain reveals the increased activity of VTA GABA neurons that result in the suppression of dopaminergic neurons. In addition, the intrathecal administration of GABA receptor agonists at the spinal cord resulted in the attenuation of neuropathic pain and neuronal hyperexcitability that suggests the decreased tone of spinal GABAergic tone following SCI. In conclusion, the present data suggests that the specific modulations of the endogenous tone of GABA are important factor for the therapeutic treatment at both sensory and psychiatric abnormality. This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP)(NRF-2015R1A5A7037508) and NRF-2014R1A1A4A01004179.
Key Words: GABA, Neuropathic Pain, Reward, Spinal cord injury

P01-01

Glycosaminoglycans increase the phagocytic ability of macrophages via the activation of AMP-activated protein kinase and cytoskeletal reorganization

Ji Hyeon Ryu[1], Jina Sung[1], Chengliang Xie[2], Jaehee Han[1], Nam-Gil Kim[3], Yeung Joon Choi[4], Byeong Dai Choi[4], Sang Soo Kang[2], Dawon Kang[1]

Departments of [1]Physiology and [2]Anatomy, College of Medicine, Gyeongsang National University, Jinju 660-751, Republic of Korea [3]Department of Marine Biology and Aquaculture and Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Republic of Korea [4]Department of Seafood Science and Technology and Institute of Marine Industry, Gyeongsang National University, Tongyeong 650-160, Republic of Korea

Glycosaminoglycans (GAGs) control a variety of physiological processes. However, the roles of GAGs in inflammatory processes are unclear. This study was performed to identify the role of GAG isolated from the sea hare Aplysia kurodai (A. Kurodai), which is an East-Asian species of marine gastropods. The role of GAG in macrophage phagocytosis was assessed. Treatment with GAG activated macrophage RAW264.7 cells, which exhibited increased cell size, cellular spreading, and vacuole formation. In addition, GAG increased the phagocytic ability of RAW264.7 cells. The GAG-induced activation and phagocytosis of macrophages were reduced with compound C, an inhibitor of AMPK, whereas AICAR, an activator of AMPK, induced the activation and phagocytosis of macrophages. However, the activation pattern was different between GAG and AICAR. GAG induced vacuolization and spreading, whereas AICAR induced primarily spreading. Disruption of cytoskeletal reorganization with nocodazole and cytochalasin D reduced the GAG-activated phagocytic ability, cellular spreading, and vacuolization of RAW264.7 cells. These results indicate that GAG increases the phagocytic ability of macrophages via AMPK activation and cytoskeletal reorganization. The AMPK activation is primarily involved in the cellular spreading. Our results suggest that GAG isolated from the sea hare A. kurodai may have potential therapeutic role in inflammatory disease.

Key Words: AMPK, Aplysia, Glycosaminoglycans, Inflammation, Phagocytosis

P01-02

Inhibitory effects of cyanidin-3-glucoside on amyloid beta (25-35)-induced neuronal cell death in cultured rat hippocampal neurons

Ji Seon Yang[1], Kee Dong Yoon[2], Yang-Hyeok Jo[1], Shin Hee Yoon[1]

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, [2]College of Pharmacy, The Catholic University of Korea, Bucheon-si 420-743, Republic of Korea

Increasing evidences implicate changes in [Ca2+]i and oxidative stress as causative factors in amyloid beta (Aβ)-induced neuronal cell death. Cyanidin-3-glucoside (C3G), a component of anthocyanin, has been reported to protect against glutamate-induced neuronal cell death by inhibiting Ca2+ and Zn2+ signaling. The present study was investigated to determine whether C3G has a protective effect against Aβ-induced neuronal cell death in cultured rat hippocampal cells and pure hippocampal neurons from embryonic day 17 fetal Sprague-Dawley rats using digital imaging methods for Ca2+, Zn2+, MMP and ROS, and MTT assay for cell survival. Pretreatment with C3G (10 μg/ml) for 30 min inhibited Aβ-induced [Ca2+]i increases in the cultured rat hippocampal neurons. C3G significantly inhibited Aβ-induced mitochondrial depolarization. C3G blocked Aβ-induced formation of ROS. C3G also significantly inhibited Aβ-induced [Zn2+]i increases. Treatment with C3G (10 μg/ml) for 48 h attenuated Aβ-induced neuronal cell death in cultured rat pure hippocampal neurons. Taken together, all these results suggest that cyanidin-3-glucoside inhibits Aβ-induced Ca2+ signaling, mitochondrial depolarization, formation of reactive oxygen species and Zn2+ signaling in cultured rat hippocampal neurons, which is involved in neuroprotection. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022015)" Rural Development Administration, Republic of Korea.

Key Words: Ca2+, flavonoid, Zn2+

P02-01

Electrophysiological profiling of cardiac myocytes underlying metabolic substrates-induced spontaneous contractions in normal and hypertensive rats

Jae Hwi Sung, Zai Hao Zhao, Sung Joon Kim, Yin Hua Zhang

Department of physiology, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

Background: Metabolic syndrome is the precursor of myocardial dysfunction, including fatal ventricular arrhythmias. Our recent results have shown that supplementation of metabolic substrates in normal Tyrode's solution (fatty acids, pyruvate and lactate, termed NF) significantly increased left ventricular (LV) myocyte contraction. Beta-adrenergic stimulation with isoprenaline (ISO) induced spontaneous myocyte contractions during diastole, which was significantly more frequent in hypertension (a condition resembles an in vitro model of cardiac "metabolic syndrome"). The phenomenon was accompanied by greater and prolonged Ca^{2+} transient amplitudes. So far, the electrophysiological properties of cardiac myocytes in NF and in NF+ISO remain elucidated. Accordingly, the present study aims to investigate action potential (AP) profile, L-type Ca^{2+} channel (ILTCC) and Na^{+}-Ca^{2+}exchanger (I_{NCX}) activities in LV myocytes from Sham and Angiotensin II-induced hypertensive rats (HTN). **Result:** 1) NF and NF+ISO did not affect the upstroke or the resting membrane potentials in either group. 2) AP duration (APD, APD_{10} - APD_{90}) were prolonged by NF and further more by NF+ISO in sham. 3) In HTN, APD_{10} - APD_{90} were reduced compared to those in sham. In addition, the effects of NF or NF+ISO on APD_{10} or APD_{20} prolongation were less prominent. 4) APD50 and APD_{90}, however, were prolonged with NF+ISO and significantly more than those in sham. 5) NF or NF+ISO significantly increased outward I_{NCX} without changing inward I_{NCX} in sham. In HTN, outward I_{NCX} was increased and similar tendency were observed for inward I_{NCX}. 6) In both Sham and HTN, I_{LTCC} was reduced by NF and was increased by NF+ISO. **Conclusion:** The present results suggest that NF significantly prolongs APD which increases the susceptibility of arrhythmia in cardiac myocytes under metabolic stress following beta-adrenergic stimulation. Additional mechanisms (apart from LTCC or NCX) those underlie arrhythmogenesis in normal and hypertensions with metabolic syndrome need to be explored further.

Key Words: hypertension, NCX, arrhythmia, metabolic syndrome, APD

P02-02

Endocytosis of KATP channels drives glucose-stimulated depolarization in pancreatic β-cell

Young-Eun Han, Young-Sun Ji, Jung Nyeo Chun, Sun-Hyun Park, Ajin Lim, Ju Hong Jeon, Sunghoe Chang, Suk-Ho Lee, Won-Kyung Ho

Department of Physiology, Seoul National University College of Medicine, Seoul, 110-799, Korea

Glucose homeostasis of the body relies primarily on the ability of pancreatic β-cells to regulate insulin secretion in response to the changes in blood glucose concentrations. Membrane depolarization by high glucose stimulation, which leads to Ca2+ entry to initiate insulin vesicle exocytosis, is mediated by inhibition of KATP currents, and ATP-dependent channel closure has been known to be responsible. Here we investigate the role of KATP channel endocytosis to glucose-stimulated membrane depolarization in INS-1 cells. High glucose stimulation (17 mM) induced a decreased in surface level of KATP channel proteins, and this decrease was abolished by inhibiting endocytosis using dynasore (a dynamin inhibitor). Interestingly, dynasore significantly inhibited glucose-stimulated membrane depolarization, while it did not affect the glucose-induced increase in intracellular ATP concentrations. Membrane depolarization could be induced under glucose-deprived conditions by reducing surface levels of KATP channels using blebbistatin (a myosin II ATPase inhibitor). These results suggest that decreased surface KATP channel density by endocytosis is a key mechanism that drives glucose-stimulated membrane depolarization, while ATP-dependent channel closure plays a minor role.
Key Words: KATP channel, pancreatic beta cell, insulin secretion, glucose

P02-03

Effects of etomoxir on lipid metabolism and oxidative stress in peripheral tissues

Hye-Jun Jo, Hye-Na Cha, Jung-Yoon Heo, Suk-Jeong Kim, So-Young Park

Department of Physiology, College of Medicine Yeungnam University, Daegu, Korea, Rep.

Fatty acids are the main energy substrates supplied to developing mammals and as fuels for energy production in tissues. Carnitine palmitoyl-transferase 1 (CPT1) is the rate-limiting enzyme in mitochondrial fatty acid oxidation. The CPT1 inhibitor, etomoxir improves sarcoplasmic reticulum function and in vivo heart performance, and delays the dilative ventricular remodelling, whereby metabolic signals appear to be involved. Recent reports indicate a shift from fatty acid oxidation to glucose oxidation leads to a reduced gluconeogenesis and improved economy of cardiac work. However etomoxir causes ATP depletion as well as oxidative stress, and oxidative stress is implicated in a variety of physiological and pathological processes. The purpose of this study was to compare the effects of etomoxir on lipid metabolism and oxidative stress in various tissues such as heart, liver, kidney, and skeletal muscle. Male C57BL/6 mice at 8 weeks of age were randomly divided into four groups: (1)saline, (2)etomoxir (1 mM), (3)lipid (20% intralipid), (4)lipid plus etomoxir. At 3-4 days before the experiments, mice were anesthetized, and an silicone catheter was inserted in the jugular vein. Mice were infused with saline, etomoxir, lipid and lipid plus etomoxir for 6h (2.5 μl/min). We analyzed gene expression using real-time PCR. The plasma levels of triglyceride and free fatty acid were increased by etomoxir and lipid. However, the plasma levels of glucose were decreased by etomoxir, when compared with lipid group. In heart, etomoxir decreased mRNA levels of sterol regulatory element-binding protein 1c (SREBP1c), fatty acid synthase (FAS), and stearoyl-CoA desaturase 1 (SCD1) involved in fatty acid synthesis. In liver, the lipid increased SREBP1c and FAS mRNA levels, whereas etomoxir decreased SREBP1c and FAS mRNA levels. Etomoxir also increased peroxisome proliferator activated receptor-α (PPAR-α) and acyl CoA oxidase (ACO) mRNA levels involved in fatty acid β-oxidation. In kidney, the lipid increased FAS mRNA levels. Etomoxir decreased the mRNA levels of SREBP1a, SREBP1c, FAS and ACC. In both liver and kidney, etomoxir increased fatty acid binding protein 1 (FABP1) mRNA levels. Lipid or etomoxir increased oxidative stress marker, 4-HNE and nitrotyrosine, only in heart. There was no differences in gene expression-related with lipid metabolism in skeletal muscle. These results suggest that etomoxir decreases gene expression related with fatty acid synthesis except skeletal muscle and induces oxidative stress only in heart.
Key Words: Etomoxir, CPT1 inhibitor, Lipid metabolism, Oxidative stress, Fatty acid synthesis

P02-04

Angiotensin II impairs β2-adrenergic receptor stimulation in Human vascular progenitor cells via regulation of β2-adrenergic receptor expression

Seon Jin Lee, Sang Mo Kwon

Laboratory for vascular medicine & stem cell biology, Medical Research Institute, Department of physiology, Pusan National University, Pusan, Korea

We investigated the relationship between angiotensin II (Ang II) and β2-adrenergic receptor (β2AR) in human vascular progenitor cells (VPCs). We firstly performed MTS assay to determine Ang II concentration. Then, we performed β2AR expression analysis after treatment with 100nM Ang II on VPCs. β2AR expression levels significantly were reduced after treatment with AngII on VPCs. Moreover, proliferation, migration, and tube formation ability were decreased in Ang II-induced VPCs when these cells were stimulated by βAR agonist. To investigate the role of Ang II on β2AR of VPCs, we inhibited angiotensin II type 1 (AT1) receptor of VPCs using AT1 receptor blocker, Telmisartan. As a results, βAR stimulation ability was restored in these cells. Take together, Our data suggested that Ang II-mediated β2AR depletion in VPCs could prevented by AT1 receptor blocker. Thus, Ang II-mediated β2AR depletion in VPCs provide important insight into the therapy of vascular disease such as hypertension.
Key Words: Angiotensin II, β-Adrenergic receptor, Hypertension, vascular progenitor cells

P02-05

Oleanolic acid modulates body fluid and blood pressure homeostasis ; suppression the renin-angiotensin system

You Mee Ahn[1,2], Yun Jung Lee[1,2], Hye Yoom Kim[1,2], Rui Tan[1,2], So Heun Lee[1,2,3], Han Sol Lee[1,2,3], Kyung Woo Cho[1,2], Ho Sub Lee[1,2,3]*, Dae Gill Kang[1,2,3]*

[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine,

Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

Oleanolic acid is known to possess beneficial effects on the regulation of the cardiovascular homeostasis. However, the exact nature of the role of oleanolic acid on the regulation of body fluid balance and blood pressure homeostasis and its mechanisms involved are not well defined. We hypothesized that oleanolic acid inhibits the renin-angiotensin system and accentuates renal function. Experiments were performed to identify the effects of oleanolic acid on the renal function, blood pressure, and the renin-angiotensin system in normotensive and hypertensive rats. Oleanolic acid (0, 20, and 30 mg/kg/day) was administered orally for 1 or 3 weeks. Here, we found that oleanolic acid suppressed the renin-angiotensin system. Oleanolic acid increased urinary volume, and urinary excretion of Na+, K+, and Cl-, concomitantly with an increase in clearance for creatinine in normotensive rats. Also, oleanolic acid decreased urinary osmolality. Oleanolic acid significantly suppressed arterial blood pressure in renal hypertensive rats. Furthermore, oleanolic acid suppressed plasma levels of renin activity, angiotensin II, aldosterone, and renal renin contents, and gene expressions by real-time PCR of renin, angiotensin converting enzyme and angiotensin II type 1 receptor in the kidney cortex from normotensive and hypertensive rats. Oleanoliac acid elicited dual effects on the angiotensin II type 2 receptor: suppression with low and accentuation with high dose. These findings suggest that oleanolic acid modulates body fluid and salt balance and blood pressure homeostasis by suppressing the systemic and intrarenal renin-angiotensin system.

Key Words: oleanolic acid, renin, angiotensin II, angiotensin I-converting enzyme, angiotensin II receptor subtype 1, natriuresis, blood pressure

P03-01(O-10)

The effect of mitochondria-targeted antioxidant (MitoQ) on the age-related impairment in vasodilatory function in human skeletal muscle feed arteries

Oh Sung Kwon, Song Young Park, Russell Richardson

Department of Internal Medicine, Division of Geriatrics, University of Utah, Salt Lake City, Utah

Aging is associated with impaired endothelium-dependent dilation in human skeletal muscle feed arteries (SMFAs). Although the cause of this dysfunction is currently not clear, it is possible that attenuated nitric oxide (NO) bioavailability induced by mitochondrial-derived oxidative stress contributes to this age-related impairment in arterial endothelial dysfunction. Therefore, the aim of this study was to determine if treatment with MitoQ can ameliorate the age-related endothelial dysfunction in human SMFAs. Endothelium-dependent and -independent vasodilation were assessed with and without MitoQ in SMFAs obtained from 12 old subjects in response to three stimuli 1) flow-induced shear stress, 2) acetylcholine (ACh), and 3) sodium nitroprusside (SNP). MitoQ significantly improved endothelium-dependent vasodilation stimulated by both flow (Control: 20±2; MitoQ: 41±8 %) and ACh (Control: 68±3; MitoQ: 122±7 %) ($p<0.05$). Meanwhile, MitoQ had no significant effect on endothelium-independent vasodilation (SNP) (Control: 67±3; MitoQ: 68±7 %). These findings suggest that mitochondria-derived oxidative stress is an important mechanism underlying the development of endothelial dysfunction in primary aging. Furthermore, MitoQ represents a promising novel strategy for the preservation of vascular endothelial function with advancing age and therefore may be a potential tool with which to help combat age-related cardiovascular disease.

Key Words: Skeletal muscle feed arteries, Mitochondria, Oxidative Stress, Endothelium, Aging

P03-02(O-11)

The impact of exercise training on vascular mitochondria and arterial function

Song-Young, Park, Russell S Richardson, Dale Abel, Matthew J Rossman, David Symons and Yi sub Kwak, Christian Riehle

[1]Geriatric Research, Education, and Clinical Center, George E. Whalen VA Medical Center, SLC, UT, [2]Department of Internal Medicine, Division of Geriatrics, University of Utah, SLC UT, [3]Department of Exercise and Sport Science, University of Utah, SLC, UT, [4]Division of Endocrinology, Metabolism, and Diabetes, University of Utah School of Medicine, SLC, UT, [5]Molecular Medicine Program, University of Utah, SLC, UT, 5Department of Physical Education Dong Eui University, [6]Fraternal Order of Eagles Diabetes Research Center Division of Endocrinology and Metabolism Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA

Exercise training is recognized to improve skeletal muscle mitochondrial respiratory capacity, however the impact of chronic exercise on vascular mitochondrial respiratory function is unknown. We hypothesized that exercise training would concomitantly increase both vascular mitochondrial respiratory capacity and vascular function. Arteries from both sedentary (SED, n=8) and swim trained (EX, 5 weeks, n=8) male mice were compared in terms of mitochondrial respiratory function, mitochondrial content, markers of mitchodondrial biogenesis, redox balance, nitric oxide (NO) signaling, and vessel function. Mitochondrial complex I and complex I+II state 3 respiration and the respiratory control ratio (RCR, complex I+II state 3 respiration / complex I state, 2 respiration) were greater in vessels from EX than SED mice, despite similar levels of arterial citrate synthase activity (CSA) and mitochondrial DNA content. Furthermore, compared to the SED mice, arteries from the EX mice displayed elevated transcript levels of PGC-1α (Ppargc1a) and the downstream targets Cox4i1, Idh2, Idh3a, increased MnSOD protein expression, increased endothelial NO synthase (eNOS) phosphorylation (Ser1177), and suppressed reactive oxygen species generation (all $P < 0.05$). Although there were no differences in EX and SED mice in terms of classically assessed endothelium-dependent and endothelium-independent vasorelaxation, phenylephrine-induced vasocontraction was blunted in vessels from EX compared to SED mice and this effect was normalized by NOS inhibition. These training-induced increases in vascular mitochondrial respiratory capacity and evidence of improved redox balance, which may, at least in part, be attributable to elevated NO bioavailability, have the potential to protect against age and disease-related challenges to arterial function.

Key Words: exercise, vascular mitochondrial function, shear stress, mitochondrial biogenesis, ROS

P03-03

The adiponectin response research of combined exercise in overweight child

Yi Sub Kwak

Department of Physical Education, Dong–Eui University, Busan 614-714, Korea

Purpose: The purpose of this study is to assess the combined exercise

program (12 weeks exercise training: resistance and aerobic) and 6 weeks detraining on the correlation of metabolic syndrome markers and plasma adiponectin level in two groups. **Methods:** Subjects were separated into two groups (exercise training group [EG: n=8] and control group [CG, n=7]). EG underwent an 12-week training (two times aerobic training per week and two times resistance training per week, more than 40 min). After 12 weeks exercise training and 6 weeks detraining, we evaluated metabolic syndrome markers and plasma adiponectin at 3 periods periods (baseline, EBP; 12 weeks exercise program, 12 EP; 12 weeks and 6 weeks detraining, 12+6 EDP) in overweight and obese children. Compared with the CG, After the 12 weeks exercise treatment, weight, body mass index, waist girth, body fat, percentage body fat, lean body mass, percentage lean body, systolic blood pressure, insulin and homeostatic model assessment (HOMA) indices were significantly lower, and plasma adiponectin level were not altered in the EG. **Results:** After the 6 weeks detraining, insulin, insulin resistance and plasma adiponectin level were significantly increased in the EG. Adiponectin between after 12 weeks exercise intervention and 6 weeks detraining were significantly positive correlations with lean body mass, percent lean body and negative correlation with percent body fat, insulin, insulin resistance. In conclusion, after 12 weeks exercise treatment, plasma adiponectin level wasn't changed significantly but, after 6 weeks detraining, it was significantly increased. **Conclusion:** These findings suggest that combined exercise training is not only an effective tool in the management of metabolic syndrome markers in the training periods but also that these change were kept or additive effecting even after 6 weeks of detraining periods.
Key Words: Exercise program, Plasma adiponectin, Insulin resistance, Metabolism syndrome markers

P03-04

Food-dependent exercise-induced anaphylaxis (FDEIAn) response research in human

Yi Sub Kwak

Department of Physical Education, Dong–Eui University, Busan 614-714, Korea

Food-dependent exercise-induced anaphylaxis (FDEIAn) is induced by different types and various intensities of physical activity, and is distinct from food allergies. It has been shown that consumption of allergenic food followed by exercise causes FDEIAn symptoms. Intake of allergenic food or medication before exercise is a major predisposing factor for FDEIAn. Urticaria and severe allergic reactions are general symptoms of FDEIAn. Dermatological tests and serum IgE assays are the typical prescreening methods, and have been used for several decades. However, these screening tests are not sufficient for detecting or preventing FDEIAn. It has been found that exercise may stimulate the release of mediators from IgE-dependent mast cells that can result in FDEIAn when a certain threshold level has been exceeded. Mast cell degradation might be a major factor to induce FDEIAn but this has not been determined. A number of foods have been reported to be involved in the onset of FDEIAn including wheat, eggs, chicken, shrimp, shellfish, nuts, fruits, and vegetables. It is also known that aspirin increases the occurrence of type I allergy symptoms when combined with specific foods. Moreover, high intensity and frequent exercise are more likely to provoke an attack than low intensity and less frequent exercise. In this paper, we present the current views of the pathophysiological mechanisms underlying FDEIAn within the context of exercise immunology. We also present a detailed FDEIAn definition along with etiologic factors and medical treatment for cholinergic urticaria (UC) and exercise-induced anaphylaxis (EIA).
Key Words: Food, Exercise, Anaphylaxis, FDEIAn

P03-05

Novel anthropometry-based calculation of the body heat capacity in the Korean population

Duong Duc Pham, Jeong Hoon Lee, Young Boum Lee, Eun Seok Park, Ga Yul Kim, Ji Yeon Song, Ji Eun Kim, Chae Hun Leem*

Department of Physiology, University of Ulsan College of Medicine, 88 OlympicRo 43-gil Songpa-gu, Seoul, Republic of Korea

Heat capacity (HC) has an important role in the temperature regulation process, particularly in dealing with the heat load. The actual measurement of the body HC is complicated and is generally estimated by body-composition-specific data. This study compared the previously known HC estimating equations and sought how to define HC using simple anthropometric indices such as weight and body surface area (BSA) in the Korean population. Six hundred participants were randomly selected from a pool of 902 healthy volunteers aged 20 to 70 years for the training set. The remaining 302 participants were used for the test set. Body composition analysis using multi-frequency bioelectrical impedance analysis was used to access body components including body fat, water, protein, and mineral mass. Four different HCs were calculated and compared using a weight-based HC (HC_Eq1), two HCs estimated from fat and fat-free mass (HC_Eq2 and HC_Eq3), and an HC calculated from fat, protein, water, and mineral mass (HC_Eq4). HC_Eq1 generally produced a larger HC than the other HC equations and had a poorer correlation with the other HC equations. HC equations using body composition data were well-correlated to each other. If HC estimated with HC_Eq4 was regarded as a standard, interestingly, the BSA and weight independently contributed to the variation of HC. The model composed of weight, BSA, and gender was able to predict more than a 99% variation of HC_Eq4. Validation analysis on the test set showed a very high satisfactory level of the predictive model. In conclusion, our results suggest that gender, BSA, and weight are the independent factors for calculating HC. For the first time, a predictive equation based on anthropometry data was developed and this equation could be useful for estimating HC in the general Korean population without body-composition measurement.
Key Words: Heat capacity, body surface area, weight-based HC , fat-free mass, mineral mass

P03-06

Effect of long-term exercise on circulating levels of Dickkpof-1 and frizzled-related protein-1 in breast cancer patients

Tae-Ho Kim[1], Jae Seung Chang[1], Hanul Kim[1], Park, jeeyeon[2], Nahyun Kim[3], In Deok Kong[1]

[1]Yonsei Univeristy Wonju College of Medicine, Wonju , Korea, [2]Keimyung University College of Nursing, Daegu, Korea, [3]Kyungsung University, Department of nursing science, Busan, Korea

Background: Dickkpof-1 (DKK1) and secreted frizzled-related protein-1 (SFRP1) are inhibitors of Wnt/β-catenin signaling. Accumulating evidence suggests that higher serum level of DKK1 is positively correlated with pathologic conditions such as cancer and atherosclerosis. Futhermore, DKK1 disrupts osteoblast differentiation and precipitates osteoporosis. Several recent studies showed that exercise reduces DKK1 level in healthy volunteers or experimental animals. We investigated the changes of serum levels of DKK1 and SFRP1, as Wnt/β-catenin inhibitors, by long-term exercise in breast

cancer patients. **Methods:** 25 breast cancer patients (50.4±11.8 years of age) after chemo- or radiotherapy were participated. Intervention groups were randomized to 12 wks of breast cancer exercise program (n = 11) or control group (n = 13). Blood samples were collected to examine the serum level of DKK1 and SFRP1. **Results:** The serum levels of DKK1 ($p < .05$) and SFRP1 ($p < .05$) were significantly reduced during 12 wks exercise program. However, those in control group were not changed during 12 wks. Exercised group showed remarkable increases in health related fitness such as hand-grip strength, muscle endurance and flexibility ($p < .05$). Also, body fat percentage and abdominal circumference were significantly decreased in exercised group compared to control group ($p < .05$). **Conclusion:** Our results suggest that long-term exercise decreases serum levels of DKK1 and SFRP1, which may contribute to the beneficial effects of exercise in cancer patients.
Key Words: DKK1, SFRP1, Breast cancer patients

P03-07

Effect of cardiorespiratory endurance training on power production in figure skaters

Seung Bo Park, Joung Kyue Han

Department of Sport Industry & Information, Graduate School of Chung-Ang University, Seoul 156-756, Republic of Korea

The aim of this study was to compare different forms of plyometric and cardiorespiratory endurance training on the vertical jump ability and 30-m sprint speed to improve motion performance capability among female figure skaters. Twenty participants were randomly allocated to either the plyometric combined with long, slow distance training group (PL) or the plyometric combined with high-intensity interval training group (PI), with 10 participants in each group. The participants performed the exercises five times per week over 12 weeks. The experimental protocol consisted of the 1) familiarization period; 2) baseline test; 3) 12-week training intervention; and 4) vertical jump and 30-m sprint speed tests. 1. Analysis of the data revealed that the vertical jump ability improved to a greater extent after PI (4.49%) as compared to that observed after PL (2.6%; $p<.05$). 2. Analysis of the data revealed that the 30-m sprint speed improved to a greater extent after PI (5.75%) as compared to that observed after PL (2.98%; $p < .05$). Thus, cardiorespiratory endurance training alters power production. Accordingly, plyometric and interval complex training are effective exercise methods for power production.
Key Words: Plyometric, HIIT training, LSD training, Power Production

P03-08

The effect of AQP3 deficiency in fuel selection during a single bout of exhausting exercise

Ju Hyun Lim, Hae-Rahn Bae

Department of Physiology, College of Medicine, Dong-A University, Busan 602-714, Korea

The aquaporin-3 (AQP3) is an integral membrane protein facilitating the transport of water and glycerol across cell membranes. However, detailed localization and function of AQP3 in skeletal muscle is currently unknown. We investigated the capacity to perform a single bout of exhausting exercise in AQP3 knockout mice and analyzed the parameters related to skeletal muscle energy metabolism at exhaustion. Both immunohistochemistry and double immunofluorescence staining revealed that AQP3 expressed at the cell surface with no evidence for colocalization with either AQP1 or AQP4 in skeletal muscle. When exposed to a single bout of treadmill running at the speed up to 12 m/min with 10° incline until exhaustion, AQP3 knock-out mice exhibited earlier fatigue with shorter average time to exhaustion than the wild-type CD1 control. After exhausting exercise, plasma and muscle glucose, muscle glycogen, and plasma and liver triglyceride levels decreased, whereas plasma and liver glycerol levels increased compared to those at rest in both AQP3 knockout and wild-type mice. However, muscle glycerol as well as liver glycogen concentrations decreased after exercise in wild-type mice, but rather increased in AQP3 knockout mice. These findings suggest that decreased glycerol efflux from skeletal muscle in AQP3 knockout mice might result in low exercise capacity due to the limitation in constant energy supply through hepatic gluconeogenesis from glycerol during the prolonged endurance exercise.
Key Words: Aquaporin-3, Glycerol, Exhausting exercise, Intramuscular triglyceride

P04-01

Transient receptor potential canonical 4 (TRPC4) channel regulation by phosphotiesterase 5 inhibitor via the cyclic guanosine 3'5'-monophosphate

Jinhong Wie, Ju-Hong Jeon, Insuk So

Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

The transient receptor potential (TRP) protein superfamily consists of a diverse group of cation channels that bear structural similarities to the Drosophila TRP. TRP superfamily is distinct from other groups of ion channels in displaying a daunting diversity in ion selectivity, modes of activation, and physiological functions. Nevertheless, they all share the common feature of six transmembrane domains, varying degrees of sequence similarity, and permeability to cations. The fourth transmembrane domain lacks the complete set of positively charged residues necessary for the voltage sensor in many voltage-gated ion channels. It is generally speculated that TRPC channels are activated by stimulation of Gq-PLC-coupled receptors and oxidation. Second messenger molecule cyclic guanosine monophosphate (cGMP) of nitric oxide was activated soluble guanylyl cyclase (sGC). cGMP then phosphorylates specific down-stream targets, such as the protein kinase G(cGK), cGMP-binding phosphodiesterases (PDEs) and ion channel. cGMP is degraded by the activity of PDE isoenzymes catalyzing the hydrolysis of cGMP to the inactive form 5'GMP. Here, we report the functional relationship between TRPC4 and cGMP. TRPC4 gene was overexpressed in HEK 293 cells that cGMP selectively activated TRPC4 channels and increased cytosolic calcium level by TRPC4 channel. We investigate to phosphorylation sites in TRPC4 channels. Thus, S688A phosphorylation site is important to associate PKG via cGMP. We have found that cGMP triggered TRPC4-like canonical current in prostate smooth muscle cell. cGMP and TRPC4 could suggest a new therapeutic agent for benign prostatic hyperplasia (BPH) syndromes.
Key Words: Ion channel, PDE inhibitor, cGMP

P04-02

Fluid flow facilitates inward rectifier K+ current by convective restoring of [K+] at cell membrane

surface

Jae Gon Kim[1,2] , Sang Woong Park[1], Hyang-Ae Lee[2], Doyoung Byun[3], Wan Soo Choi[4], Kyung Chul Shin[1], Ki-Suk Kim[2], kyung Kim[1], Sung Il Cho[1], Young Min Bae[1,*]

[1]Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, Chungbuk 380-701, South Korea [2]Next-Generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, 141 Gajeong-Ro, Yuseong-Gu, Daejeon, South Korea [3]Department of Mechanical Engineering, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-Gu, Suwon, Gyeonggi 440-746, South Korea. [4]Department of Immunology, School of Medicine, Konkuk University, Chungju 380-701, South Korea

Inward rectifier K+ (Kir) 2.1 channel functions as typical Kir channel and is expressed in many cells such as ventricular cardiomyocytes, vascular endothelial cells, and blood cells. It was reported that Kir2.1 current (IKir2.1) was facilitated by fluid flow. However, the mechanism is uncertain. We hypothesized that K+ concentration [K+] just near the outer mouth of Kir2.1 channel may decrease below the average [K+] of bulk extracellular solution during K+ influx and that fluid flow restores the decreased [K+], resulting in the apparent facilitation IKir2.1. We recorded the IKir2.1in RBL-2H3 cell using whole-cell patch-clamp technique. Fluid flow immediately increased the IKir2.1, which was not prevented either by pretreating inhibitors of various protein kinases (genistein, AG213, AG1478, H7, and staurosporine) or by modulating cytoskeleton and caveolae. We assumed an artificial cell of which membrane capacitance and K+ conductance are similar to those of RBL-2H3 cell. Simulation with Nernst-Plank mass equation indicated that [K+] near membrane surface decreased markedly below the average of bulk extracellular solution during K+ influx. Moreover, fluid flow restored the decreased-[K+] at the cell surface with flow rate-dependent manner. These results support the 'convection-regulation hypothesis' and define a novel interpretation of the fluid flow-induced modulations of ion channels including Kir2.1.
Key Words: Fluid flow, Shear force, RBL-2H3 cell, inward rectifier K+2.1 channel, Nernst-Plank mass equation, Convection

P04-03

Nicardipine inhibits hERG Channel

Eun Yong Chung[1], Hea-Young Cho[1], Ji-Hun Cha[2], Kyoung-Jin Kwon[2], Seol-Hee Jeon[2], Su Hyun Jo[3], Eun-Jung Kim[2], Hye Soo Kim[1], Hye Ju Chung[1]

[1]Korea Food and Drug Administration, [2]National Institute of Food and Drug Safety Evaluation, Seoul 122-704, [3]Department of Physiology and Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, School of Medicine, Kangwon National University, Chuncheon 200-701, Republic of Korea

Drug-induced long QT syndrome is known to be associated with the onset of torsades de pointes (TdP), resulting in a fatal ventricular arrhythmia. QT interval prolongation can result from blocking the human ether-a-go-go-related gene (hERG) channel, which is important for the repolarization of cardiac action potential. Nicardipine, a Ca-channel blocker and antihypertensive agent, has been reported to increase the risk of occasional serious ventricular arrhythmias. We studied the effects of nicardipine on hERG K+ channels expressed in HEK293 cells and Xenopus oocytes. The cardiac electrophysiological effect of nicardipine was also investigated in this study. Our results revealed that nicardipine dose-dependently decreased the tail current of the hERG channel expressed in HEK293 cells with an IC50 of 0.43 μM. On the other hand, nicardipine did not affect hERG channel trafficking. Taken together, nicardipine inhibits the hERG channel by the mechanism of short-term channel blocking. Two S6 domain mutations, Y652A and F656A, partially attenuated (Y652A) or abolished (F656A) the hERG current blockade, suggesting that nicardipine blocks the hERG channel at the pore of the channel.
Key Words: Nicardipine, HERG, LQTS, HEK293, Xenopus oocyte

P04-04

Phosphodiesterase 9A controls nitric-oxide-independent cGMP and hypertrophic heart disease

Dong I. Lee[1], Guangshuo Zhu[1], Takashi Sasaki[2], Su-Hyun Jo[3], Nazha Hamdani[4], Ronald Holewinsky[5], Thomas Danner[1], Manling Zhang[1], Peter P. Rainer[1], Djahida Bedja[1], Jonathan Kirk[1], Wolfgang R. Dostmann[6], Hideki Uosaki[1], Chulan Kwon[1], Kenneth B. Margulies[7], Jennifer Van Eyk[5], Walter Paulus[4], Eiki Takimoto[1], David A. Kass[1]

[1]Division of Cardiology, Department of Medicine, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205 (USA), [2]Advanced Medical Research Laboratories, Research Division, Mitsubishi Tanabe Pharma Corporation, Yokohama, Kanagawa 227-0033, Japan, [3]Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea, [4]Department of Physiology and Cardiology, Institute for Cardiovascular Research, VU University Medical Center, Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands ,[5]Heart Institute and Advanced Clinical Biosystems Research Institute, Cedar Sinai Medical Center, 8700 Beverly Blvd, AHSP A9229 Los Angeles, CA 90048 (USA), [6]Department of Pharmacology, University of Vermont, Burlington, VT 05405 (USA), [7]Department of Medicine, Division of Cardiovascular Medicine, Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 (USA)

Cyclic guanosine monophosphate (cGMP) is a second messenger molecule transducing nitric oxide (NO) and natriuretic peptide (NP) stimulated signaling. Enhancing cGMP synthesis or blocking its degradation by phosphodiesterase type 5A (PDE5A) protects against cardiovascular disease1,2. However, cGMP stimulation alone is limited by counter-adaptions including PDE upregulation3, and though PDE5A regulates NO-generated cGMP4,5, NO-signaling is often depressed by heart disease6, and PDEs controlling NP-coupled cGMP remain uncertain. Here we show that cGMP-selective PDE9A7,8 is expressed in mammalian heart including human, and is upregulated by hypertrophy and cardiac failure. PDE9A regulates NP rather than NO-stimulated cGMP in heart myocytes and muscle, and its genetic or selective pharmacological inhibition protects against pathological responses to neuro-hormones in vitro, and sustained pressure-overload stress in vivo. Pre-established heart disease is reversed by oral PDE9A inhibition independent of NO-synthase (NOS) activity, whereas PDE5A inhibition is effective only with active NOS. Transcription factor and phospho-proteome analysis of myocytes with each PDE selectively inhibited reveals substantial differential targeting, with phosphorylation changes from PDE5A inhibition being more sensitive to NOS activation. Thus, PDE9A regulates cGMP signaling independent of the NO-pathway, contributes to stress-induced cardiac disease, and may provide a useful therapeutic target for heart disease.
Key Words: PDE9A, cGMP, cardiac hypertrophy, TRPC, NO

P04-05

Shear stress induces longitudinal Ca^{2+} wave via autocrine activation of P2Y1 purinergic signaling in atrial myocytes

Joon-Chul Kim, Sun-Hee Woo

Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, 34134, South Korea

Atrial myocytes are exposed to shear stress during cardiac cycle and hemodynamic disturbance. It is known that shear stress elicits longitudinal global Ca^{2+} wave ("L wave") in atrial myocytes (1). Here, we investigated cellular mechanisms for shear-mediated Ca^{2+} response in atrial myocytes using two-dimensional confocal Ca^{2+} imaging. We applied shear stress to single myocytes using pressurized micro flow system. Atrial myocytes were enzymatically isolated from male Sprague-Dawley rats (230–300 g) and from wild-type (WT) and type 2 inositol 1,4,5,-trisphosphate receptor (IP3R) knockout (KO) mice (C57/B6, 24-28 g). Shear stress of ~16 dyn/cm^2 aperiodically induced L wave 1.2±0.26 times for 8 s-long exposure (n=39), with a delay of 0.2-3 s. Shear-induced L wave was restituted after 3-4-min resting period after the first occurrence. Pharmacological blockade of either ryanodine receptor (RyR by using 1 mM tetracaine) or IP3R (3 μM 2-APB) abolished the L wave occurrence under shear stress. In type 2 IP3R KO cells, shear stress failed to induce L wave. Consistent with these results, inhibition of phospholipase C (PLC) using U73122 (5 μM) removed shear-induced L wave, although its inactive analogue U73343 (5 μM) did not affect it. These observations indicate that PLC-IP3-IP3R signaling and Ca^{2+}-induced Ca^{2+} release via RyRs play a role in the generation of L wave under shear. Pre-treating atrial cells with the blockers for stretch-activated channel, TRPM4 or NADPH oxidase did not alter the occurrence of L wave under shear. Suramin (10 μM), the inhibitor of purinergic receptor, suppressed the L wave occurrence under shear stress. Antagonist of P2Y1 receptor MRS2179 (200 nM), but not P2X receptor antagonist (iso-PPADS 1~50 μM), eliminated the L wave generation under shear. Suppression of connexon that releases ATP using carbenoxolone (50 μM), or extracellular application of apyrase (2 U/mL) that metabolizes ATP inhibited the occurrence of L wave under shear. Our data suggest that longitudinal Ca^{2+}m wave is triggered by type 2 IP3R-mediated Ca^{2+} release that is activated by connexon-mediated ATP release and subsequent activation of P2Y1 receptor-PLC signaling in atrial myocytes under shear stress.

Key Words: Atrial myocyte, Shear stress, Ca^{2+} wave, ATP

P04-06

Shear stress enhances Ca^{2+} spark occurrence in rat ventricular myocytes via mitochondrial NOX-ROS signaling

Jun Wang, Joon-Chul Kim, Sun-Hee Woo

Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, 34134, South Korea

We have previously reported that shear stress enhances Ca^{2+}-induced Ca^{2+} release during depolarization in ventricular myocytes. To know molecular basis for the increase in global Ca^{2+} releases we assessed the effects of shear stress (~16 dyn/cm^2) on the frequency of Ca^{2+} sparks (unitary Ca^{2+} release events in cardiac myocytes) and examined cellular mechanism for the shear-mediated Ca^{2+} spark regulation using confocal Ca^{2+} imaging in rat ventricular myocytes. The frequency of Ca^{2+} sparks was immediately increased by shear stress to about 1.5-fold (within 1 s), and further increased to about 2-fold by more prolonged (20 s) shear exposure. Inhibition of nitric oxide synthetase (NOS) and interference of cytoskeletal integrity using L-NAME (1 mM) and colchicine (10 μM), respectively, did not affect the shear-mediated enhancement in spark frequency. When Na^+-Ca^{2+} exchange and L-type Ca^{2+} channel were suppressed by KB-R7943 (5 μM) or Ni^{2+} (5 mM), the effect of shear stress on the spark frequency was not altered, although these blockers significantly decreased the resting spark occurrences. Interestingly, when NADPH oxidase (NOX) was inhibited by its selective inhibitor diphenyleneiodonium (3 μM), the immediate and prolonged effects of shear stress on the spark occurrence were reduced by about 50-60%, which was washable. Pretreatment of reducing agent dithiothreitol (2 mM) also significantly reduced the shear-mediated spark enhancement (to about 50%). Mitochondiral uncoupling using carbonyl cyanide 3-chlorophenylhydrazone plus oligomycin (1 μg/ml) removed the shear-induced spark enhancement. Measurement of intracellular reactive oxygen species (ROS) using its specific dye 2',7'-dichlorofluorescein revealed increase in ROS level by shear stress. Sarcoplasmic reticulum (SR) Ca^{2+} concent was not altered immediately after the application of shear stress, but significantly increased after 20-s long shear exposure. Our data suggest that shear stress enhances the frequency of Ca^{2+} sparks by producing ROS via NOX, and that prolonged enhancement in spark frequency by shear stress may be partly mediated by increase in the SR Ca^{2+} loading. These mechanisms may partly explain the shear-mediated enhacement in Ca^{2+} transient in ventricular myocytes.

Key Words: shear stress, ventricular myocytes, Ca^{2+} spark, NOX, ROS

P04-07

Changes of K^+ channel currents in skeletal arterial smooth muscle by exercise training in sciatic nerve-injured rats

Ming Zhe Yin, Eun Young Seo, Sung Joon Kim

Department of Physiology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

K^+ channel currents determine the plasma membrane potential of vascular myocytes, influencing arterial tone. Voltage-gated K^+ channel current (I_{Kv})counterbalances the depolarization and voltage-operated Ca^{2+} channel (VOCC) activation. Moderate increase in extracellular [K^+] ([K^+]$_e$) induces relaxation of small arteries via augmenting inwardly rectifying K^+ channel current (I_{Kir}) and membrane hyperpolarization. The K^+-vasodilation is one of the mechanisms for the regional control of skeletal blood flow in response to exercise. In the rats underwent endurance exercise training (ET, rodent treadmill running) for two weeks, both I_{Kv} and I_{Kir} are increased by almost two fold in the skeletal arterial (SkASMC) and cerebral arterial smooth muscle cells (CASMCs) [Jin CZ et al 2011]. We also tested whether unilateral sciatic nerve injury and paralysis of the lower hindlimb affects I_{Kir} and I_{Kv} in rat SkASMCs. Atrophy of gastrocnemius and tibialis posterior were confirmed in the injured leg, and the amplitude of I_{Kir} was decreased in the SkASMCs from the injured legs. However, the amplitude of IKv was increased in the SkASMCs from the counter lateral legs. When 2-4 weeks of ET were combined with the sciatic nerve injury model, IKv was increased in both injured and intact legs. Interestingly, the decreased I_{Kir} in the injured leg was increased to the level even higher than normal condition. The recovery of I_{Kir} and I_{Kv} by ET might be one of the mechanisms for the beneficial effects of regular exercise on the rehabilitation of motor nerve injury.

Key Words: sciatic nerve injured, exercise training, I_{Kv}, I_{Kir}, skeletal arterial smooth muscle cell

P04-08

Ion channel gene expression predicts survival in glioma patients

Donghee Lee, Young-Won Kim, Jeongyoon Choi, Misuk Yang, Hyemi Bae, Inja Lim, Hyoweon Bang, Jae-Hong Ko

Department of Physiology, College of Medicine, Chung-Ang University, Seoul 156-756, South Korea

Ion channels are important regulators in cell proliferation, migration, and apoptosis. The malfunction and/or aberrant expression of ion channels may disrupt these important biological processes and influence cancer progression. In this study, we investigate the expression pattern of ion channel genes in glioma. We designate 18 ion channel genes that are differentially expressed in high-grade glioma as a prognostic molecular signature. This ion channel gene expression based signature predicts glioma outcome in three independent validation cohorts. This signature is independent of traditional clinical, molecular, and histological factors. Resampling tests indicate that the prognostic power of the signature outperforms random gene sets selected from human genome in all the validation cohorts. More importantly, this signature performs better than the random gene signatures selected from glioma-associated genes in two out of three validation datasets. This study confirms the central role of ion channels in brain cancer. Individualized profiling of ion channel gene expression serves as a superior and independent prognostic tool for glioma patients. This study also suggests that due to their central role in the cancer disease process, ion channels may serve as potential drug targets in cancer therapy.
Key Words: ion channel, glioma, gene expression, molecular signature, microarray

P04-09

Tonic inhibition of TREK-2 K2P channels by intrinsic PI(4,5)P_2 is the physiological mode of regulation

Joohan Woo[1], Dong Hoon Shin[3], Yin-Hua Zhang[1], Joo Hyun Nam[2], Sung Joon Kim[2]

[1]Department of physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea [2]Department of physiology & Ion Channel Disease Research Center, College of Medicine, Dongguk University, Kyungju, 780-714, Korea [3]Division of Natural Medical Sciences, Chosun University, College of Health Science, Gwang-Ju, 501-759, Korea

TWIK-related two-pore domain K+ channels (TREK) are activated by various physicochemical conditions including anionic phospholipids such as PI(4,5)P_2. However, there has been controversy over the direction of regulation by PI(4,5)P^2 that are dynamically generated by PI kinases using ATP. Here we investigated heterologously and intrinsically expressed TREK-2 in HEK293T, COS-7, WEHI-231 B cells, and primary astrocytes. Also, TREK-1 in HEK293T were compared. TREK-1 and TREK-2 current (I_{TREK-1} and I_{TREK-2}) commonly increased spontaneously by dialysis with ATP-free solution or by membrane excision for inside-out (i-o) patch clamp. The I_{TREK-1} and I_{TREK-2} were inhibited by ATP in all types of cells. The ATP-dependent inhibition was prevented by wortmannin, a PI kinase inhibitor. Consistently, I_{TREK-1} and I_{TREK-2} were totally inhibited by 10μM PIP2. The ATP-dependent inhibition of I_{TREK-2} was more prominent in HEK293T and WEHI-231 than COS7 and astrocytes. Confusingly, poly-L lysine (poly-L, polycationic agent) treatment also abolished I_{TREK-2}, which was reversed by treatment with polyanionic heparin. In HEK293T cells coexpressing TREK-2 and voltage-sensitive lipid phosphatase (Dr-VSP), I_{TREK-2} was increased by depolarisation initially, then suppressed with sustained depolarization. The inhibition of I_{TREK-2} by the unspecific charge screening (poly-L) or by the excessive stimulation of Dr-VSP suggested dual modes of regulation by PI(4,5)P_2. Physiologically, we suggest that the ATP-dependent intrinsic PI(4,5)P_2 induces tonic inhibition of TREK-2, the level of which appears different between the cell types.
Key Words: TREK-1, TREK-2, PIP2, ATP, VSP

P04-10

Inhibitory modulation of hERG K^+ channels by endogenous polyunsaturated fatty acid-derived electrophiles, 4-HNE and 4-ONE

Seong Woo Choi[1], Hyang-Ae Lee[1,2], Yin-Hua Zhang[1], Sung Joon Kim[1]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology

Oxidative stress occurs in the pathphysiological conditions such as inflammation, reperfusion after ischemia and aging. Lipid peroxidation products 4-Hydroxynonenal (4-HNE) and 4-Oxononenal (4-ONE) are peroxidation products of ω6-polyunsaturated fatty acid peroxidation, and have been studied as endogenous electrophiles that are potential harmful signaling molecules by formation of 4-HNE(4-ONE)-protein adducts. Previously we have reported the modulation of several ion channels including Kv, SOCE and hERG channels by exogenous electrophile molecules (e.g. curcumin). Here we investigate the effects of 4-HNE and 4-ONE on hERG current overexpressed in HEK-293 cells and the action potentials of guinea-pig ventricular myocytes (GPVMs). Acute application of 4-HNE (30~100 μM) and 4-ONE (1~10 μM) gradually but significantly decreased the tail current of hERG (IhERG,tail). The half-activation voltage (actV1/2) was shifted to the left by 4-HNE, while not by 4-ONE. The action potential duration (APD) of GPVMs was prolonged by 100 μM 4-HNE and 10 μM 4-ONE. Long-term incubation (> 1 h) with lower concentrations of 4-HNE (10 μM) induced time-dependent suppression of IhERG,tail. Western blot analysis revealed that membrane expression of hERG protein was reduced by the long-term incubation with 10 μM 4-HNE while not by 4-ONE. Consistently, the incubation with 10 μM 4-HNE significantly prolonged the APD of GPVMs as well as QT prolongation of ECG. Taken together, the suppression of hERG by higher dose of 4-HNE and 4-ONE may participate in cytotoxic and proarrhythmic effects. Sustained exposure to 4-HNE could impair hERG trafficking.
Key Words: hERG channel, lipid peroxidation product, 4-hydroxynonenal, 4-oxononenal

P04-11

TLR3-/4-Priming Differentially Promote Ca2+ Signaling and Cytokine Expression and Ca2+-Dependently

Kyoung Sun Park[1], Sun Hwa Kim[1], Kyoung Hwa Jung[1], Mi Kyung Kim[2], Yangmi Kim[3], Hyun Jin Kim[2], YoungGyu Chai[1]

[1]Department of Molecular and Life Sciences, Hanyang University, Ansan, Korea, [2]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea, [3]Department of Physiology, College

of Medicine, Chungbuk National University, Cheongju, 361-763, Korea

Toll-like receptor 3 (TLR3) and TLR4 in human mesenchymal stem cells (hMSCs) act as key players in tissue repair process by recognizing their ligands and stimulating their downstream activities including cytokine release. Mechanisms of TLR3- and TLR4-mediated cytokine releases from hMSCs are still uncertain. We now show that exposure to the TLR3 agonist polyinosinic-polycytidylic acid (poly (I:C)) or incubation with the TLR4 agonist lipopolysaccharide (LPS) increased mRNA expression of TLR3, TLR4 and cytokines in hMSCs. Poly (I:C) exposure rather than LPS incubation not only elevated inositol 1,4,5-triphosphate receptor (IP_3R) expression and IP_3R-mediated Ca2+ release, but also promoted Orai and STIM expression and store-operated Ca2+ entry in hMSCs. Both poly (I:C) and LPS exposure enhanced cytokine release from hMSCs. The enhanced cytokine release vanished upon chelation of intracellular Ca2+. The data demonstrate that TLR3- and TLR4-priming differentially enhance Ca2+ signaling and cytokine expression and Ca2+-dependently potentiate cytokine release in hMSCs.
Key Words: MSCs, TLR3, TLR4, LPS, Poly(I:C)

P04-12

Close Spatio-Association of Transient Receptor Potential Canonical (TRPC4) channel with Gαi in TRPC4 activation process

JongYun Myeong, Misun Kwak, Jae-Pyo Jeon, Chansik Hong, Ju-hong Jeon and Insuk So

Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

TPRC channels are Ca2+-permeable nonselective cation channels that are activated by a wide variety of stimuli, including G-protein-coupled receptors (GPCRs). TRPC4 is commonly assumed to be activated by Gq/phospholipase C-coupled receptors. However, the other molecular mechanisms by which Gα proteins regulate TRPC4 remain unclear. Here, we found that Gαi2 regulates TRPC4 activation by direct binding. To investigate this mechanism, we used whole patch clamp and FRET. We tagged an isoform of mTRPC4 and G protein with CFP and YFP, respectively, and transiently transfected cells with the FRET pair. The FRET efficiency between TRPC4β and the constitutively active mutant form of Gαi2 was nearly 15% and was greater than that observed with wild-type Gαi2 (nearly 5%). Gβγ and the TRPC4 channel showed a fluorescence resonance energy transfer (FRET) efficiency lower than 6%. In HEK293 cells transfected with the M2 muscarinic receptor, the application of carbachol (CCh) increased the FRET efficiency between TRPC4β and Gαi2 from 4.7 ± 0.4% (n = 7) to 12.6 ± 1.4% (n = 7). We also found that the TRPC4 channel directly interacts with Gαi2 but not with Gαq when the channel is open. We analyzed the calcium levels in HEK293 cells expressing the channels and Gαi2 or Gαq using the calcium indicator YC6.1 (yellow cameleon-6.1). In response to the muscarinic agonist carbachol, M2-, Gαi2- and TRPC4-expressing cells showed a prolonged Ca2+ influx, compared with cells expressing only M2. Together, these data suggest that Gαi2 activates the TRPC4 channel by direct binding, which then induces Ca2+ entry.
Key Words: TRPC4, G protein, FRET, Calicum

P04-13

ATP sensitive potassium currents on Human Periodontal ligament cells

Tran Thi Huyen Phuong, Soo Joung Park, Seong Kyu Han

Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

Adenosine-5′-triphosphate (ATP) has been mentioned in dental research on multiple levels, such as inflammation, mechanical strain and pain, making the system particularly relevant for the specific challenges in the oral cavity. There are studies showing human periodontal ligament cell respond to mechanical stress by increasing ATP release, which participates in bone resorption or bone homeostasis. So, in this study, we used the RT-PCR and patch-clamp techniques to investigate the presence of KATP channel subunits and influence of ATP on the KATP channel opening on PDL cells. We observed transcripts for Kir6.1, Kir6.2 and Sur2B in mRNA isolated from the PDL cells. In inside-out patches, the single channel conductance of 163 pS at symmetrical K+ concentration of 140 mM, as well as an outward rectification at voltages positive to +60 mV was recorded at the ATP- free bath solution. Single channel currents were almost inhibited when adding 5 mM ATP in the bath solution. However, the currents did not respond with 100 μM glibenclamide a subunit specific ATP channel blocker. Further, reversal potential was found to be 0 mV at symmetrical concentration (140 mM) of K+ in bath solution. These results support that human PDL cells express KATP channels. This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1D1A3A01018700)
Key Words: human periodontal ligament cell, Inside-out patch, KATP channels

P04-14

Physiological temperature increase the calcium sensitivity and current activation of TMEM16F (ANO6)

Haiyue Lin[1], Joo Hyun Nam[2], Sung Joon Kim[1]

[1]Department of Physiology, Seoul National University College of Medicine, 28 Yongon-dong, Chongro-gu, Seoul 110-799, Republic of Korea, [2]Department of Physiology, Dongguk University College of Medicine, 123 Dongdae-ro, Kyungju 780-714, Republic of Korea

TMEM16F, also known as ANO6, belongs to a family of putative Ca^{2+}-activated Cl· channel (CaCC). Different to other classic CaCC such as TMEM16A (ANO1), activation of ANO6 requires very high cytosolic Ca^{2+} concentration ($[Ca^{2+}]c>3\mu M$) and strong positive membrane potentials. Moreover, even with the high $[Ca^{2+}]c$, ANO6 current appears very slowly; >10 min to reach a steady-state. These properties were confirmed in all the three functioning variant (V1, 2, and 5) of ANO6. The low Ca^{2+}-sensitivity and slow kinetics of ANO6 poses some doubt about the physiological roles as an ion channel. In the present study, using whole-cell patch recordings, we identified that the three types of ANO6 isoforms (V1, 2 and 5) transiently transfected in HEK293T cells are activated by lower $[Ca^{2+}]c$ under physiological temperature (37°C). Among them, however, V2 and V5 showed significantly higher sensitivity; activated by 400nM $[Ca^{2+}]c$ whereas V1 by 1μM $[Ca^{2+}]c$. Furthermore, V2 and V5 showed significantly more shortened lag time of activation than V1; after making the whole-cell configuration, the currents reached maximal size after 543.3 ± 72.48 s, 56.67 ± 9.55

s and 62.5 ± 4.53 s for V1, V2 and V5, respectively. The temperature-dependent effect was reversible. In inside-out patch, all isoforms of ANO6 exhibit higher calcium sensitivity as well as channel activity at 37°C than RT. V3 still did not show any current activity in the inside-out configuration. All these results suggest that almost all isoforms ANO6 can be activated at low Ca^{2+} concentration (≤1μM) when conditioned at physiological temperature even though they showed different kinetics. This study may provide the possibility of ANO6 functional action as ion channel in variable tissues and cells depending on the variant forms of ANO6.

Key Words: ANO6, variant forms, temperature, TMEM16F

P04-15

Electrophysiological Characterization of Novel KCNQ4 Mutant Channels

Hyun Been Choi[1], Mina Park[2], Min-Young Kim[3], Ah-Reum Kim[3], Byung Yoon Choi[3], Tong Mook Kang[1]

[1]Department of Physiology, SBRI, Sungkyunkwan University School of Medicine, Suwon, Korea, [2]Department of Otorhinolaryngology-Head and Neck Surgery and Healthcare Research Institute, Seoul National University Hospital, Healthcare System Gangnam Center, [3]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, Korea

KCNQ4 encodes a voltage-gated K+ channel (Kv7.4), and is highly expressed in the hair cells of the cochlea and plays a pivotal role in maintaining cochlear K+ homoeostasis. It has been known that many mutations in the KCNQ4 gene induce autosomal dominant non-syndromic hearing loss (ADNSHL) prominent in the high frequencies and associate with deafness mapping the DFNA2 locus. Here, we provide two novel KCNQ4 mutants that found in Korean families, p.R331Q and c.811_816 deletion mutant. To evaluate electrophysiological consequences and to confirm the pathogenicity of the mutations, we expressed each mutant in HEK cells and compared them with wild type KCNQ4 channel (WT-KCNQ4). To explore whether the mutants play a role of dominant negative, each mutant (MT) was co-expressed with WT-KCNQ4 at the ratio of 3:1. With a conventional whole-cell patch-clamp technique, the current densities of KCNQ4-mediated K+ current were compared. For pharmacological separation of KCNQ4 current, linoperdine (10 μM) and retigabine (30 μM) were treated to inhibit and activate KCNQ4-mediated current, respectively. No appreciable amounts of linoperdine-sensitive KCNQ4 current was measured from the cells expressed with either p.R331Q or c.811_816 del mutant, confirming their pathogenic potential. Half activation voltages of the linoperdine-sensitive current from p.R331Q and c.811_816 del mutant (-17 mV) were significantly different from that of WT-KCNQ4 (-26 mV), but similar to that of GFP-transfected HEK cells (-15 mV). Both mutants abolished KCNQ4-mediated current when they were co-expressed with WT-KCNQ4 (WT:MT=3:1), suggesting their dominant negative roles. Interestingly, KCNQ4 activator retigabine can activate KCNQ4-mediated K+ current in p.R331Q-expressed cells, but not in cells with c.811_816 del mutant. Retigabine-activated p.R331Q current showed the same voltage dependence with WT-KCNQ4 current. Taken together, both p.R331Q and c.811_816 del mutant are estimated as loss-of-function mutations with a dominant negative effect. However, R331Q still preserves response to KCNQ4 activator (retigabine), and therefore being activated on demand.

Key Words: KCNQ4, mutation, ADNSHL, DFNA2, hearing loss

P04-16

Influence of Bisphenol-A on ion channel activities on Gonadotropin Releasing Hormone Neurons

Janardhan P. Bhattarai, Seong Kyu Han

Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

Bisphenol-A (BPA), a monomer of polycarbonate plastics, and epoxy resins is an environment pollutant which has been considered as endocrine disrupter. So, in this study, we investigated the effects of the BPA on gonadotropin-releasing hormone (GnRH) neurons using single cell electrophysiology on GnRH-green fluorescent protein (GnRH-GFP) transgenic mice. Conventional whole cell patch-clamp recordings were performed. In this study, we showed that 100% of GnRH neurons responded to 300 μM of BPA with a markedly prolonged inward current. The effect of BPA not only persisted in the presence of tetrodotoxin, but also in the presence of amino acid receptor antagonists, indicating the direct site of action is on postsynaptic GnRH neurons. Further, BPA-induced inward currents were concentration dependent. In addition, these inward currents were not blocked by gabazine, a synaptic GABAA receptor antagonist but were completely blocked by bicuculline, a broad (synaptic and extrasynaptic) GABAA antagonist. Apart from direct action of BPA as a GABAA receptor agonist, it significantly blocked the voltage gated potassium, calcium and sodium ion channels on GnRH neurons suggesting the direct influence on ion channel physiology of GnRH neurons. These results demonstrate that BPA can act directly on GnRH neurons to induce excitation via extra synaptic GABAA receptors and may influence GnRH neuron signaling via inhibition of voltage gated potassium, calcium and sodium ion channels on GnRH neurons. This research was supported by Basic Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2058356) and (2014R1A1A2054241)

Key Words: Endocrine disrupting chemicals, Bisphenol-A, GnRH neurons, patch-clamp, GABAergic

P04-17

The Conserved Gating Elements in CIRB domain of TRPC4 Channel

Chansik Hong[1], Jongyun Myeong[1], Joo Hyun Nam[2], Young-Cheul Shin[1], Misun Kwak[1], Insuk So[1]

[1]Department of Physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea, [2]Department of Physiology, College of Medicine, Dongguk University, Gyeongju, 780-714, Korea

The activity of transient receptor potential (TRP) channel complexes is regulated via interactions with various binding partners. The structures of TRPV1 (vanilloid subfamily) and TRPA1 (ankyrin repeats subfamily) have been recently defined using electron cryo-microscopy techniques underlying functional architecture in the activity of TRP proteins. However the activation mechanism of classical TRP (TRPC) channel in gating property still remains controversial. Based on a low level of homology in cytoplasmic region following the coiled-coil domain among TRP proteins, we focused on the highly conserved TRP motif and CIRB (CaM- and IP3R-binding) domain near pore helix. To investigate structural activity of TRPC4 channel, we mutated positively charged amino acids physically adjacent to CIRB domain. The basal activity of the alanine mutants was analyzed by perfusion of external Cs+ solution with high permeability. We found that replacing a conserved K715

or R716 within CIRB domain was activated by 2- to 5-fold increases in open probability and steady-state. Double mutant of K715/R716 (KR/AA) showed increased activity induced by the infusion of GTPγS or the stimulation of muscarinic (Gαq, or Gαi/o-coupled) receptor at most fivefold compared to wild-type (WT) of TRPC4β. In the KR/AA mutant using intracellular Ca2+-buffered solution with EGTA, nominally free [Ca2+]i of 0 or 200 nM induced inward currents of 135 ± 29 or 441 ± 75 pA/pF, respectively (WT; 0.9 ± 0.3 or 20 ± 15 pA/pF). Although the double mutant of TRPC4β channel was also increased in inside-out patches than wild-type channel, the mutant exhibited single-channel conductance and surface expression similar to wild-type channel. While the basal activity of histidine mutant (KR/HH) at a neutral pH of internal solution was increased to Cs+ current of 92 ± 29 pA/pF similar to that of KR/AA, internal solution at pH 5.4 significantly decreased Cs+ current due to the protonated (i.e. positively charged) histidine at an acidic pH. The negatively charged (KR/DD) mutant completely lost activity induced by constitutively active mutant of inhibitory Gα proteins (Gαi/o) and significantly decreased the interaction with Gαi2 by FRET. The function of KR region of TRPC4α channel is similar to that of TRPC4β channel. Collectively, the neutralization of KR charged amino acids activates TRPC4 channel by competing with binding proteins in CIRB domain of TRPC4. The structural and mechanistic roles of the CIRB domain are responsible for TRPC4 activation acting as an inner helix gate and G loop gate.

Key Words: TRPC, Gi, Charge, Gating, Neutralization

P04-18

KCNQ2/3 channel inhibition by ethanol is regulated by plasma membrane PI(4,5)P2 level

Kwon-Woo Kim, Dongil Keum, Hae-Jin Kweon, Byung-Chang Suh

Department of Brain and cognitive science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, South Korea

KCNQ2/3 channel is known that M-type channel which is one of the voltage-gated potassium channel. It was known that KCNQ2/3 channel is regulated by plasma PI(4,5)P2 level. The membrane PI(4,5)P2 play as cofactor for activating KCNQ2/3 channel. KCNQ2/3 channel is generally expressed in central and peripheral neurons. Also, KCNQ2/3 channels are expressed in Ventral tegmental area (VTA) dopamine neuron, which is associated with brain reward system. It was known that excitability of VTA neuron could reinforce the brain reward system, which is critically induced by ethanol. Although it was known that M-channel in VTA neuron is inhibited by ethanol, molecular mechanism of M-channel inhibition has not been studied. In this study, we investigated that alcohol affects KCNQ2/3 channels and alcohol inhibition mechanisms is related with PI(4,5)P2 level. We tested the inhibition in living tsA201 cells using pharmacology, electrophysiology, FRET, confocal microscopy methods. We found that alcohol inhibition was occur transiently and the inhibition was related by carbon chain length and conformation of alcohols. Also, the inhibition was related with plasma membrane PI(4,5)P2 level, the inhibition has the KCNQ channel subunit specificity. Therefore, even though alcohols have low specificity to KCNQ2/3 channel, these results indicate that alcohols could play an important physiological role as an inhibitor. Also, understanding this mechanism will give some evidences to develop alcohol-selective therapy for overcoming or preventing alcohol addiction.

Key Words: KCNQ2/3 channel, PI(4,5)P2, electrophysiology, alcohol

P04-19

Verapamil inhibits TRESK current in trigeminal ganglion neurons independently of the blockade of Ca2+ influx

Dawon Kang, Eun-Jin Kim, Ji Hyeon Ryu, Jaehee Han

Department of Physiology, College of Medicine and Institute of Health Sciences, Gyeongsang National University, Jinju 660-751, South Korea

The TWIK-related spinal cord K+ (TRESK; K2P18.1) channel is the only member of the two-pore domain K+ (K2P) channel family that is activated by an increase in intracellular Ca2+ concentration ([Ca2+]i) and linked to migraine. However, little is known about the effect of Ca2+ channel blockers on the TRESK channel. Here, we identified the effect of verapamil, an L-type Ca2+ channel blocker and a prophylaxis for migraine, on the TRESK channel in trigeminal ganglion (TG) neurons. Verapamil, nifedipine and NiCl2 inhibited the whole-cell currents in HEK293 cells overexpressing mTRESK with IC50 values of 5.0, 54.3, and >100 μM, respectively. The inhibitory effect of verapamil on TRESK channel was reduced in a TRESK double mutant (F156A/F364A). Verapamil (10 μM) inhibited TRESK single-channel and whole-cell currents in TG neurons. In the absence of extracellular CaCl2, TRESK channel activity was lower than that in the presence of extracellular CaCl2, but the difference was insignificant. The inhibitory effect of verapamil on the TRESK channel remained despite the absence of extracellular CaCl2. These findings show that verapamil directly inhibits the TRESK current independently of the blockade of Ca2+ influx. These results suggest that verapamil could affect the excitability of TG neurons through blockage of TRESK channel, and thus we should carefully consider using verapamil for prophylaxis and treatment for migraine.

Key Words: background potassium channel, calcium, trigeminal ganglions, verapamil

P04-20

Stable interaction of Ca^{2+} channel β subunit with high voltage-activated Ca^{2+} channels α1 subunit revealed by translocatable CaV β subunit systems

Jun-Hee Yeon, Byung-Chang Suh

Department of Brain and Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology, Daegu, 711-873, South Korea

The ancillary β subunit of voltage-gated Ca^{2+} (Ca_V) channel is known to play an important role in regulating cell surface expression and biophysical gating properties of high-voltage activated (HVA) Ca^{2+} channels via a type-specific interaction with Ca_V α1 subunit. However, the molecular mechanism of Ca_V channel regulation by β subunit is not clearly determined. Several recent studies showed that the interaction between Ca_V α1 and Ca_V β subunit is not dynamic but stable. Here we developed the rapamycin-induced translocatable systems with Ca_V β subunit systems to reveal the molecular interaction properties between Ca_V α1 and Ca_V β subunit. We found that cytosol-localized Ca_V β subunits were well translocated to the intracellular target membranes by rapamycin application, whereas plasma membrane-bound Ca_V β subunits were not moved. When the Ca_V β subunit was expressed together with Ca_V α1, it was not translocated to the target membranes by rapamycin, probably due to the irreversible Ca_V α1-Ca_V β interaction. Confocal imaging experiments showed that in the presence of Ca_V α1 subunits, translocation of Ca_V β subunits to endoplasmic reticulum (ER) triggered artificial ER-PM junctions and then formed puncta

in the plasma membrane. When the cytosolic Ca_V β subunits were translocated to plasma membrane, they did not enhance the current density, suggesting that β subunits don't interact with Ca_V α1. In order to further understand the functional role of Ca_V α1-Ca_V β interaction in the channel gating activity, we constructed several Ca_V β mutants which have low affinity to Ca_V α1 subunits. Electrophysiological experiments showed that dissociation of Ca_V β mutants from Ca_V α1 subunits decreased whole-cell currents, slowed inactivation, and shifted voltage dependent I-V curve to the right. Taken together, our data demonstrate that the Ca_V α1-Ca_V β interaction is very stable and not reversible.
Key Words: Voltage-gated calcium channel, Ca_V β subunits, Chemically-inducible dimerization (CID) system, protein-protein interaction, ER-PM junction

P04-21

Functional role of GABA as a gliotransmitter in epileptic hippocampus

Sudip Pandit, Hyang-Joo Lee, Hyun-Sill Cho, Yoon-Hyung Pai, Jin Bong Park

Department of Physiology, Brain Research Institute, School of Medicine, Chungnam National University, 6 Munhwa-Ro, Jung-gu, Daejeon, 301-131, Republic of Korea

Nowadays, it is claimed that GABA present in astrocyte is the major source of tonic inhibition in cerebellum and other brain areas. Although, GABAA tonic current in hippocampal pyramidal neurons are targeted for anti-epileptic drug (AED's) in seizures or epilepsy, source of GABA mediating them in epileptic condition remains unclear. Loss of GABAergic interneurons and less presynaptic GABA release in pyramidal neurons are the ones that lead to neuronal hyper excitability during seizure or epilepsy, saying that the GABAA tonic current is maintained in these conditions. Using whole cell patch clamp recording, here we show that bestrophin1 (Best1) mediated GABA release and its component of the GABAA tonic current in mouse model of seizure using 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), which blocks recombinant Best1 channels. Intracerebroventricular (i.c.v) injection of Kainic acid (KA) produced typical seizure activity in mice. Time schedule of 1, 3, 7 and 15 days after kainate injection was made in order to see its long lasting effect on GABAA tonic current mediated by best1 released GABA. ICR and BALB/c mice exhibited the NPPB sensitive current after the kainate injection which was gradually decreased with the time, in contrast we did not see any portion of NPPB sensitive current in best1 knock out (KO) mice with the kainate injection. N0-711, a GABA transporter (GAT-1) blocker induced a larger inward current in 3 day kainate injected mice compared to wild type (WT) control but 3 day kainate injected KO mice had similar response to WT. Further, through the immunohistochemical staining we see the increased GABA and best1 in GFAP positive cells from kainate injected mice. To find out the functional significance of best1 expression in pyramidal layer astrocytes from kainate injected mice, best1 KO and WT mice were injected with kainate then compared the seizure response and latent period in the groups. We did not see the latent period difference between the groups, yet seizure activity was lasted long in KO mice on the injected day and remained hyper activate until 11th day after the kainate injection compared to WT. Maximal electroshock seizure (MES) test was conducted where KO mice displayed higher seizure sensitivity in compared to WT control after 3 day kainate injection. In conclusion, enhanced GABA and best1 in pyramidal layer astrocytes in kainate model mediate and contribute to maintain GABAA tonic current in compensation of decreased GABA level in epilepsy. Also it may also play a role in suppression of the seizure.
Key Words: tonic GABAA current, bestrophin1, hippocampus

P04-22

Gαi-mediated TRPC4 activation by Polycystin-1 contributes to cystic disease via STAT1 activation

Misun Kwak, Chansik Hong, Kotdaji Ha, Ju-Hong Jeon, Insuk So

Department of Physiology, Seoul National University College of Medicine

Polycystin-1 (PC1) regulates a number of cellular processes (ex. heterotrimeric G protein, transcription factor etc.) through the formation of complexes with the polycystin-2 (PC2) ion channel or with other signal transduction proteins. Although Ca2+ modulation by polycystins has been reported between transient receptor potential (TRP) channels, the function with TRPC subfamily regulated by G-protein signaling has remained elusive. We have previously reported that TRPC4/C5 channel can be activated by Gαi through direct interaction1. Here, we identified that PC1 dominantly interacts with Gαi3 using co-immunoprecipitation. Thus we recorded the activity of TRPC4/C5 heterologously co-expressed with PC1 in HEK293 cells. PC1 activated TRPC4β channel ($4 \pm 1 \rightarrow 41 \pm 14$ pA/pF) by modulating G-protein signaling without change in TRPC4 translocation. Intracellular 0.2 mM GTPγS-induced TRPC4 activation was not significantly different in the presence or absence of PC1. C-terminal fragment (CTF) of PC1 did not affect TRPC4/C5 activity due to loss of N-terminus containing G-protein coupled receptor proteolytic site (GPS). Dominant negative Gαi3 (G202T) mutant inhibited PC1-activated TRPC4 current. TRPC5 also was activated by full-length PC1 ($54 \pm 8 \rightarrow 114 \pm 16$ pA/pF). We, next, investigated whether TRPC4 induces activation of STAT (signal transduction and transcription) proteins, leading to cell proliferation or death. We observed that STAT1 and STAT3, but not STAT6 activation by PC1 is independent on Src kinase cascades. Interestingly, TRPC4 promoted STAT1 activation. When PC1 co-expressed with TRPC4, STAT1 activation was further increased compared to each sole expression, causing cystic cell death. Our findings indicated an important function between PC1 and TRPC4/C5 in modulation of intracellular Ca2+ signaling and provided a new potential therapeutic approach targeting TRPC4/C5 channel in polycystic kidney disease.
Key Words: polycystin, TRPC, STAT

P04-23

Single channel recordings of the positive pressure-specific mechanosensitive piezo2 ion channels in human MCC-13 Merkel cell line

Kyung Chul Shin, Sang Woong Park, Hyunji Park, Young Min Bae

Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Chungju, Chungbuk, 380-701, South Korea

Recently, it was shown that Merkel cells and piezo2 ion channels that are expressed in Merkel cells and their A afferent fibers play critical roles in the gentle or light-touch somatosensation. However, there is no study reporting the single channel recordings of piezo2 ion currents, hampering the clear understanding of piezo2 characteristics. Here, we investigated whether the Merkel cell carcinoma-13 (MCC-13) cell line express the functional piezo2 ion channels. Further, we also tried to record the single channel current of the piezo2 ion channels in the MCC-13 cells. Under the cell-attached configuration of the patch-clamp technique, application of weak positive pressure (≥ 5mmHg) markedly activated a non-selective cation current (reversal potential of 00 mV, single channel conductance of ~00 pS), whereas application of negative pressure (up to 25 mmHg) failed to evoke the currents in MCC-13 cells.

In contrast, either positive or negative pressure similarly activated non-selective cation currents in neuro2A cells, which reportedly express mechanosensitive piezo1 ion channel. Immunocytochemistry and Western blotting data showed that the MCC-13 cells express piezo2, whereas neruo2A cells do not. Knock-down of piezo2 by siRNA inhibited both the protein level of piezo2 and the positive pressure-activated ion currents in MCC-13 cells. When expressed in HEK293T cells, the piezo2 ion channel displayed similar positive pressure-specific mechanosensitivity. These results indicate that MCC-13 Merkel cells express mechanosensitive piezo2 ion channels and that the piezo2 channel is low-threshold, positive pressure-sensing ion channel without negative pressure sensitivity. We suggest that piezo2 ion channel is a mechanotransducer optimized for light-touch somatosensation
Key Words: mechanosensitive ion channel, piezo2, merkel cell

P04-24

Proton modulates common gate of ClC-1 chloride channel via helix O

Ju Yong Seong, Kotdaji Ha, Insuk So

Department of Physiology, College of medicine, Seoul National Univeristy

The ClC proteins play many important physiological roles by transporting anion across plasma membrane. The ClC family consists of two subfamilies, channels and antiporters, that share similarity in their structures and mechanism; a homodimer with two gating residues. Proton, similar to its role in antiporter, provides energy in ClC channels and affects common gate that regulates the closing of both pore simultaneously. Conserved glutamate (Gluin) at the intracellular part of the protein is known to provide proton pathway in ClC antiporters. Unlike the antiporter, Gluin is replaced by valine in ClC-1 channels, and the mechanism underlying how proton modulates the common gate is largely unknown. Previously, myotonic mutations in human ClC-1, G523D and G499R in helix O and N were respectively reported to exhibit reversed voltage dependency in the common gate, similar to that of wild type under a low extracellular pH condition. In our study, molecular modelling data suggest that those two mutants are related to fixation of helix O at the extracellular part, and we tentatively concluded that these mutants resemble the conformational change caused by low extracellular pH. We also identify that serine (S537) residue at the C-terminal of helix O locates near the central pore of the channel and hypothesized that it regulates the proton transport in ClC-1 channels. Mutating the serine residue to negatively charged amino acids largely diminished the effect of proton at low extracellular pH condition, displaying no significant difference compared to wild type. Double mutant G523D and S537E also showed retained voltage dependency in the common gate. Replacing the valine residue (V292) corresponding to Gluin in the ClC antiporters to glutamate reduced the common gate. Collectively, these data reveal that proton transport across the ClC-1 channel is largely linked to the common gate via S537 residue, and suggest that a role swapping between S537 and V292 is a key evolutionary step in ClC proteins.
Key Words: ClC-1, pH, Common gate, Chloride, Proton

P04-25

The role of PIP_2 signaling in NALCN regulation

Jungeun Hong[1,3], Tae Jung Ahn[2], KyeongJin Kang[2,3], Hana Cho[1,3]

[1]Department of Physiology, [2]Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, [3]Samsung Biomedical Research Institute, Suwon, 440-746, Republic of Korea

NALCN (Sodium leak channel, non-selective) is predominantly expressed in neurons where it regulates the resting membrane potential and neuronal excitability. Both in mammals and invertebrates, animal models revealed an involvement of NALCN in many biological processes such as locomotor behaviors, sensitivity to volatile anesthetics, and respiratory rhythms. Although NALCN was reported to be activated either by M3 muscarinic receptor or by lowering $[Ca^{2+}]_e$, the underlying mechanisms of this NALCN activities remain poorly characterized. In this study, I examined the regulation of NALCN by phosphatidyl-inositol 4,5-bisphosphate (PIP_2). As previously reported, I first confirmed that NALCN was activated by M3 muscarinic receptor and also by lowering $[Ca^{2+}]_e$, using the conventional whole-cell patch clamp technique. To examine whether phosphoinositide is implicated in the regulation of the NALCN, I tested the effects of the Phospholipase C (PLC) inhibitor, U73122 on NALCN activity, which blocked muscarinic receptor-induced activation of NALCN. This result was also confirmed by the confocal imaging technigue, Acetylcholine (ACh) induced PIP_2 hydrolysis. Thus, one possibility is that PIP_2 depletion might lead to augmentation of NALCN activity. In line with this hypothesis, supplementing PIP_2 to cells blocked the activation of NALCN by M3 muscarinic receptor. Consistently, the depletion of PIP_2 by intracellular application of a PIP_2 antibody strongly increased the NALCN activity whereas PIP_2 reduced the NALCN activity in a dose dependent manner. In addition, the increased channel activity by the PIP_2 antibody was inhibited by 10 μM Gd^{3+}, a NALCN inhibitor. Taken together, these data suggest that membrane PIP_2 negatively regulates NALCN at rest. This inhibition was released by PIP_2 depletion resulting from the PIP_2 antibody or a muscarinic agonist, leading to NALCN activation.
Key Words: NALCN, Phosphoinositide, PLC, PIP_2, muscarinic receptor

P04-26

The Role of Kv Channels in Osteoblast Differentiation

Ji Eun Yang, Min Seok Song, Pan Dong Ryu, So Yeong Lee

Department of Veterinary Pharmacology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-742, Korea

In the present study, we focused on the role of Kv channels in osteogenic differentiation using human mesenchymal stem cells (hMSCs) and osteoblast-like MG-63 and SaoS-2 cells. hMSCs, MG-63 cells and SaOS-2 cells were used for the experiments. RT-PCR, real-time RT-PCR, western blot assay were performed. To induce osteoblastic differentiation, ascorbic acid-2-phosphate, beta-glycerophosphate, and dexamethasone were added to the maintaining medium. Osteoblastic differentiation was confirmed by Alizarin Red S staining and quantification of calcium deposits. We confirmed that overall blockade of Kv channels by tetraethylammonium (TEA) augmented the mineralization of bone marrow-derived human mesenchymal stem cells (hMSCs) during osteogenic differentiation. We identified that Kv7.3 was expressed in MG-63 and Saos-2 cells at the mRNA and protein levels. Inhibition of Kv7.3 by linopirdine or XE991 increased the matrix mineralization during osteoblast differentiation. This was confirmed by alkaline phosphatase, osteocalcin, and osterix in MG-63 cells, whereas the expression of Runx2 showed no significant change. Furthermore, the extracellular glutamate secreted by osteoblasts was measured to investigate its effect on MG-63 osteoblast differentiation. Blockade of

Kv7.3 promoted the release of glutamate via the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2-mediated up-regulation of synapsin. On the other hand, activation of Kv7.3 by flupirtine did not produced notable changes in matrix mineralization during osteoblast differentiation. In conclusion, Kv7.3 may be one of the important regulators in osteoblastic differentiation.
Key Words: Kv channels, Osteoblast Differentiation, Matrix mineralization

P04-27

Enhancements of contraction and L-type Ca^{2+} current by murrayafoline-A via protein kinase C in rat ventricular myocytes

Bojjibabu Chidipi, Min-Jeong Son, Nguyen Manh Cuong[1], Sun-Hee Woo

College of Pharmacy, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, South Korea, [1]Department of Bioactive Products, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Rd., Hanoi, Vietnam

It has been recently reported that murrayafoline-A (1-methoxy-3-methylcarbazole, Mu-A), which is isolated from the dried roots of *Glycosmis stenocarpa* increases Ca^{2+} transients by directly activating protein kinase C (PKC), and that its positive inotropic effect is dependent on enhancement of Ca^{2+} influx through the L-type Ca^{2+} channels ($I_{Ca,L}$) in rat ventricular myocytes. In the present study, we further examined whether the enhancements of contractility and $I_{Ca,L}$ by Mu-A are mediated by its effect on PKC or not. Cell shortenings and $I_{Ca,L}$ were measured using the video edge detection method and perforated patch-clamp technique, respectively. The positive inotropic effect of Mu-A (25 μM) reached a maximum after about 2-min exposures, and then decayed after a about 1-min steady-state. Mu-A transiently enhanced the $I_{Ca,L}$ with a similar time course. The positive inotropic effect of Mu-A was not inhibited by pre-treatment of β-adrenergic receptor inhibitor propranolol, protein kinase A inhibitors KT5720 or H89, Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII) blocker KN93, or phospholipase C (PLC) inhibitor U73122. Interestingly, the Mu-A-mediated positive inotropy was eliminated by preincubation of PKC inhibitors GF109203X or calphostin C. Consistently, the PKC blocker prevented the Mu-A-induced $I_{Ca,L}$ enhancement, although the inhibition of β-adrenergic receptor, CaMKII, PKA, or PLC failed to suppress the stimulatory effect of Mu-A on $I_{Ca,L}$. These results indicate that Mu-A-mediated positive inotropy may be mediated by increase in $I_{Ca,L}$ via PKC in rat ventricular myocytes.
Key Words: Murrayafoline-A, positive inotropy, PKC, L-type Ca^{2+} current, ventricular myocytes

P04-28

Enhancing skin barrier homeostasis via modulation of calcium ion channels by topical botanical products

Mi-Ok Lee[1], Joo Hyun Nam[1,2], Woo Kyung Kim[2,3]

[1]Department of physiology, College of Medicine, Dongguk University, Kyungju, 780-714, Korea, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, 27 Dongguk-ro, Goyang 410-773, Korea, [3]Department of Internal Medicine Graduate School of Medicine, Dongguk University, 27 Dongguk-ro, Goyang 410-773, Republic of Korea

Intracellular Ca^{2+} signaling via various calcium channels, such as Orai1, Transient receptor potential (TRP)A1 and TRPV3, has been shown to directly modulate epidermal proliferation, differentiation, barrier homeostasis, and inflammation. Ca^{2+} influx through these channels eventually generates intracellular Ca^{2+} signaling that results in different outcomes dependent on the individual Ca^{2+} channel type. For example, keratinocyte proliferation and migration through Orai1, epidermal barrier formation and keratinocyte differentiation through TRPA1, and keratinocyte cornification through TRPV3. Therefore, a specific agonist/antagonist for each calcium channel is required for maintaining skin barrier homeostasis and for the treatment of dermatological diseases. To identify botanically derived chemicals for topical use in functional cosmetics or agents for dermatological diseases. Novel modulators of Orai1, TRPA1 and TRPV3 were identified by screening the extracts (plus their fractions) of 30 medicinal herbs and their constituents. The potencies of the activating or inhibiting compounds on the channels were determined by an automated patch clamp system. Biophysical properties of the channel modulation by the hit products were reanalyzed using conventional whole-cell patch clamp. We prepared 30 medicinal herb extracts, and performed bioassay-guided fractionation of the active extracts to isolate and identify the bioactive constituents. With combination of automated patch and conventional whole-cell patch clamp studies, we found eight medicinal herb fractions for Orai1, two for TRPA1, and three for TRPV3 that showed >50% inhibition rates at 30 μg/mL. We also found three fractions with TRPA1 agonist activity. Chemical constituents of the agonists or antagonists will be discussed.
Key Words: skin barrier, TRPV3, TRPA1

P04-29

Epidermal growth factors activate TRPC4 and TRPC5, reducing desensitization of TRPC5 channel

Seungjoo Jeong, Jinhong Wie, Ju-Hong Jeon, Insuk So

Department of Physiology and Institute of Dermatological Science, Seoul National University College of Medicine, Seoul, Republic of Korea

Receptor-operated Ca^{2+} (ROC) channels are activated via ligand-mediated activation of receptor tyrosine kinase (RTK) and G protein-coupled receptors (GPCR). The evidence for activation of classical transient receptor potential (TRPC) channels in ROC entry is linked to G_q-PLC, G_i and EGF (epidermal growth factor). A recent study suggested the involvement of TRPC4 in the activation of a store-operated current in corneal epithelial cells by EGF. However, the primary activation mechanism of TRPC4 remains controversial. Although we previously reported that TRPC4 and TRPC5 channels are activated by $G_{\alpha i}$ proteins, we further investigated the effect of EGF on TRPC4 and TRPC5 activity in our hands. We recorded the currents by whole-cell patch clamp on HEK293 cells transiently transfected with TRPC4 or TRPC5. The external application of EGF activated TRPC4β channel, but not TRPC4α. Under the serum deprived conditions, EGF significantly enhanced TRPC4β activation (43.4 ± 13.9 pA/pF). Similarly, TRPC5 channel was activated by EGF. EGF induced an increase in La^{3+}-sensitive TRPC5 current. Interestingly, the treatment of EGF blocked desensitization of the activated current. Taken together, these results confirmed that EGF activates TRPC4β and TRPC5 channels. It needs to be further studied to unveil the mechanism how EGF activates TRPC4β and attenuates the desensitization of TRPC5 channel, respectively.
Key Words: TRPC4, EGF, leptin

P04-30

Inhibition of the extrinsic aging-related ion channels TRPV1 and ORAI1 by constituents of the fruits of Foeniculum vulgare

Joo Hyun Nam[1], Dong-Ung Lee[2]

[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 780-714; and Channelopathy Research Center (CRC), Dongguk University College of Medicine, 27 Dongguk-ro, Ilsan Dong-gu, Goyang 410-773, Republic of Korea, [2]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea

Ultraviolet (UV) exposure due to the solar radiation is the most important cause of extrinsic skin aging (photoaging), which is characterized clinically by deep skin wrinkling and pigmentation. These phenomena are due to the increasing of metalloproteinases-1 (MMP-1) expression in keratinocytes and tyrosinase activation in melanocytes. In a recent study, it was reported that two Ca^{2+} channels, a transient receptor potential vanilloid type-1 (TRPV1) and a calcium release-activated calcium channel protein 1 (ORAI1), are involved in UV-induced MMP-1 expression and tyrosinase activity, respectively. In the present study, we evaluated whether the fruits of Foeniculum vulgare have inhibitory effects on TRPV1 and ORAI1 using the whole-cell patch-clamp technique and intracellular Ca^{2+} measurements. In our electrophysiological study of the extract and its fractions, the methylene chloride and hexane fractions were found to strongly block capsaicin-induced TRPV1 and ORAI1 currents in HEK293T cells overexpressing TRPV1 or a combination of ORAI1 and STIM1. Furthermore, of the 18 compounds isolated from above fractions, trans-anethole in the hexane fraction had inhibitory effects on both ORAI1 and increases in cytoplasmic Ca^{2+} concentrations in response to ORAI1 activation (both by ~70% at 100 μM). Our findings suggest that the fruit extract of F. vulgare provides a possible novel approach for treating and preventing UV-induced skin aging.
Key Words: Foeniculum vulgare, TRPV1, ORAI1, photoaging, trans-anethole

P04-31

The rhizomes of Cyperus rotundus and its active component valencene inhibit skin photoaging related ion channels, TRPV1 and Orai1

Joo Hyun Nam[1], Dong-Ung Lee[2]

[1]Department of Physiology, Dongguk University College of Medicine, 123 Dongdae-ro, Kyungju 780-714, Republic of Korea, [2]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea

Ultraviolet (UV) irradiation deeply penetrates into skin and causes skin inflammation, immune response changes and pigmentation that promote skin photo aging. Recently, accumulated evidence suggests that several calcium ion channels such as transient receptor potential vanilloid 1 (TRPV1) and calcium release-activated calcium channel protein 1 (ORAI1) mediate diverse skin process including melanogenesis, skin wrinkling and inflammation. The rhizome of Cyperus rotundus (C. rotundus) is a traditional medicinal herb that has been reported to treat inflammatory diseases such as dermatitis and arthritis. However, its effects on UV-induced photo aging related ion channels are still not known. The aim of this study was to evaluate whether C. rotundus extract and its constituents confer antagonistic activity on TRPV1 and Orai-1. Electrophysiological analysis revealed that the hexane fraction (100 μg/mL) inhibited capsaicin-induced TRPV1 (95 ± 3% at -60 mV) and ORAI1 (89 ± 4% at -120 mV) currents. Furthermore, valencene, which was one of five constituents isolated from the hexane fraction, showed potent inhibitory effects on TRPV1 (69 ± 15% at -60 mV) and ORAI1 (97 ± 2% at -120 mV) currents. Our results provide insight of the potential therapeutic effects of C. rotundus in the contexts of UV-induced photoaging. The therapeutic and cosmetic applications of valencene need further investigation.
Key Words: Cyperus rotundus, TRPV1, Orai1, valencene

P04-32

Cryopreservation method of isolated adult cardiac myocytes of rat

Ga Yul Kim, Ji Yeon Song, Jeong Hoon Lee, Young Boum Lee, Pham Duc Doung, Chae Hun Leem

Department of Physiology, University of Ulsan College of Medicine, 88 OlympicRo 43-gil Songpa-gu, Seoul, Republic of Korea

Cardiomyocytes in a physiologic condition are useful model for cardiac physiological research. However, there are inevitable problems to isolate cell; 1) need to sacrifice the animal, 2) need to find the appropriate experimental conditions and adequate enzymes, and 3) difficult to maintain the quality of the isolated myocytes in each isolation. Especially, these problems are the biggest obstacles for isolating human cardiomyocytes. To resolve the above problems, cryopreservation method for long-term cell preservation should be established. In this study, we tried to find the cryopreservation method that can store and recover the cardiomyocytes in a physiologic condition. We tested the several cryoprotective agents, the freeze-thawing conditions, the used solution compositions, and the other agents like 2,3-butanedione monoxime (BDM). The viability and the shape of cardiac myocytes were checked using trypan blue staining. We found dimethyl sulfoxide (DMSO) was the best cryoprotective agents than the others (methanol, formamide, ethanol, ethylene glycol, glycerol). Among the test of different percentage of DMSO, 15 % DMSO condition produced the best cell survival rate. Cell viability is about 7 percent higher when the rat serum was used instead of fetal bovine serum. Freezing cardiac myocytes in -80°C in deep freezer generated better survival rate than -20°C or -196°C. Using Iscove's Modified Dulbecco's Medium (IMDM) for storage solution makes the cell survival rate higher than using Dulbecco's Modified Eagle's Medium (DMEM). The survival rate has clearly increased when thawing in 37°C water bath than room temperature. Pretreatment of BDM definitely improved the cell shape and the survival rate. In conclusion, DMSO reduced cell damage from the procedure of freezing or thawing. Using rat serum helped the cell survival rate. IMDM improved the cell yield and the survival rate after the cryopreservation. The higher concentration of glucose and Mg^{2+} may contribute for that effect. From the above results, we could obtain more than 75% survival rate after cryopreservation compared to the survival rate of the initial isolation by optimizing the cryopreservation conditions.
Key Words: Cardiomyocyte, Cryopreservation, Cryoprotective agents, DMSO, Cell viability

P04-33

Unusual acid- and voltage-dependency of a prokaryotic CLC, ecCLC-2: A marginal ion channel or broken transporter?

Kun Woong Park, Jung Ha Kim, Hee Soon Choi, Hyun-Ho Lim

Lab. of Membrane Biochemistry and Biophysics, Dept. of Structure & Functional Neural Network, Korea Brain Research Institute (KBRI), Daegu, Korea 701-300

The CLC superfamily can be classified into Cl^- channels and Cl^-/H^+ antiporters and be found in virtually all organisms. Genetic mutations in the CLC genes are linked to the various diseases such as myotonia deafness, epilepsy, leukodystrophy, kidney malfunctions and lysosomal storage disease. CLC proteins also play key roles in the physiology of other organisms: NO3- uptake for nitrogen fixation in plants, extreme acid-resistance in enteric bacteria. In enteric bacterium, two CLC proteins, CLC-1 and CLC-2 are both functionally important for surviving extreme acid environment such as in the stomach: doubly knock-outed E.coli cannot survive at pH 2.5 (in the stomach), but either one of CLC gene is enough to rescue the KO mutant. Intriguingly, reconstituted ecCLC-2 (and ckCLC-2) shows much lower Cl^- transport activity than ecCLC-1, which immediately raise a question: how does ecCLC-2 have such a physiological contribution with much lower activity than its paralogue, ecCLC-1? Here, we present a line of evidences, suggesting a plausible explanation: ecCLC-2 is activated below pH 3, where ecCLC-1 begins to shut down, and ecCLC-2 is also activated by transmembrane voltage stimulus to which ecCLC-1 marginally respond. Interestingly, ecCLC-2 swaps 1 H^+ with 10 Cl^- in the reconstituted membrane and the exchanging stoichiometry can be changed to 1 : 4 (H^+/Cl^-) by both acidification and transmembrane voltage change. To reveal the operating mechanisms of ecCLC-2, we are currently devoting our time to solve the crystal structure of ecCLC-2 (and ckCLC-2).

Key Words: CLC protein, Ion channel, Transporter, Extreme acid-resistance

P04-34

Alcohol impair intracellular calcium oscillation in mouse pancreatic acinar cell

Mi Na Yoon, Min Jae Kim, Ye Jin Jo, Yeong Umg Baek, Dong Kwan Kim, Se hoon Kim, Hyung Seo Park

Department of Physiology, College of Medicine, Konyang University, Daejeon 35365, Korea

The major function of pancreatic acinar cells is to synthesize and secrete a variety of digestive enzymes, and this function is tightly regulated by intracellular calcium. The intracellular calcium can be mobilized from extracellular space or intracellular calcium stores. Physiological concentration (500 nM) of carbamylcholine (CCh) could generate repetitive calcium transients termed calcium oscillation. These oscillations are known to be initiated by calcium release from intracellular calcium store and be sustained by calcium entry from extracellular medium. In this study, we were aimed to investigate the role of alcohol on CCh-induced calcium oscillation and the mechanisms of alcohol on calcium mobilization in dispersed pancreatic acinar cell. Pancreatic acinar cells were isolated from Balb/C mice by collagenase digestion. To measure the cytosolic and the stored calcium, acinar cells were loaded with fura-2 AM, and then fluorescence ratio of 340/380 was measured using Till-Photonics imaging system. The gradual increase of alcohol significantly inhibited CCh-induced calcium oscillation dose-dependently, and maximum inhibition was observed at a concentration of 100 mM alcohol. Pretreatment of alcohol has no effect on the store-operated calcium entry induced by high concentration (10 μM) of CCh. 100 mM alcohol reduced the initial calcium peak induced by CCh. As a result, the CCh-dose response curves of initial calcium peak were shift to the right by 100 mM alcohol treatment. These results provide evidences that alcohol has a regulatory role on CCh-induced calcium mobilization through reduction of calcium release from intracellular stores in pancreatic acinar cell.

Key Words: alcohol, acetylcholine, calcium oscillation, pancreatic acinar cell

P04-35

Ketamine inhibits KCNQ2/3 channels and modulates excitability in hippocampal dentate gyrus granule cells

Seul Yi Lee, Xianlan Wen, Hana Cho

Department of Physiology and Samsung Biomedical Research Institute, School of Medicine, Sungkyunkwan University, Suwon, Korea

Recent studies showed that ketamine, an ionotropic glutamatergic NMDAR (N-methyl-D-aspartate receptor) antagonist, produces a fast-acting antidepressant response in patients with major depressive disorder. However, little is known about the effects of ketamine in the regulation of ion channels in the brain. KCNQ channels regulate neuronal excitability and KCNQ channel inhibitor XE991 reverts cognitive impairment. We tested the action of ketamine on KCNQ2/3 channels in HEK293 cells and hippocampal neurons using patch clamp technique. Ketamine inhibits KCNQ2/3 currents heterologously expressed in HEK293 cells. Current inhibition by ketamine was voltage independent but concentration-dependent. The IC50 for current inhibition was 50.7±13.4 μM. The voltage-dependent activation of the channel was not modified. The powerful effects of ketamine on cloned KCNQ channels imply that ketamine action on KCNQ channels didn't involve NMDAR. The effects of MK801 and DL-2-amino-5-phosphonopentanoic acid (AP-5), NMDAR blockers that are structurally similar to and distinct from ketamine, respectively, were also examined. MK801 had similar inhibitory effects on KCNQ2/3 channels, but AP-5 showed no effects on KCNQ2/3 activity, suggesting the direct effects of ketamine and MK801 on KCNQ2/3 channels. In hippocampal neurons, which endogenously express KCNQ2/3 channels, application of ketamine produced an increase in neuronal excitability and input resistance. Taken together, these data suggest that ketamine is a KCNQ2/3 channel modulator and the modulation of the neuronal excitability by ketamine may contribute to the fast-acting antidepressant action of ketamine.

Key Words: depressive disorder, K channel, ketamine

P04-36

Voltage gated sodium channel 1.7 as therapeutic target for treatment of neuropathic pain

Sung-Young Kim

New Drug Laboratory, Daewoong 72, Dugye-ro, Pogok-eup, Cheoin-gu, Yongin-si, Gyeonggi-do, 449-814, Korea

A novel class of voltage gated sodium channel 1.7 (Nav1.7) inhibitors has been identified. Our compounds showed potent human Nav1.7 inhibitory activities with fair subtype selectivity over Nav1.5. Compounds successfully demonstrated analgesic efficacy in animal models comparable to that of the currently used drug, gabapentin.

Key Words: voltage gated sodium channel, Pain, ion channel

P04-37

Autocrine insulin stimulates plasma membrane trafficking of KATP channel via PI3K-VAMP2 pathway in MIN-6 cells

Shanhua Xu[1,2], Ji-Hee Kim[1], Kyu-Hee Hwang[1,2], Ranjan Das[1], Xianglan Quan[1], Tuyet Thi Nguyen[1], Soo-Jin Kim[1,2], Seong-Woo Jeong[1], In-Deok Kong[1], Seung-Kuy Cha[1], Kyu-Sang Park[1]

[1]Department of Physiology, [2]Department of Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, 220-701, Korea

Regulation of ATP sensitive inwardly rectifying potassium (KATP) channel plays a critical role in metabolism-secretion coupling of pancreatic β-cells. Released insulin from β-cells has been known to inhibit insulin and glucagon secretion with autocrine and paracrine modes. However, molecular mechanism for inhibition of hormone exocytosis by insulin still remains unclear. In this study, we investigated the effect of released insulin on surface abundance of KATP channel suppressing further insulin exocytosis in mouse clonal β-cell line, MIN-6. Surface biotinylation assay and immunostaining experiments revealed that incubation with high glucose increased plasmalemmal sulfonylurea receptor 1 (SUR1) protein composing KATP channel. Acute exposure of insulin also increased cell surface abundance of SUR1. High glucose or exogenous insulin-triggered SUR1 trafficking was blocked by inhibition of Phosphoinositide 3-kinase (PI3K) with wortmannin. Pretreatment with brefeldin A, which inhibits protein transport from the ER to the Golgi, or silencing of vesicle-associated membrane protein 2 (VAMP2) abolished insulin-mediated upregulation of surface SUR1. Glucose-stimulated cytosolic Ca^{2+} ($[Ca^{2+}]_i$) increase was blunted by insulin pre-incubation similar to the effect of diazoxide, which was reverted by pretreatment with GSK1838705, an insulin receptor blocker. Taken together, these results suggest that released insulin by nutrients recruits plasmalemmal KATP channel via PI3K-VAMP2 pathway to impede depolarization-induced $[Ca^{2+}]_i$ oscillation and exocytosis, which may participate in a feedback regulation to avoid sustained insulin secretion.

Key Words: MIN-6 cells, insulin, KATP channel , PI3K, VAMP2

P04-38

Effects of nitric oxide on voltage-dependent K^+ currents in human cardiac fibroblasts

Hyemi Bae, Donghee Lee, Misuk Yang, Youngwon Kim, Jeongyoon Choi, Jaehong Ko, Hyoweon Bang, Inja Lim

Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

The aim of this study is to know the effects of nitric oxide (NO) on voltage-dependent K^+ currents and underlying signaling pathways in human cardiac fibroblasts. We found three types of voltage-dependent K^+ currents in human cardiac fibroblasts by whole-cell mode patch clamp techniques, RT-PCR and Western blots; 1) severe oscillated, non-inactivating, and outward rectifying Ca^{2+}-activated K^+ (K_{Ca}) currents, 2) fast activating and non-inactivating, or slowly inactivating delayed rectifier outward K^+ (KDR) currents, and 3) fast activating and fast inactivating transient outward K^+ (KTO) currents. In whole-cell configuration, S-nitroso-N-acetylpenicillamine (SNAP, NO donor) significantly increased K_{Ca}, K_{DR}, and K_{TO} currents. KT5823 (protein kinase G blocker) and 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, soluble guanylate cyclase blocker) blocked the stimulatory effect of SNAP on K_{Ca}, K_{DR} and K_{TO}. In addition, 8-bromo-cGMP increased these currents. KT5720 (protein kinase A blocker) also inhibited the SNAP stimulating effect on K_{Ca} and K_{DR} currents but not K_{TO} currents. 8-bromo-cAMP (cell-permeable cAMP analogue) and forskolin (adenylate cyclase activator) increased K_{Ca} and K_{DR} currents. On the contrary, the stimulating effect of SNAP on KTO currents was not blocked by KT5720. 8-bromo-cAMP also did not increase K_{TO} currents. These findings suggest PKG and PKA pathways involved in the SNAP stimulating effect on K_{Ca} currents and K_{DR} currents in human cardiac fibroblasts. On the other hand, the SNAP stimulating effect on K_{TO} currents were through PKG pathway but not PKA pathway.

Key Words: Human Cardiac Fibroblast , Nitric oxide, PKA Pathway, PKG Pathway, Voltage-dependent K^+ currents

P04-39

WNK1 promotes tumor progression via TRPC6 activation in clear cell renal cell carcinoma

Ji-Hee Kim[1,3], Kyu-Hee Hwang[1,3], Minseob Eom[2,3], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Kyu-Sang Park[1,3,4], Seung-Kuy Cha[1,3,5]

Departments of [1]Physiology, [2]Pathology, [3]Global Medical Science, [4]Institute of Lifestyle Medicine and [5]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

WNK1 kinase regulates renal ion homeostasis and causes hypertension and hyperkalemia when it overexpressed in the renal tubules by genetic mutation. WNK1 stimulates PLCβ signaling leading TRPC6 channel activation. However, its signaling functions in regulating tumor progression remain elusive. Here, we show that the WNK1 expression is positively correlated with TRPC6 expression and WNK1 stimulation of TRPC6 is important for clear cell renal cell carcinoma (ccRCC) cell proliferation and migration. The expression of WNK1 and TRPC6 in tumor tissues was significantly higher than those in the adjacent normal parenchymal tissues. TRPC6 is a major downstream effector of Gαq-coupled receptor-PLCβ signaling pathway in ccRCC confirmed by pharmacological inhibition and siRNA knockdown of TRPC6. Knockdown of WNK1 reduced whole-cell and single channel current of TRPC6 activated by Gαq-coupled receptors supporting the notion that WNK1 is a novel regulator of PLCβ signaling and its biosensor TRPC6. Silencing WNK1 and TRPC6 in ccRCC cells significantly blunted cell proliferation and migration. TRPC6-mediated Ca^{2+} influx and the activation of calcineurin and its substrate NFATc1 are crucial for proliferation of ccRCC. Accordingly, NFATc1 and its target genes including Cox2, CyclinD1 and myc involving tumor progression are highly expressed in tumor tissues compared to that of normal parenchymal tissues. These findings provide a new perspective of WNK1 signaling functions involved in the tumorigenesis. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)]

Key Words: WNK1, TRPC6, renal cell carcinoma, cancer, Ca^{2+}

P04-40

Klotho inhibits tumor progression via targeting Orai1 channels in cancer cells

Ji-Hee Kim[1], Kyu-Hee Hwang[1], Seong-Woo Jeong[1,2], In Deok Kong[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2,3,¶]

Departments of [1]Physiology and Global Medical Science, [2]Institute of Lifestyle Medicine, and [3]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

An anti-aging protein Klotho exerts a tumor suppressor that is produced in the kidneys. Despite Klotho suppresses tumor progression with diverse mechanism, little insight has been provided for Klotho function on Ca^{2+} signaling, which plays a crucial role in cancer hallmarks. Store-operated calcium entry (SOCE) is the principal Ca^{2+} influx mechanism in non-excitable cells including cancer cells. However, the molecular mechanism by which Klotho suppresses tumor progression through SOCE regulation remains largely elusive. Here, we show that Klotho regulation of Orai1, a pore-forming subunit of SOCE, is a common molecular mechanism for tumor suppression. Orai1 is a predominant molecular component of SOCE, which participates in cancer cell migration and proliferation in diverse breast, lung and renal cancers. Klotho blunts not only Orai1-mediated SOCE in those cancer cell lines but also Orai1 currents without altering its intrinsic channel properties. Klotho inhibition of Orai1 is mediated by phosphoinositide-3-kinase- and VAMP-2-dependent exocytosis of the channel that leads cancer cell migration and proliferation. These results provide a novel common molecular basis of Klotho effects on tumor progression. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A2060764)]
Key Words: Klotho, Orai1, cancer, store-operated Ca^{2+} channel, VAMP-2

P04-41

Inhibition of N-type Ca^{2+} currents in rat peripheral sympathetic neurons by Imidazoline I_2 receptors activation

Soo-Yeon Lee, Eun Jeong Kim, Ji-Hyun Joeng, Young-Hwan Kim, Duck-sun Ahn, Seungsoo Chung

Department of Physiology, Yonsei University College of medicine, Seoul, 120-752, Republic of Korea Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea

Agmatine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_2 receptors (IR2) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_2-induced inhibition of noradrenaline (NA) release is still unknown. To investigate this possibility, we investigated the effect of IR_2 activation on N-type Ca^{2+} currents ($I_{Ca\text{-}N}$) in isolated neurons of the celiac ganglion (CG), which is involved in the sympathetic regulation of mesenteric artery vascular tone. In the present study, agmatine diminished voltage-gated Ca^{2+} currents (I_{Ca}), measured using the patch-clamp method, in an irreversible manner in rat CG neurons, while, thrombin had little effect on I_{Ca}. This agmatine-induced inhibition was nearly completely prevented by ω-CgTx, a potent N-type Ca^{2+} channel blocker, suggesting involvement of N-type Ca^{2+} channel in the PAR-2-induced inhibition. In addition, agmatine inhibited $I_{Ca\text{–}N}$ in a voltage-independent manner in rat CG neurons. Moreover, agmatine reduced action potential firing frequency measured using the current-clamp method in rat CG neurons and this inhibition of AP firing induced by agmatine nearly completely prevented by ω-CgTx, indicating IR2 activation may regulate the membrane excitability of peripheral sympathetic neurons through modulation of N-type Ca^{2+} channels in rat CG neurons. In conclusion, the present findings demonstrate that the activation of IR2 suppresses peripheral sympathetic outflow by modulating N-type Ca^{2+} channel activity located in peripheral sympathetic nerve terminals, which appear to be involved in IR2-induced hypotension. This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009525)
Key Words: Imidazoline I_2 receptors, N-type Ca^{2+} currents, celiac ganglion

P04-42(O-2)

TRPC6 induces hepatic stellate cell activation causing liver fibrosis

Kyu-Hee Hwang[1,2,3], Ji-Hee Kim[1,2,3], Soo-Jin Kim[1,2,3], Ranjan Das[1], Seong-Woo Jeong[1,2,3], In Deok Kong[1,2,3], Kyu-Sang Park[1,2,3], Seung-Kuy Cha[1,2,3,4]

Departments of [1]Physiology and [2]Global Medical Science, [3]Institute of Lifestyle Medicine, and [4]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

Hepatic stellate cells (HSCs) activation is the leading cause of liver fibrosis and portal hypertension. In response to injury, HSCs are activated by phospholipase-linked receptors such as Gαq-protein coupled receptors and receptor tyrosine kinases whose activation evokes Ca^{2+} influx termed receptor-operated Ca^{2+} entry (ROCE). The Ca^{2+} mediated signaling pathways are implicated directly or indirectly activating HSCs causing de novo expression of α-smooth muscle actin (αSMA) and/or profibrotic ligand TGFβ. However, the molecular identity and underlying mechanism of ROCE involving HSC activation are ill-defined. Here, we report that TRPC6 channel is an essential molecular component of ROCE mediating HSC activation. Among TRPCs, TRPC6 expression was significantly increased in bile duct ligation- and thioacetamide-induced liver cirrhosis animal models. Functionally, TRPC6-stimulated Ca^{2+} influx was blunted by specific blockade of TRPC6 with the siRNA against Trpc6 and pharmacological inhibitor SKF96365 in cultured human HSCs. Overexpression of TRPC6 by gene delivery in mice induced de novo expression of αSMA and TGFβ suggesting that TRPC6-mediated Ca^{2+} influx may involve in HSCs activation and liver fibrosis. These results provide a new perspective on the pathogenesis of liver fibrosis and may provide clues for treatment of the liver cirrhosis. [This research was supported by Basic Science Research program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)]
Key Words: TRPC6, liver fibrosis, ROCE, HSC activation

P04-43

The organellar Ca^{2+} channel TRPML3 regulates early autophagosome biogenesis by interaction with phosphoinositides

Mi Kyung Kim, So Woon Kim, Hyun Jin Kim

Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, 440-746, Korea

TRPML3 is a Ca^{2+} permeable cation channel expressed in multiple organelles including autophagosomes. Although TRPML3 induces autophagy and increases autophagy upon cell stress, how TRPML3 regulates autophagy is not known. By using a fusion protein containing

GCaMP6, a genetically encoded calcium indicator to C-terminus of TRPML3, we observed an organellar Ca^{2+} efflux through TRPML3 in the autophagy process. Colocalization studies revealed that TRPML3-GCaMP6, a functional TRPML3 is localized in the sites for early autophagosome biogenesis, indicating that TRPML3 provides Ca^{2+} for the process. Lipid binding assay showed that TRPML3 interacts with phosphatidylinositol-3-phosphate (PI3P) which is essential for the initiation of autophagy and enriched in autophagosomes. Moreover, we found that TRPML3-GCaMP6 could be activated by PI3P as well as by induction of autophagy. These results suggest that TRPML3 is a key regulator for autophagy process, activating by PI3P and providing Ca^{2+} during early steps of autophagosome formation.
Key Words: TRPML3, phosphoinositide, Autophagy, GCaMP6

P04-44

Trafficking-dependent N-glycan structure regulates cell surface expression of potassium channel Kv3.1b

Paul Christian Vicentea[1], Jin Young Kim[2], Ji Seon Shim[1], Jeong-Ju Ha[1], Dong-Hyeon Kim[3], Min-Young Song[1], Jin-Sung Choi[3], Kang-Sik Park[1]

[1]Department of Physiology, Kyung Hee University School of Medicine, Seoul 02447, Korea, [2]Division of Mass Spectrometry Research, Korea Basic Science Institute, Cheongju, Chungcheongbuk-do 28119, South Korea, [3]College of Pharmacy, Catholic University of Korea, Bucheon, Gyeonggi-Do 14662, Korea

The potassium ion channel Kv3.1b, member of the third group of maturely glycosylated voltage-gated ion channel family, allows high frequency firing of neurons through a controlled modulation of outward currents when functional and physiologically expressed on the cell surface. N-glycosylation is known to regulate several functions of different ion channels including trafficking to the cell surface, targeted localization, gating, and stability. However, the mechanisms of N-glycosylation-dependent function to Kv3 channels remain to be elucidated. Here, we show the principal roles of glycosylation at specific asparagine sites to the diverse functions of the potassium channel Kv3.1b. We observed that the N-glycosylation at N229, similar to wild-type, predominantly mediates the cell-surface trafficking and localization of Kv3.1b proteins whereas high quantities of wild-type (WT) and N229-glycosylated Kv3.1b channel proteins reach the cell surface. Both N220-glycosylated and unglycosylated Kv3.1b channel proteins do also reach the cell surface but in very minute amounts. Mass spectrometric analyses revealed a complex GlcNAc2Man3GlcNAc2Fuc1 as the predominant glycan composition of N229-glycosylated Kv3.1b proteins; as compared to Man8GlcNAc2 for N220-glycosylated Kv3.1b proteins which are of the high-mannose type. Additionally, all forms of ER-retained Kv3.1b channel proteins especially of the unglycosylated types are susceptible to degradation when coexpressed with calnexin, in comparison to plasma membrane Kv3.1b pools which are generally resistant, which suggest distinctions in Kv3.1b protein localization. Taken together, our findings suggest that the various trafficking functions of the potassium channel Kv3.1b are majorly N229-glycosylation site-dependent and shed significant knowledge on N-glycosylation-dependent molecular mechanisms of the Kv3.1b potassium channel.
Key Words: Potassium channel, Kv3.1b, glycosylation, trafficking, masspectrometry

P04-45(O-1)

Tyrosine phosphorylation of Kv2.1 channel contributes to neuronal cell death in brain ischemia

Min-Young Song[1], Eun Ji Bae[1], Hye-Min Kang[2], Chan Park[2], Kang-Sik Park[1]

[1]Department of Physiology, Department of Anatomy, [2]Kyung Hee University School of Medicine, Seoul 02447, Korea

Oxidative stress induces neuronal apoptosis and is involved in brain ischemia. Oxidant induced apoptosis enabling intracellular K^+ efflux is mediated by increase of Kv2.1 channel activity. It has been shown that the increas of channel activity is triggered by tyrosine phosphorylation of Kv2.1 and Y686 and 810 of the cytoplasmic domains of Kv2.1 are phosphorylated by the Src kinase. However, the functional roles of Kv2.1 tyrosine phosphorylation in brain ischemia are not fully known. Here we provide the evidences that the oxidative stress-induced brain ischemia is regulated by tyrosine phosphorylation of Kv2.1. The tyrosine phosphorylation levels of Kv2.1 were increased by oxidant induced neuronal ischemia. Mutation of the tyrosine phosphorylation sites (Y686F and Y810F) of Kv2.1 channel results in a decrease of cleaved PARP-1 level, which indicates the suppression of the neuronal cell death. In a brain ischemia model, the tyrosine phosphorylation of Kv2.1 is also increased after brain ischemia. A sustained increase of tyrosine phosphorylation of Kv2.1 was observed for at least 2h after reperfusion. Our results show that the tyrosine phosphorylation of Kv2.1 channel in brain play a critical role in regulating neuronal ischemia and may be a potential therapeutic target for brain ischemia.
Key Words: Kv2.1, tyrosine phosphorylation, brain ischemia, oxidative stress

P04-46

The phosphorylation sites of potassium channel Kv2.1 determine cell background specific differences in function between cerebellum and cerebrum

Ji Yeon Hwang, Eun Ji Bae, Ji Seon Shim, Kang-Sik Park

Department of Physiology, School of Medicine, Kyung Hee University, Seoul 02447, Korea

The voltage-gated potassium channel Kv2.1 is highly phosphorylated in mammalian brain, and its variable phosphorylation sites modulate the activity-dependent regulation of the channel functions. Previous our studies reported that the differential phosphorylation subsets of Kv2.1 affect differences in its physiological properties in different cell types under basal conditions. Here we found the differences of Kv2.1 phosphorylation status between cerebellum and cerebrum in brain. To understand whether these differences are due to different phosphorylation states of the same sites or the different sets of phosphorylation sites on Kv2.1 in brain, we used nano-LC tandem mass spectrometry (nano-LC MS/MS) for the qualitative and quantitative analysis of phosphorylation of Kv2.1 purified from cerebellum and cerebrum. We identified a total of 14 phosphorylation sites on Kv2.1 proteins that exhibits different levels or phosphorylation sites in cerebellum and cerebrum. Of these, four sites were only found phosphorylated in cerebellum, on the other hands, two sites were identified in cerebrum. Taken together, our data suggest that phosphorylation may play a critical role in determining the physiological properties of neurons in cerebellum and cerebrum.
Key Words: Potassium channel, Kv2.1, phosphorylation, mass

spectrometry, cerebellum, cerebrum

P05-01

MicroRNA-200a/210 controls proliferation and Osteogenic differentiation of human adipose tissue stromal cells

Young Suk Kim[1], Hee Jeong Park[1], Keun Koo Shin[1], Seon Young Lee[1], Yong Chan Bae[2], Jin Sup Jung[1]

[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan (626-870), Korea, [2]Department of Plastic Surgery, School of Medicine, Pusan National University, Pusan (602-739), Korea

Background/Aims: The elucidation of the molecular mechanisms underlying the differentiation and proliferation of human adipose tissue–derived mesenchymal stem cells (hADSCs) represents a critical step in the development of hADSCs-based cellular therapies. We determined the role of microRNA-210 (miR-210) and microRNA-200a (miR-200a) in hADSCs functions. **Methods:** The microRNAs and microRNA's target genes levels were regulated by oligonucleotides transfection. The osteogenic differentiation was induced for 14 days in an osteogenic medium and assessed by using an Alizarin Red S stain. The regulation of miRNAs on target genes expressions were determined by western blot, real-time PCR and luciferase reporter assay. **Results:** Overexpression of miR-200a increased the proliferation and osteogenic differentiation of hADSCs, while causing downregulation of the levels of ZEB2 protein and mRNA. Inhibition of miR-200a with a 2′O methyl antisense RNA inhibited the proliferation and osteogenic differentiation of hADSCs. Overexpression of miR-210 was found to inhibit the proliferation of hADSCs but increase the osteogenic differentiation. In addition, it caused downregulation of the levels of IGFBP3 protein and mRNA. Inhibition of miR-210 with a 2′O methyl antisense RNA increased the proliferation but inhibited the osteogenic differentiation of hADSCs. Analysis of the luciferase reporter activity of the constructs containing the miR-200a target site within the ZEB2 3′ untranslated region and the miR-210 target site within the IGFBP3 3′ untranslated region revealed lower activity in the miR-200a- or miR-210-transfected hADSCs than in control miRNA-transfected hADSCs. RNA interference-mediated downregulation of ZEB2 or IGFBP3 in the hADSCs showed similar effects on both their proliferation and osteogenic differentiation with that of miR-200a and miR-210 overexpression, respectively. **Conclusion:** The results of the current study indicate that miR-200a and miR-210 regulate the osteogenic differentiation and proliferation of hADSCs through the direct targeting of IGFBP3 and ZEB2, respectively.
Key Words: hADSC, osteogenic differentiation, proliferation, miRNA-200a, miRNA-210

P05-02

MicroRNA-4284 controls proliferation and Adipogenic and Osteogenic differentiation of human adipose tissue stromal cells

Hee Jeong Park[1], Young Suk Kim[1], Keun Koo Shin[1], Sun Young Lee[1], Yong Chan Bae[2], Jin Sup Jung[1]

[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan (626-870), Korea, [2]Department of Plastic Surgery, School of Medicine, Pusan National University, Pusan (602-739), Korea

Elucidation of the molecular mechanisms underlying the differentiation and proliferation of human adipose tissue–derived mesenchymal stem cells, or adipose tissue stromal cells (hADSCs), represents a critical step in the development of hADSC-based therapies. Regarding the role of microRNA-4284 (miR-4284) in the functioning of hADSCs, we found that overexpression of miR-4284 decreased proliferation and increased adipogenic and osteogenic differentiation of these cells while downregulating the levels of HDAC1 protein and mRNA. Inhibition of miR-4284 with a 2′-O-methyl antisense RNA had the opposite effect, increasing proliferation and inhibiting differentiation of hADSCs. Analysis of luciferase reporter activity of the constructs containing the miR-4284 target site within the HDAC1 3′ untranslated region revealed lower activity in the miR-4284-transfected hADSCs than in control microRNA-transfected hADSCs. The effects of RNA interference–mediated downregulation of HDAC1 on both the proliferation and adipogenic and osteogenic differentiation of hADSCs were similar to those of miR-4284 overexpression. Our results indicate that miR-4284 regulates the adipogenic and osteogenic differentiation and proliferation of hADSCs through direct targeting of HDAC1.
Key Words: hADSC, adipogenic differentiation, osteogenic differentiation, proliferation, miRNA-4284

P05-03(O-12)

mTOR signaling in the insular cortex modulates neuropathic pain

Minjee Kwon[1,2,*], Jeongsoo Han[1,2,*], Myeounghoon Cha[1], Un Jeng Kim[1], Bae Hwan Lee[1,2,†]

[1]Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea, [2]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

The insular cortex (IC) has recently been associated with chronic pain, but the underlying molecular mechanisms remain unclear. Because the IC was thought to store pain-related memories, determining the role that translational regulation plays in neuronal plasticity may identify novel targets for controlling chronic pain. The mammalian target of rapamycin (mTOR) is known to control mRNA translation and influence synaptic plasticity and dendritic growth. There have been many studies that have investigated mTOR signaling at the spinal level, but mTOR signaling in the IC in neuropathic pain remains unknown. Therefore, we investigated the role of mTOR signaling in the modulation of neuropathic pain and assessed the potential therapeutic effects of rapamycin, an inhibitor of the mTORC1, in the IC of neuropathic rats. Adult male Sprague-Dawley rats were used for neuropathic surgery, and the development of neuropathic pain was evaluated by measuring mechanical allodynia. Western blot analysis and microinjection of rapamycin into the IC were performed on post-operative day 3 (POD 3). Microinjection of rapamycin into the IC reduced mechanical allodynia and altered the activation of mTOR signaling, which is activated by nerve injury. Furthermore, rapamycin inhibited the development of synaptic plasticity via downregulation of postsynaptic density protein 95 (PSD95). These findings suggest that inhibition of supraspinal mTOR signaling may be a critical molecular mechanism that modulates neuropathic pain. Therefore, rapamycin may be a potential therapeutic agent for the treatment of chronic pain. This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (No.2015021989).
Key Words: Neuropathic pain, mTOR, insular cortex, rapamycin, synaptic plasticity

P05-04

Nerve injury-induced neuroplasticity in the insular cortex contribute to pain hypersensitivity

Jeongsoo Han, Minjee Kwon, Motomasa Tanioka, Bae Hwan Lee

Department of Physiology, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

The insular cortex (IC) is involved in important functions linked with pain and emotion. According to recent reports, neural plasticity in the brain including the IC can be induced by nerve injury and may contribute to chronic pain. Protein kinase Mζ (PKMζ) is considered to maintain the late phase of long-term potentiation (L-LTP). This study was conducted to determine the role of PKMζ in the IC which may be involved in the modulation of neuropathic pain. Behavioral test for neuropathic pain development, immunohistochemistry (IHC) for zif268, analgesia test after ζ-pseudosubstrate inhibitory peptide (ZIP, a selective inhibitor of PKMζ) injection, and immunoblotting of PKMζ, phospho-PKMζ, GluR1 and GluR2 subunits of AMPA receptor after ZIP injection were performed. IHC data showed that zif268 expression significantly increased after nerve injury. Mechanical allodynia was significantly decreased by ZIP microinjection into the IC. The analgesic effect lasted for 12 hours. Moreover, the levels of GluR1, GluR2 and, p-PKMζ were decreased after ZIP microinjection. These results suggest that peripheral nerve injury induces neural plasticity related to PKMζ and ZIP has potential effects for relieving chronic pain. This research was support by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (No. 2015021989)
Key Words: PKMζ, insular cortex, neuronal plasticity, ZIP, neuropathic pain

P05-05

Alteration of cardiac hypertrophic marker gene expression by PCB 126 and PCB 77

Mi-Hyeong Park , Su-Hyun Jo

Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea

Polychlorinated biphenyls (PCBs) and other halogenated aromatic hydrocarbons elicit a variety of adverse biological effects on the cardiovascular systems of mammalian, piscine and avian species. However, there are a limited number of studies on hypertrophic marker gene induction by these persistent organic pollutants (POPs) in the heart. We compared the effects of two PCB congeners, 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) on cytotoxicity and gene expression related with cardiac hypertrophy using primary culture of rat adult ventricular myocytes, cytotoxicity assay, and real-time RT-PCR. PCB 126 and PCB 77 increased cytotoxicity, and PCB 77 had ~ 2-fold higher potency than PCB 126. PCB 126 at 10 μM increased the expression of the hypertrophic marker genes, ANP and BNP, and decreased the related gene, SERCA2A. PCB 77 at 1~10 μM increased the expression of the hypertrophic marker genes, ANP and BNP, and decreased the related genes, SERCA2A and PLB. We explored the possible mechanism underlying the PCB 126 and PCB 77-induced cardiac hypertrophy by examining gene expression related with TRPC-PKG signaling. PCB 126 increased the expression of RCAN and TRPC1, but decreased PDE5A. PCB 77 increased the expression of RCAN, TRPC1, TRPC3, and TRPC6, but didn't change the expression of PDE5A. 8Br-cGMP, a PKG activator, blunted PCB126- and PCB 77-induced increase of gene expressions of ANP, BNP, and RCAN, however, TRPC3 blocker, pyrazole, failed to negate the PCB126 and PCB 77-induced increase of gene expressions of ANP and BNP. The present data indicate that the environmental toxicants, PCBs, can modulate the expression of genes coding for programmes of cellular differentiation and stress (ANP and BNP) and induce cardiac hypertrophy by the mechanism related with RCAN, PKG, and TRPC.
Key Words: cardiomyocytes, cytotoxicity, hypertrophy, PCB 126, PCB 77

P05-06

Impaired cholesterol homeostasis increases the secretion of beta-amyloid peptide in Familial Alzheimer's disease-associated presenilin mutant

Yoon Young Cho, Oh-Hoon Kwon, Sungkwon Chung

Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

Alzheimer's disease (AD) is a major neurodegenerative disorder characterized by the accumulation of β-amyloid peptide (Aβ) and formation of neurofibrillary tangles. The highly amyloidogenic 42-residue Aβ (Aβ42) is the first species to be deposited in both sporadic and familial AD (FAD). PS mutations lead to several key cellular phenotypes, including alterations in proteolysis of β-amyloid precursor protein (APP) and Ca2+ entry. It is also reported that PS mutant elevates cholesterol levels due to the upregulated expression of CYP51, which plays a critical role for the cholesterol synthesis (Tomboli et al., 2008 저널명, vol, pages?). Since elevated cholesterol is a risk factor for AD, it may contribute to the increased Aβ production in PS1 mutant cells. In this study, we tested whether there exists a functional link between the impaired cholesterol homeostasis and the elevated Aβ levels in PS mutant cell. We confirmed that the expression of CYP51, and cholesterol level were elevated in CHO cells transfected with PS1 delta E9 mutant, compared to cells transfected with PS1 wild type (WT). A CYP51 specific inhibitor, tebuconazole, decreased the cholesterol level in PS1 delta E9 cells to the comparable level with PS1 WT cells, and significantly reduced secreted Aβ42 from PS1 delta E9 cells. It suggests that the elevated cholesterol in PS1 delta E9 cells is directly linked to the increased Aβ production. When cholesterol was depleted by incubating cells with lipid depleted serum, larger amount of Aβ42 was decreased in PS1 delta E9 cells than in PS1 WT cells. Combined together, our results demonstrate that the elevated cholesterol in PS1 mutant contributes to the increased Aβ42 production and the pathology of a genetic basis of AD.
Key Words: Alzheimer's disease, β-amyloid peptide, Familial AD (FAD), Presenilin, Cholesterol

P05-07

Trichostatin A inhibits Angiotensin II-induced Hypertension in Vasocontraction and Blood Pressure via Inhibiting p66shc and Reactive Oxygen Species

Yu Ran Lee[1], Gun Kang[1], Hee Kyoung Joo[1], Myoung Soo Park[2], Cuk-Seong Kim[1], Sunga Choi[1], Byeong Hwa Jeon[1,2]

[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, [2]Preclinical Research Center, Chungnam

National University Hospital, Daejeon, 301-747, Republic of KOREA

Histone deacetylase (HDAC) has been recognized as a potentially useful therapeutic target for cardiovascular disorders. However, the effect of the HDAC inhibitor, trichostatin A (TSA), on vasoreactivity and hypertension remains unknown. We performed aortic coarctation at the inter-renal level in rats in order to create a hypertensive rat model. Hypertension induced by abdominal aortic coarctation was significantly suppressed by chronic treatment with TSA (0.5 mg/kg/day for 7 days). Nicotinamide adenine dinucleotide phosphate-driven reactive oxygen species production was also reduced in the aortas of TSA-treated aortic coarctation rats. The vasoconstriction induced by angiotensin II (Ang II, 100 nM) was inhibited by TSA in both endothelium-intact and endothelium-denuded rat aortas, suggesting that TSA has mainly acted in vascular smooth muscle cells (VSMCs). In cultured rat aortic VSMCs, Ang II increased p66shc phosphorylation, which was inhibited by the Ang II receptor type I (AT1R) inhibitor, valsartan (10 μM), but not by the AT2R inhibitor, PD123319. TSA (1~10 μM) inhibited Ang II-induced p66shc phosphorylation in VSMCs and in HEK293T cells expressing AT1R. Taken together, these results suggest that TSA treatment inhibited vasoconstriction and hypertension via inhibition of Ang II-induced phosphorylation of p66shc through AT1R.

Key Words: Angiotensin II, Angiotensin receptor type I, Hypertension, p66shc, Trichostatin A

P05-08

Regulation of basal autophagy and Aβ clearance by TRPM7

Hyun Geun Oh, Oh Hoon Kwon, Sungkwon Chung

Dept. of Physiol., Sungkyunkwan Univ. Sch. of Med., Suwon, Republic of Korea

Autophagy is a conserved process for degradation of cytoplasmic components using lysosomal machinery, and the dysfunction of autophagy is related to many neurodegenerative diseases including Alzheimer's disease (AD). As a ubiquitous Ca2+ channel, TRPM7 channel underlies the constitutive Ca2+ influx, and related to many neurodegenerative diseases. Since intracellular Ca2+ level is known to regulate autophagy, we set out to test whether Ca2+ influx through TRPM7 channel regulates the basal autophagy. When TRPM7 channel expression is elevated, basal autophagy and AMPK phosphorylation (a main regulator for autophagy by Ca2+) are increased. In contrast, basal autophagy and AMPK phosphorylation were decreased when TRPM7 channel expression is down-regulated by shRNA and specific TRPM7 blocker. Recently, autophagy has been implicated as the Aβ clearance mechanism. We have reported that the activation of TRPM7 channel is chronically suppressed by the presence of familial AD mutants via PIP2 imbalance. Consistent with close relationship between TRPM7 channel activity and autophagy, we demonstrated that the basal autophagy is down-regulated via AMPK phosphorylation in PS1ΔE9 mutant cells. In these mutant cells, PIP2 supplement increases autophagy level and decreases secreted Aβ level. Moreover, secreted Aβ level is increased by down-regulating TRPM7 using specific shRNA. Combined together, these results suggest that TRPM7 channel contributes Aβ clearance via regulating basal autophagy.

Key Words: autophagy, TRPM7, PIP2, beta-amyloid

P05-09

The 18-kDa translocator protein inhibits vascular cell adhesion molecule-1 expression via inhibition of mitochondrial reactive oxygen species

Hee Kyoung Joo[1], Yu Ran Lee[1], Myoung Soo Park[2], Su Hyeon Kim[1], Sunga Choi[1], Byeong Hwa Jeon[1]

[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, Daejeon, Republic of Korea, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Republic of Korea

Translocator protein 18 kDa (TSPO) is a mitochondrial outer membrane protein and is abundantly expressed in a variety of organ and tissues. To date, the functional role of TSPO on vascular endothelial cell activation has yet to be fully elucidated. In the present study, the phorbol 12-myristate 13-acetate, an activator of protein kinase C (PKC), was used to induce vascular endothelial activation. Adenoviral TSPO overexpression inhibited PMA-induced vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1) expression in a dose dependent manner. PMA-induced VCAM-1 expressions were inhibited by Mito-Tempo, a specific mitochondrial antioxidants, and cyclosporine A, a mitochondrial permeability transition pore inhibitor, implying on an important role of mitochondrial reactive oxygen species (ROS) on the endothelial activation. Moreover, adenoviral TSPO overexpression inhibited mitochondrial ROS production and manganese superoxide dismutase expression. On contrasts, gene silencing of TSPO with siRNA increased PMA-induced VCAM-1 expression and mitochondrial ROS production. Midazolam, TSPO ligands, inhibited PMA-induced VCAM-1 and mitochondrial ROS production in endothelial cells. These results suggest that mitochondrial TSPO can inhibit PMA-induced endothelial inflammation via suppression of VCAM-1 and mitochondrial ROS production in endothelial cells.

Key Words: TSPO, ROS, VCAM-1, Mitochondria, Vascular endothelium

P05-10

O-GlcNAcylation-induced GPAT Expression is Critical for Anti-apoptosis under Hypoxia

Hyun Jik Lee, Eun Ju Song, Ki Hoon Lee, Dah Ihm Kim , Jeong Yeon Kim, So Hee Ko, Gee Euhn Choi, Ji Young Oh, Ho Jae Han*

Department of Veterinary Physiology, BK21 PLUS Creative Research Center and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, 08826, Seoul, Korea

Oxygen signaling is critical for stem cell regulation, and oxidative stress-induced stem cell apoptosis decreases the efficiency of stem cell therapy. Hypoxia activates O-linked β-N-acetyl glucosaminylation (O-GlcNAcylation) of stem cells, which contributes to regulation of cellular metabolism as well as cell fate. Our study investigated the role of O-GlcNAcylation via glucosamine in the protection of hypoxia-induced apoptosis of mouse embryonic stem cells (mESCs). Hypoxia increased mESCs apoptosis in a time-dependent manner. Moreover, hypoxia also slightly increased the O-GlcNAc level. Glucosamine treatment further enhanced the O-GlcNAc level and prevented hypoxia-induced mESC apoptosis, which was suppressed by O-GlcNAc transferase inhibitors. In addition, hypoxia regulated several lipid metabolic enzymes while glucosamine increased expression of glycerol-3-phophate acyltransferase-1 (GPAT1), a lipid metabolic enzyme producing

lysophosphatidic acid (LPA). In addition, glucosamine increased O-GlcNAcylation of Sp1, which subsequently leads to Sp1 nuclear translocation and GPAT1 expression. Silencing of GPAT1 by gpat1 siRNA transfection reduced glucosamine-mediated anti-apoptosis in mESCs and reduced mammalian target of rapamycin (mTOR) phosphorylation. Indeed, LPA prevented mESCs from undergoing hypoxia-induced apoptosis and increased phosphorylation of mTOR and its substrates (S6K1 and 4EBP1). Moreover, mTOR inactivation by rapamycin (mTOR inhibitor) increased pro-apoptotic proteins expressions and mESC apoptosis. Furthermore, transplantation of non-targeting siRNA and glucosamine-treated mESCs increased cell survival and inhibited flap necrosis in mouse skin flap model. Conversely, silencing of GPAT1 expression reversed those glucosamine effects. In conclusion, enhancing O-GlcNAcylation of Sp1 by glucosamine stimulates GPAT1 expression, which leads to inhibition of hypoxia-induced mESC apoptosis via mTOR activation.
Key Words: O-GlcNAcylation, GPAT1, embryonic stem cells

P05-11

Amyloid β-Induced abnormal Autophagolysosome formation leading defective mitochondrial accumulation causes neuronal cell death

Dah Ihm Kim, Jung Min Ryu, Ki Hoon Lee, Jeong Yeon Kim, Gee Euhn Choi, Ing Ing Chai, Ho Jae Han

Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea

Recent studies support that mitochondria became a significant controller affecting the cell fates in aging related diseases such as Alzheimer's disease (AD). Mitochondria are dynamic organelles changing their morphology which is tightly related to mitochondrial function. Mitochondrial dysfunction is one of causative factor inducing neuronal cell death. Therefore, we investigated the effect of Aβ on mitochondrial dynamics regulating cellular functions and maturation of autophagy pathway. In this report, Aβ induced neuronal cell death in a dose-dependent manner, which was accompanied with increase in caspase-9 and -3 activities. Consistently, the length of mitochondria are highly dependent on the concentration of Aβ showing excessive mitochondrial fragmentation. Aβ increased the mRNA and protein expression of pro-fission protein, Drp1 and Fis1. Whereas, pro-fusion protein (Opa1 and Mfn2) did not significantly affected by Aβ. Furthermore, we found abnormal autophagolysosome formation using Acridine orange staining which has been decreased the red-to-green ratio. These results indicate that lysosomal degradation of dysfunctional mitochondria is lacking. Taken together, the disruption of autophagosome-lysosome fusion leads to accumulation of damaged mitochondria causing neuronal cell death.
Key Words: Amyloid β, mitochondria, Fis1, neuronal cell death

P05-12

Aβ-Induced mTOR Activation is Important for Tau hyperphosphorylation through Regulation of Expression and Autophagy of CDK2 and CDK4

Ki Hoon Lee, Jung Min Ryu, Dah Ihm Kim, Jeong Yeon Kim, Gee Euhn Choi, Ho Jae Han

Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea

Alzheimer's Disease (AD) is neurodegenerative disorder induced by Aβ resulting in Tau hyperphosphorylation, which is associated with neuronal cell apoptosis. As there has been no defined therapeutic target for AD, we investigated critical signaling pathway regulating Aβ-induced apoptosis in SK-N-MC. In our results, Aβ increased cleaved caspase 3 expression and apoptosis in a dose- dependent manner. We also demonstrated that Aβ increased ROS generation, followed by HIF-1α expression. In addition, Aβ stimulated mTOR phosphorylation, which is inhibited by HIF-1α siRNA transfection. Our results show that Aβ-induced mTOR activation increased CDK2 and CDK4 mRNA expressions, but not CDK1. Moreover, we confirmed that inhibition of autophagy by Aβ-induced mTOR activation increased CDK2 and CDK4 accumulation, which are blocked by autophagy inducer. mTOR-induced CDKs upregulation contributed to Tau hyperphosphorylation. Furthermore, we confirmed that both mTOR and CDKs inhibition and mTOR-independent autophagy induction prevented Aβ-induced apoptosis. Especially, mTOR inhibition had more protective effect than either CDKs inhibition or autophagy induction. In conclusion, we demonstrated that Aβ-activated mTOR by HIF-1α regulated both transcription and autophagy of CDK2 and CDK4 which are essential for tau hyperphosphorylation leading neuronal cell apoptosis.
Key Words: Amyloid beta, mTOR, CDK, Alzheimer's Disease

P05-13

Essential Role of Vibrio (V.) vulnificus VvpE in Promoting the Pyroptosis of Intestinal Epithelial Cells

Sei-Jung Lee[1], Hyeon Su Lim[1], Eun Ju Song[1], Jun Sung Kim[1], Kyung Ku Jang[2], Sang Ho Choi[2], Ho Jae Han[1,*]

[1]Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 08826, Korea, [2]National Research Laboratory of Molecular Microbiology and Toxicology, Department of Agricultural Biotechnology, and Center for Food Safety and Toxicology, Seoul National University, Seoul 08826, Korea

In the present study, we investigate the cellular mechanism of Vibrio (V.) vulnificus, VvpE with regard to host cell death and the inflammatory response of human INT-407 cells. The recombinant protein (r) VvpE caused cytotoxicity mainly via necrosis coupled with IL-1β production. The necrotic cell death induced by rVvpE is highly susceptible to the knockdown of ANXA2 (full?) and the sequestration of membrane cholesterol. We found that rVvpE induces the recruitment of NOX2 (full?) and NCF1 (full?) into membrane lipid rafts coupled with ANXA2 to facilitate the production of ROS (Full?) and phosphorylation of NF-κB. rVvpE induced hypomethylation and region-specific transcriptional occupancy by NF-κB in the IL-1β promoter and has ability to induce pyroptosis via NLRP3 inflammasome. In a mouse model, the mutation of the vvpE gene negated the pro-inflammatory responses and maintained the physiological levels of the proliferation and migration of enterocytes. These results demonstrate that VvpE induces the hypomethylation of the IL-1β promoter and the transcriptional regulation of NF-κB through lipid raft-dependent ANXA2 recruitment and ROS signaling to promote IL-1β production in intestinal epithelial cells.

Key Words: VvpE, NF-κB, IL-1β, intestinal epithelial cells, ROS, Vibrio vulnificus

P05-14

EphB2-ephrinB2 signaling-induced Nanog expression is critical for maintaining the differentiation potential of umbilical cord blood derived mesenchymal stem cells

Young Hyun Jung, Sei-Jung Lee, Eun Ju Song, Hyeon Su Lim, Jun Sung Kim, Ho Jae Han*

Department of Veterinary Physiology, College of Veterinary Medicine, Research Institute for Veterinary Science, and BK21 PLUS Creative Veterinary Research Center, Seoul National University, Seoul 151-742, Korea

Eph (full name)/ephrin system as a possible regulator of stem cell functions is involved in cell-cell and cell-microenvironment interactions in the stem cell niche. Therefore, the present study investigated the role of EphB signaling as a regulator of maintaining MSCs (full name?) functions. We found that EphB2 and ephrinB2 are expressed mainly in umbilical cord blood derived MSCs (UCB-MSCs). Treatment with pre-clustered ephrinB2-Fc (0.5 μg/mL) induced the phosphorylation of EphB2 and increased Nanog expression known as one of the pluripotency genes in maintaining MSCs properties. Pre-clustered ephrinB2-Fc also induced phosphorylation of Akt (Ser 473 and Thr 308)/mammalian target of rapamycin (mTOR), and inhibition of Akt/mTOR attenuated EphB2-ephrinB2 signaling axis-mediated Nanog expression in UCB-MSCs. In addition, the differentiation potential of UCB-MSCs into adipogenic, chondrogenic, and osteoblastogenic lineages was reduced by EphB2 silencing. Notably, EphB2-facilitated Nanog expression contributed to increasing the expression of Dnmt1 and the Dnmt1-induced hypermethylation of p21 promoter. These findings identify the EphB2 signaling as a target of enhancing therapeutic benefits in regard to maintaining UCB-MSCs functions. In conclusion, the EphB2-ephrinB2 signaling regulates Nanog expression to support the differentiation potential of UCB-MSCs.
Key Words: Ephrin, EphB2, Stem cell niche, Umbilical cord blood derived mesenchymal stem cell, Nanog

P05-15

Cdo regulates surface expression of the Kir2.1 K+ channel in myoblast differentiation

Jewoo Koh[1,4,*], Young-Eun Leem[2,4,*], Hyeon-Ju Jeong[2,4,*], Hyun-Ji Kim[1,4,*], Kyungjin Kang[3,4], Jong-Sun Kang[2,4,#], Hana Cho[1,4,#]

[1]Department of Physiology, [2]Department of Molecular Cell Biology, [3]Department of Anatomy and Cell Biology, Sungkyunkwan University School of Medicine, [4]Samsung Biomedical Research Institute, Suwon, 440-746, Republic of Korea

Kir2.1-associated membrane hyperpolarization is required for myogenic differentiation. However the molecular regulatory mechanisms modulating Kir2.1 channel activities in early stage of myogenesis are largely unknown. A cell surface protein, Cdo functions as a component of multiprotein cell surface complexes to promote myogenesis. In this study, we investigate the crosstalk between Cdo and Kir2.1 channels in myoblast differentiation. Cdo depleted or deficient myoblasts show defects in differentiation without a clear correlation with Kir2.1 protein levels. Interestingly, Cdo-depleted or deficient myoblasts exhibit a declined Kir2.1 channel activity, correlating with decreased differentiation. Kir2.1 is coprecipitated with Cdo which correlated with the increased membrane-resident Kir2.1. Cdo is known to be associated with various signaling pathways including p38MAPK, Akt and ERK/Stim1/Ca2+ pathways leading to activation of MyoD, NFATc3 and downstream target genes in myoblast differentiation. We found that the inward rectifying K+ currents induced upon differentiation were sensitive to p38MAPK inhibition and it seems to be due to Kir2.1 surface trafficking. These data suggest that Cdo-mediated signaling might be involved in regulation of Kir2.1 trafficking and activation.
Key Words: Kir2.1, Cdo, p38MAPK

P05-16

Anti-adhesive activity of the ethanol extracts of Ulmus davidiana var japonica in cultured endothelial cells

Ki Mo Lee[1,*], Hee Kyoung Joo[1,*], Yu Ran Lee[1], Myoung Soo Park[2], Gun Kang[1], Sunga Choi[1], Kwon Ho Lee[3], Byeong Hwa Jeon[1]

[1]Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Republic of KOREA, [3]Department of Physical Therapy, Joongbu University, 201 Daehak-Ro, Chubu-Myeon, Geumsan-Gun, Chungnam 312-702, Korea

Background/Objectives: Ulmus davidiana var japonica Rehder (UD) has long been used for traditional folk medicine. This study is designed to investigate the anti-adhesive activity of UD and its underlying mechanisms in the cultured endothelial cells. **Subjects/Methods:** The dried root bark of UD was extracted with 80% ethanol. The anti-adhesive activity of the ethanol extracts of UD (UDE) was investigated in cultured human umbilical vein endothelial cells (HUVEC) and HEK293 cells with stable transfected with VCAM-1-luc. The anti-adhesive activity was evaluated with monocyte-endothelial cell adhesion and vascular cell adhesion molecule-1 (VCAM-1) expression. Promotor activity of VCAM-1 is visualized with in vivo optical imaging system. **Results:** The exposure of UDE (3~30 μg/ml) for 24 h showed no cytotoxicity in HUVECs. UDE (3~30 μg/ml) treatment significantly inhibited TNF-a-induced monocyte adhesion and VCAM-1 expression in HUVECs. Luciferase activity of VCAM-1 promotor is increased by the TNF-α, its activity was inhibited with UDE. Also, UDE inhibited TNF-a-induced ROS generation, NF-κB nuclear translocation, and IκBα degradation in HUVECs. **Conclusion:** Our results indicate that the UDE inhibited TNF-α-induced monocyte adhesion in endothelial cells, suggesting that UD may be potentially useful to vascular endothelial inflammation.
Key Words: Ulmus davidiana var japonica, endothelial cells, vascular cell adhesion molecule-1, monocyte adhesion

P05-17

FBXO11 represses cellular response to hypoxia by destabilizing hypoxia-inducible factor-1α mRNA

Uk-Il Ju[1], Jong-Wan Park[1,2], Hyoung-Sook Park[1], Sang Jeong Kim[1,2,3], Yang-Sook Chun[1,2,3]

[1]Department of Biomedical Sciences, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of

Korea [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea [3]Department of Physiology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul 110-799, Republic of Korea

The transcriptional factor hypoxia-inducible factor-1α (HIF-1α) is induced under hypoxia and plays crucial roles in cancer progression and angiogenesis. Protein arginine methyltransferases (PRMTs), 11 isoforms of which have been identified so far, modulates the functions of diverse proteins by catalyzing arginine methylation in post-translational level. PRMT9 (alternatively named FBXO11) and PRMT11 (FBXO10) are expected to have the E3 ubiquitin ligase activity through their F-box domains as well as the methyltrasferase activity. Given previous studies examining roles of 8 PRMT isoforms (PRMT1-8) in the HIF-1 signaling pathway, PRMT1 and PRMT5 were demonstrated to regulate HIF-1α expression in opposite ways. We herein examined if FBXO10 and FBXO11 participate in the HIF-1 signaling pathway. Consequently, the siRNA-mediated knockdown of FBXO11 facilitated HIF-1α expression in various cancer cells and HIF-1-driven gene expressions, but the FBXO10 knockdown did not. Mechanistically, FBXO11 was found to inhibit de novo synthesis of HIF-1α protein by destabilizing HIF-1α mRNA. Since a FBXO11 mutant lacking F-box failed to reverse the HIF-1α expression by FBXO11 knockdown, the FBXO11 regulation of HIF-1α may be attributed to the ubiquitination of some proteins controlling HIF-1α mRNA stability. Considering the oncogenic roles of HIF-1α, FBXO11 is suggested to act as a tumor suppressor andalso to be a potential target for cancer therapy.

Key Words: FBXO11, Hypoxic signaling, HIF-1a, mRNA stability

P05-18

Fatty acid modulates nitric oxide synthase activity in hypertensive rat atrium

Yu Na WU, Ji Hyun Jang, Sung Joon Kim, Yin Hua Zhang

Department of Physiology & Biomedical Sciences, Seoul National University, College of Medicine, Seoul, Republic of Korea

Atrial fibrillation (AF) is the most common cardiac arrhythmias. Hypertension (HTN) and hyperlipidemia (obesity) are the major risk factors for the initiation and the recurrence of AF. In cardiac myocytes, nitric oxide (NO) from constitutive nitric oxide synthases (eNOS & nNOS) is well established to prevent arrhythmogenesis and contractile dysfunction under stress. So far, whether and how fatty acids affect cardiac NOS activities which are involved in the pathogenesis of AF is not known. Since high plasma level of palmitic acid (PA), one of the saturated fatty acids, is associated with increased incidence of AF, we aim to investigate 1) whether PA supplementation affects the protein expressions of eNOS and nNOS in rat atrial myocardium; 2) whether PA regulates phosphorylations of nNOS and eNOS; 3) whether total protein or phosphorylation of NOSs are changed by PA in angiotensin II-induced hypertension. Our result showed that PA (100 μM, 1 hr) failed to affect total protein levels of eNOS in left atrial (LA) tissue in both groups. In contrast, PA tended to increase eNOS-Ser^{1177}in sham rats but significantly reduced it in HTN. This is in contrast to the effect of PA on ventricular myocytes, where PA did not change the total amount of eNOS protein or its phosphorylation at Ser^{1177}. Furthermore, PA did not affect total protein level or Ser^{1417} of nNOS in sham. Nevertheless, despite that nNOS protein expression was increased in HTN, nNOS-Ser^{1417} was significantly reduced by PA, which is in contrast to the fact that PA increased nNOS-Ser1417 in LV myocytes in hypertension. Taken together, our results show that PA reduces phosphorylations of eNOS and nNOS in hypertension. Reduced NO bioavailability may underlie myocardial arrhythmias induced by PA.

Key Words: left atrium, left ventricular myocytes, NOS, palmitic acid.

P05-19

TGFBIp targeting peptide evaluation

Haeuk Jung[1,3,4], Hye-Nam Son[2], Soyoun Kim[2,4], In-San Kim[5], Ha-Jeong Kim[1,3,4]

[1]Department of Physiology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea [2]Department of Biochemistry and Cell Biology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea [3]BK21 Plus KNU Biomedical Convergence Program, School of medicine Kyungpook National University, Daegu 700-842, Korea [4]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea [5]Center for Theragnosis, Biomedical Research Institute, Korea Institute of Science and Technology, Seoul, Republic of Korea

TGFBIp (full name?) is an extracellular matrix protein, and secreted from several cells, including fibroblasts. It supports cell adhesion, migration, proliferation of various cells. It is also reported that TGFBIp is increased in the tumor. We hypothesized that TGFBIp is a biomarker of tumor, and if it is so, we would like to detect TGFBIp in tumor using TGFBIp targeting peptide. We identified 5R18 peptide by T7 DMID phage display. The phage, we used, is DMID (designed modular immunodiagnostics) protein scaffold fused into the capsid. Seven random peptide sequences from a peptide library were displayed in the exposed loop region of the protein scaffold for specific selection of target protein. We found 4 candidates of TGFBIp binding peptides by T7 DMID phage display and validated the binding specificity using ELISA. One of them is 5R18. We cloned and purified DMID-5R18 in E.coli expression system and tested its specificity against TGFBIp. We provided an evidence that 5R18 recognizes TGFBIp in dose-dependent manner. Its binding is mediated by the fasciclin domain in TGFBIp. It specifically binds to Fastatin, 4th fasciclin domain of 4 fasciclin domains in TGFBIp. We will check whether 5R18 detects TGFBIp in tumor site in vivo model. If it works well, we will develop it as a diagnostic marker of tumor.

Key Words: TGFBIp, Designed modular immunodiagnostics, T7 phage display, tumor

P05-20(O-4)

The regulatory role of phosphodiesterase 4 inhibitor rolipram in lipopolysaccharides-induced signaling in submandibular glands

Dong Un Lee, Wanhee Suk, Jeong Hee Hong

Department of Physiology, College of Medicine, Gachon University, 191 Hambakmeoro, Yeonsu-gu, Incheon, 406-799, South Korea

Innate immunity is triggered after microbial invasion in response to conserved structures present in the groups of microorganism such as lipopolysaccharides (LPS). Salivary glands were exposed to LPS in bacterial invasion and induced inflammatory signals. However, it is unclear LPS-induced intracellular Ca2+ signaling and reactive oxygen species (ROS) formation in salivary glands. Additionally, we elucidate the anti-oxidative role of rolipram as phosphodiesterase 4 inhibitor

in LPS-induced signaling. Primarily isolated mouse submandibular glands (SMG) and human salivary submandibular glands cell line (HSG) were measured Ca2+ signals by fura-2, AM fluorescence imaging technique and the levels of ROS production by quantification of DCFDA fluorescence. Our results revealed that LPS induced Ca2+ signaling and ROS production in SMG, which expressed toll-like receptor 4. The treatment of rolipram blocked LPS-induced Ca2+ increase and ROS production in SMG. Application of histamine as an inflammatory agonist mediated Ca2+ increase and ROS production, also attenuated by rolipram in both SMG and HSG cells. Our study showed that inflammatory signals can be diminished by rolipram and may provide potential therapeutic strategy for LPS-induced inflammation or other inflammatory signals in salivary glands.
Key Words: rolipram, lippolysaccharides, calcium signaling, reactive oxygen species, submandibular glands

P05-21

TRPC6 as a critical regulator in osteoclastogenesis

Jung Yun Kang, Yu-Mi Yang, Dong Min Shin

Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea

The transient receptor potential canonical type 6 (TRPC6) channel is a Ca^{2+}-permeable nonselective cation channel and widely expressed in brain, smooth muscle containing tissues, kidney, and so on. TRPC6 channels are permeable for Ca^{2+} than for Na^{+} and are directly activated by diacylglycerol and regulated by specific tyrosine or serine phosphorylation. However, the role of TRPC6 in Ca^{2+} signaling during osteoclastogenesis is not well known. In the present work, we investigated the functional role of TRPC6 channel in bone metabolism using TRPC6 knockout ($TRPC6^{-/-}$) mice. Depletion of TRPC6 markedly decreased the bone density of the tibias. However, TRPC6 deletion did not affect osteoblast formation. RANKL-induced intracellular Ca^{2+} oscillations were generated 24 h after RANKL treatment in the $TRPC6^{-/-}$ bone marrow-derived macrophages (BMMs). Finally, RANKL treatment of $TRPC6^{-/-}$ BMMs significantly increased induction of multinucleated cell formation and bone resorption. These results suggest that TRPC6 is a critical negative regulator in osteoclasts differentiation.
Key Words: TRP channel, Calcium signaling, osteoclastogenesis, RANKL, bone

P05-22

Homer2/3 modulate RANKL-induced NFATc1, osteoclastogenesis and bone metabolism

Yu-Mi Yang, Aran Son, Jung Yun Kang, Dong Min Shin

Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea

Ca^{2+} signaling and NFATc1 activation are essential for RANKL-induced osteoclastogenesis through the induction of Ca^{2+} oscillation, calcineurin activation, and translocation of NFATc1 into the nucleus. Homer proteins are scaffold proteins that have been proposed to modulate multiple Ca^{2+} signaling channels and proteins, including inositol 1,4,5-triphosphate receptors, ryanodine receptors, transient receptor potential channels, and NFAT family of transcription factors in skeletal muscle myocytes and T cells. However, the role of Homer proteins in Ca^{2+} signaling during osteoclast differentiation is not known. In the present work, we investigated the role of Homer2 and Homer3 in bone metabolism using *Homer2/Homer3* (*Homer2/3*) double-knockout (DKO) mice. Deletion of *Homer2/3* markedly decreased the bone density of the tibias, resulting in bone erosion. However, *Homer2/3* deletion did not affect osteoblast formation and RANKL-induced Ca^{2+} oscillation. Rather, 48 hours RANKL treatment of *Homer2/3* DKO bone marrow-derived monocytes/macrophages (BMMs) facilitated greatly osteoclast differentiation through increased NFATc1 expression and translocation of NFATc1 into the nucleus. Notably, the interaction of Homer proteins with NFATc1 was inhibited by RANKL treatment, but restored by cyclosporine A treatment to inhibit calcineurin. Finally, RANKL treatment of *Homer2/3* DKO BMMs significantly increased ~3.0-fold induction of multinucleated cells formation. These findings suggest that Homer2/3 regulate NFATc1 function by interacting with NFATc1 to sequester it in the cytosol and thus modulate the NFATc1 pathway and RANKL-induced osteoclastogenesis and bone metabolism.
Key Words: Homer protein, bone metabolism, osteoclastogenesis, Calcium signaling

P05-23

Osmo-mechanosensitive TRP channels regulate Ca^{2+}-mediated RANKL expression in mouse osteoblastic cells

Yu-Mi Yang[1], Jung Yun Kang[1], Aran Son[1], Hyo Jin Yang[2], Dong Min Shin[1]

[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, [2]Division of AIDS, Center for Immunopathology, Korea National Institute of Health, Chengju 28160, Korea

Mechanical stress plays an important role in the regulation of bone turnover. However, the intracellular mechanisms of mechanical stress under osteoblast differentiation and proliferation are not well understood. In this study, we investigated the effects of osmo-mechanosensitive transient receptor potential (TRP) channels-induced calcium signaling in primary mouse osteoblasts and MC3T3-E1 cells. Hypotonic stress induced significant increases of RANKL mRNA expression but not OPG. In addition, hypotonic stress-induced increases of intracellular calcium concentration ($[Ca^{2+}]_i$) and RANKL expression persisted in the presence of non-specific Ca^{2+} channel blockers or Ca^{2+}-free bath solution. Furthermore, we examined hypotonic stress-induced effects on agonists and antagonists of osmo-mechanosensitive TRP channels in order to determine the cellular mechanism of hypotonic stress-mediated increases on $[Ca^{2+}]_i$ and RANKL. We found that antagonists of TRPV4 and TRPM3 decreased hypotonic stress-mediated increases on $[Ca^{2+}]_i$ and protein expression levels of RANKL and NFATc1. We also identified that hypotonic stress-induced effects reduced by the genetic suppression of TRPV4 and TRPM3. Taken together, our results indicate that hypotonic stress activates the expression of RANKL and NFATc1 by $[Ca^{2+}]_i$ increases through TRPV4 and TRPM3 in osteoblasts. These effects may be important for the differentiation and proliferation of bone cells on bone remodeling that are mediated via mechanosensitive TRP channels.
Key Words: TRP channel, osteoblast, mechanical stress, Calcium signaling, RANKL

P05-24

Endothelin stimulates inflammatory bone loss in periodontitis

Sue Young Oh[1], So Yun Lee[1,2], Ga-Yeon Son[1], Inik Chang[1], Dong Min Shin[1,2]

Department of [1]Oral Biology, [2]BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, South Korea

Periodontitis is a very common oral inflammatory disease and results in the destruction of supporting connective and osseous tissues of tooth. Although the etiology is still unclear, Gram-negative *Porphyromonas gingivalis* in subgingival pockets has been thought as one of the major etiologic agent. It has been known that endothelin is involved in the occurrence and progress of various inflammatory process and diseases. However, functional roles of endothelin in periodontitis are still unclear. In this study, we explored cellular and molecular mechanisms of ET-1 actions in periodontitis using human gingival epithelial cells (hGECs) and human gingival fibroblasts (hGFs). ET-1 and ET_A, but not ET_B were abundantly expressed in both hGECs and hGFs. Stimulation of hGECs with *P. gingivalis* LPS increased the expression of ET-1 and ETA suggesting the activation of endothelin signaling pathway. Production of pro-inflammatory cytokines, IL-1β, IL-6, and IL-8 was significantly enhanced by exogenous ET-1 treatment in both hGECs and hGFs. Moreover, ET-1 augmented the number of multinucleated osteoclasts implicating the acceleration of alveolar bone loss. Together, our study showed that activation of ET-1/ET_A signaling pathway by *P. gingivalis* may exacerbate periodontitis by stimulating production of pro-inflammatory cytokines in hGECs and hGFs and provoking the alveolar bone loss through the increment of multinucleated osteoclasts at the same time. To directly examine the endothelin antagonism as a potential therapeutic approach for periodontitis, the inhibitors for ET receptors will be applied to the animal periodontitis model. Infiltration of immune cells, production of pro-inflammatory cytokines, and alveolar bone loss will be evaluated.

Key Words: Endothelin, inflammation, periodontitis, human gingival epithelial cells

P05-25(O-15)

Disarrangement of regulated exocytosis in TRPML1 knock-out mice

Soonhong Park[1], Min Seuk Kim[3], Shmuel Muallem[2], Dong Min Shin[1]

[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul 03722, Korea, [2]Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Bethesda, MD 20892, USA, [3]Department of Physiology, Lab of Oral Biology, College of Dentistry, Wonkwang University, Iksan, Jeonbuk 54538, Korea

TRPML1 is expressed in late endosomes and lysosomes and control lysosomal pH. Deletion or loss-of-functional mutations in TRPML1 cause mucolipidosis type IV that is characterized by a psychomotor retardation, corneal opacity, retinal degeneration, and achlorhydria. In vitro studies of constitutive membrane trafficking concluded that TRPML1 plays a role in delivery or fusion of late endosomes and lysosomes, resulting in accumulation of material in the lysosomes and induction of autophagy. However, the role of TRPML1 in regulated exocytosis is not known. Our previous work with the $Trpml1^{-/-}$ mice showed that $Trpml1^{-/-}$ mice recapitulate many features of the human disease, including neuronal degeneration and achlorhydria. Achlorhydria was associated with a permanent stimulated morphology of the parietal cells, suggesting that TRPML1 may have role in regulated exocytosis, perhaps in membrane retrieval. To further explore the role of TRPML1 in regulated exocytosis we are studying salivary gland and pancreatic exocytosis in vivo and in isolated acini. In the present study, we found amylase secretion was increased in parotid gland in vivo and in isolated acini also. And in the isolated acini, the agonist-related amylase secretion was increased in time-dependent and dose-dependent manner. Interestingly, TEM (full name?) images both of pancreas and parotid gland show enlarged vesicles. Some of the enlarged vesicle shows fusion of the lysosome and vesicles. We found acid phosphatase activity, it is lysosomal enzyme located in the lumen of lysosome, was increased in the saliva and pancreatic fluids. Also, we found enlarged vesicles in neuronal synapse and glutamate release in the synapse was increased in $Trpml1^{-/-}$ cells both resting-state and activating-state. However, in the similar lysosomal storage disease model, such as Niemann-Pick type C1 mice model, have not represented this phenotype. From these results, we suggest that Trpml1 deletion may relate with exocytosis of the secreting vesicles or related with fusion of the vesicles with the lysosome.

Key Words: TRP channel, mucolipidosis, exocytosis, lysosome

P05-26

Calcium ion regulates WNK/OSR1/NKCC1 pathway in HSG cell-line

Soonhong Park[1], Sang Kyun Ku[2], Hye Won Ji[1], Jong-Hoon Choi[2], Dong Min Shin[1]

[1]Department of Oral Biology, BK21 PLUS Project and [2]Department of Oral Medicine, Yonsei University College of Dentistry, Seoul 03722, Korea

Cell volume homeostasis is important to cell survival. Our body strictly maintains the osmosis in the body fluids for keeping the cell volume. Sodium-Potassium-Chloride co-transporter 1 (NKCC1) is the key components of volume maintaining process by using the chloride ion transport. When cells are located in the hyperosmotic condition, chloride ion is getting into the cytosol, and when cells are located in the hypoosmotic condition, chloride ion is coming out to the extracellular fluids, and cell reduce cytosol osmotic concentration. In the present study, we found that HSG cell-line expressed molecules participated in WNK-OSR1-NKCC pathway, such as Wnk1, Wnk4, OSR1, SPAK, and NKCC1. HSG cell-line showed intraceullular Ca^{2+} concentration ($[Ca^{2+}]_i$) increase in the hypotonic stimulation, and it was synchronized with the phosphorylation of OSR1. Interestingly, when we inhibited hypotonic-induced $[Ca^{2+}]_i$ increase with non-specific Ca^{2+} channel blockers such as 2-aminoethoxydiphenyl borate, gadolinium, and lanthanum, phosphorylated OSR1 was also diminished. Moreover, cyclopiazonic acid-induced passive $[Ca^{2+}]_i$ elevation evoked to phosphorylation of OSR1, and amount of phosphorylated OSR1 was decreased when treated with BAPTA, a Ca^{2+} chelator by using human salivary gland (HSG) cell-line as a model. Finally, through this process, NKCC1 activity also reduced to maintain the cell volume in the HSG cell-line. These results indicate that Ca^{2+} may affect to regulate WNK-OSR1 pathway and NKCC1 activity in the HSG cell-line, and this is the first demonstration that indicates upstream Ca^{2+} regulation of WNK-OSR1 pathway in the intact cell.

Key Words: Calcium signaling, NKCC1, WNK/OSR

P05-27

Hypotonic stress induces RANKL via transient receptor potential melastatin 3 (TRPM3) and vaniloid 4 (TRPV4) in human PDL cells

Ga-Yeon Son, Dong Min Shin

Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

Bone remodeling occurs in response to various types of mechanical stress. The periodontal ligament (PDL) plays an important role in mechanical stress-mediated alveolar bone remodeling. However, the underlying mechanism at the cellular level has not been extensively studied. In this study, we investigated the effect of shear stress on the expression of bone remodeling factors, including receptor activator of nuclear factor-kappa B (NF-κB) ligand (RANKL) and osteoprotegerin (OPG), and its upstream signaling pathway in primary human PDL cells. We applied hypotonic stress to reproduce shear stress to PDL cells. Hypotonic stress induced the mRNA and protein expression of RANKL but not OPG. It also increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Extracellular Ca^{2+} depletion and non-specific plasma membrane Ca2+ channel blockers completely inhibited the increase in both $[Ca^{2+}]_i$ and RANKL mRNA expression. We identified the expression and activation of transient receptor potential melastatin 3 (TRPM3) and vaniloid 4 (TRPV4) channels in PDL cells. Pregnenolone sulfate (PS) and 4α-phorbol 12, 13-didecanoate (4α-PDD), which are agonists of TRPM3 and TRPV4, augmented Ca^{2+} influx and RANKL mRNA expression. Both pharmacological (2-aminoethoxydiphenyl borate [2-APB], ruthenium red [RR], ononetin [Ono], and HC 067047 [HC]) and genetic (small interfering RNA [siRNA]) inhibitors of TRPM3 and TRPV4 reduced the hypotonic stress-mediated increase in $[Ca^{2+}]_i$ and RANKL mRNA expression. Our study shows that hypotonic stress induced RANKL mRNA expression via TRPM3- and TRPV4-mediated extracellular Ca^{2+} influx and RANKL expression. This signaling pathway in PDL cells may play a critical role in mechanical stress-mediated alveolar bone remodeling.
Key Words: Hypotonic stress, calcium signaling, RANKL, TRP channels, human periodontal ligament cells

P05-28

Induction of IL-6 and IL-8 by activation of thermosensitive TRP channels in human PDL cells

Ga-Yeon Son, Dong Min Shin

Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

The oral cavity is often exposed to not only diverse external pathogens but also dramatic temperature changes. In this study, we investigated the effect of thermal stress on PDL cells with a focus on the inflammatory responses and bone homeostasis. The PDL cells were isolated from healthy premolar extracted for orthodontic reasons, and examined using intracellular calcium concentration ($[Ca^{2+}]_i$) measurement and reverse transcription-polymerase chain reaction for pro-inflammatory cytokines and bone remodeling mediators. We detected the expression of thermosensitive transient receptor potential (TRP) channels, such as TRPV1, TRPV2, TRPV3, TRPM8, and TRPA1. Functional activation of the channels by thermal stress and their specific agonists increased $[Ca^{2+}]_i$ and interleukin (IL)-6 and IL-8 mRNA expression. A selective Ca^{2+} chelator, BAPTA-AM, prevented TRP channel agonists-mediated IL-6 and IL-8 induction. Unlike pro-inflammatory cytokines, the expression of bone remodeling mediators, including receptor activator of nuclear factor-kappa B ligand and osteoprotegerin, was not altered by treatment with TRP channel agonists. The activation of thermosensitive TRP channels induced IL-6 and IL-8 expression by increasing $[Ca^{2+}]_i$ in human PDL cells. Therefore, thermal stress may play a critical role in the inflammatory responses of PDL cells.
Key Words: Calcium signaling, human periodontal ligament cells, TRP channels, cytokines

P05-29

Bacterial PAMPs and allergens trigger increase in $[Ca^{2+}]_i$-induced cytokine expression in human PDL fibroblasts

Ga-Yeon Son, Dong Min Shin

Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

An oral environment is constantly exposed to environmental factors and microorganisms. The periodontal ligament (PDL) fibroblasts within this environment are subject to bacterial infection and allergic reaction. However, how these condition affects PDL fibroblasts has yet to be elucidated. PDL fibroblasts were isolated from healthy donors. We examined using reverse transcription-polymerase chain reaction and measuring the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). This study investigated the receptors activated by exogenous bacterial pathogens (Lipopolysaccharide and peptidoglycan) and allergens (German cockroach extract and house dust mite) as well as these pathogenic mediators-induced effects on the intracellular Ca^{2+} signaling in human PDL fibroblasts. Moreover, we evaluated the expression of pro-inflammatory cytokines (interleukin (IL)-1β, IL-6, and IL-8) and bone remodeling mediators (receptor activator of NF-κB ligand and osteoprotegerin) and intracellular Ca^{2+}-involved effect. Bacterial pathogens and allergic mediators induced increased expression of pro-inflammatory cytokines, and these results are dependent on intracellular Ca^{2+}. However, bacterial pathogens and allergic mediators did not lead to increased expression of bone remodeling mediators, except lipopolysaccharide-induced effect on receptor activator of NF-κB ligand expression. These experiments provide evidence that a pathogens and allergens-induced increase in $[Ca^{2+}]_i$ affects the inflammatory response in human PDL fibroblasts.
Key Words: Calcium signaling, human periodontal ligament fibroblasts, inflammation

P05-30

Corn Silk Extract Prevents Carrageenan-Induced Inflammatory Edema by Suppressing Expression of P-Selectin Glycoprotein Ligand-1

Han Na Choi[1], Yong Hwan Kim[1], Soo Jin Kim[1], Yun A Kim[2], Byeong Hwa Jeon[1], Hyun Woo Kim[1], Dong Woon Kim[2], Sang Do Lee[1]

Department of [1]Physiology, Departmeent of [2]Anatomy, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

Corn silk extract (CSE) has been used as traditional medicine for edema, cystitis, gout, kidney stones, nephritis and prostatitis. Although CSE was shown to be effective in oxidative stress, inflammation and diabetes, preventive effect of CSE is not known well. So, we have investigated

the preventive effect of CSE in carrageenan-induced inflammatory edema. CSE administered orally for a week not only suppressed initial formation of edema but also enhanced recovery through inhibiting infiltration of immune cells. Pretreatment of CSE to human umbilical vein endothelial cells (HUVECs) for a day has shown no effect on the monocyte-endothelial adhesion. However, pretreatment of CSE to THP-1 cells inhibited adhesion between monocytes and endothelial cells. Expression of lymphocyte function-associated antigen-1 (LFA-1) and very late antigen-4 (VLA-4) on THP-1 cells was not changed by CSE. Whereas P-selectin glycoprotein ligand-1 (PSGL-1), involved in affinity between LFA-1 and intercellular adhesion molecule-1 (ICAM-1), was significantly reduced by CSE. These results suggest that CSE prevents inflammatory edema through the suppression of PSGL-1 expression on monocytes.
Key Words: Corn silk, Inflammation, Edema, Adhesion, PSGL-1

P05-31

Airborne allergens induce protease activated receptor-2-mediated production of inflammatory cytokines in human gingival epithelium

Ga-Yeon Son, Dong Min Shin

Department of Oral Biology, Yonsei University College of Dentistry, Seoul 03722, Korea

In reaching the airways inhaled allergens pass through and contact with the oral mucosa. Although they are often responsible for initiating asthmatic attacks, it is unknown whether airborne allergens can also trigger chronic inflammation of gingival epithelial cells leading to chronic periodontitis. In this study, we investigated the inflammatory responses of human gingival epithelial cells (HGECs) to airborne allergens, particularly German cockroach extract (GCE) with a focus on calcium signaling. HGECs isolated from healthy donors were stimulated with GCE. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with Fura-2-acetoxymethyl ester (Fura-2/AM) staining. Expression of inflammatory cytokines interleukin (IL)-8, IL-1β, IL-6, and NOD-like receptor family, pyridine domain-containing (NLRP) 3 was analyzed using reverse transcription-polymerase chain reaction (RT-PCR). GCE promoted increase in the $[Ca^{2+}]_i$ in a dose-dependent manner. Depletion of endoplasmic reticulum (ER) Ca^{2+} by the ER Ca^{2+} ATPase inhibitor thapsigargin (Tg) but not the depletion of extracellular Ca^{2+} abolished the GCE-induced increase in $[Ca^{2+}]_i$. Treatment of phospholipase C (PLC) inhibitor (U73122) or 1,4,5-trisinositolphosphate (IP3) receptor inhibitor (2-APB) also prevented GCE-induced increase in $[Ca^{2+}]_i$. Protease activated receptor (PAR)-2 activation mainly mediated the GCE-induced increase in $[Ca^{2+}]_i$ and enhanced the expression of IL-8, NLRP3, IL-1β, and IL-6 in HGECs. GCE activates PAR-2, which can induce PLC/IP3-dependent Ca^{2+} signaling pathway, ultimately triggering inflammation via the production of pro-inflammatory cytokines in HGECs.
Key Words: Inflammation, calcium signaling, PAR2, human gingival epithelial cells

P05-32

Macrophages programmed by apoptotic cells inhibit epithelial-mesenchymal transition in lung alveolar epithelial cells via PGE2, PGD2, and HGF

Young-So Yoon, Ye-Ji Lee, Jihee Lee

Department of Physiology, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans Uniersity, Seoul, 158-710, Korea

Apoptotic cell clearance results in the release of growth factors and the action of signaling molecules involved in tissue homeostasis maintenance. Previously, we demonstrated that macrophages exposed to apoptotic cells counteract TGF-β1-induced epithelial-mesenchymal transition (EMT) in lung epithelial cells. Here, we investigated how macrophages programmed by apoptotic cells inhibit the TGF-β1-induced EMT process in lung alveolar epithelial cells. Exposure of macrophages to cyclooxygenase (COX-2) inhibitors (NS-398 and COX-2 siRNA) or RhoA/Rho kinase inhibitors (Y-27632 and RhoA siRNA) and LA-4 cells to antagonists of prostaglandin E2 (PGE2) receptor EP4 [AH-23848]), PGD2 receptors (DP1 [BW-A868C] and DP2 [BAY-u3405]), or the hepatocyte growth factor (HGF) receptor c-Met (PHA-665752), reversed EMT inhibition by the conditioned medium. Additionally, we found that apoptotic cell instillation inhibited bleomycin-mediated EMT in primary mouse alveolar type II epithelial cells in vivo. Our data suggest a new model for epithelial cell homeostasis, by which the anti-EMT programming of macrophages by apoptotic cells may control the progressive fibrotic reaction via the production of potent paracrine EMT inhibitors.
Key Words: EMT, TGF-β1, PGE2, PGD2, HGF

P05-33

Diverse effects of a 445 nm diode laser on isometric contraction of the rat aorta

Sang Woong Park, Kyung Chul Shin, Hyun Ji Park, Young Min Bae*

Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, Chungbuk 380-701, South Korea

The usefulness of visible lasers in treating vascular diseases is controversial. It is probable that multiple effects of visible lasers on blood vessels and their unclear mechanisms have hampered the usefulness of this therapy. Therefore, elucidating the precise actions and mechanisms of the effects of lasers on blood vessels would provide insight into potential biomedical applications. Here, using organ chamber isometric contraction measurements, western blotting, patch-clamp, and en face immunohistochemistry, we showed that a 445 nm diode laser contracted rat aortic rings, both by activating endothelial nitric oxide synthase and by increasing oxidative stress. In addition to the effects on the endothelium, the laser also directly relaxed and contracted vascular smooth muscle by inhibiting L-type Ca2+ channels and by activating protein tyrosine kinases, respectively. Thus, we conclude that exposure to 445 nm laser might contract and dilate blood vessels in the endothelium and smooth muscle via distinct mechanisms.
Key Words: Laser, e-NOS, Aorta, L-type Ca channel

P05-34

Suberoylanilide hydroxamic acid enhances apoptotic effect of TNF-α in human lung cancer cells via TNFR1 upregulation

Bo Ra You, Bo Ram Han, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim and Woo Hyun Park*

Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, JeonJu, 561-180,

Republic of Korea

Suberoylanlide hydroxamic acid (SAHA) is a histone deacetylase (HDAC) inhibitor to have an anti-cancer effect. In the present study, we evaluated the anti-growth effect of SAHA in lung cancer (A549, SK-LU-1, HCC-95, HCC-1588, NCI-H460, NCI-H1299, Calu-6, HCC-33 and NCI-H69) and human small airway epithelial cells (HSAEC). SAHA inhibited the growth of lung cancer cells and induced apoptosis in these cells. However, this agent did not affect cell growth and apoptosis in HSAEC. All the tested caspase inhibitors markedly prevented lung cancer cell death induced by SAHA. Treatment with TNF-α and SAHA synergistically enhanced apoptosis in lung cancer cells, which was accompanied by caspase-8 activation. In addition, SAHA increased the expression of TNF-α receptor 1 (TNFR1) in lung cancer cells. The down-regulation of TNFR1 suppressed apoptotic cell death in TNF-α and SAHA-treated lung cancer cells. In conclusion, SAHA inhibited the growth of lung cancer cells via caspase-dependent apoptosis. SAHA also enhanced apoptotic effect of TNF-α in human lung cancer cells through TNFR1 upregulation and caspase-8 activation. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2008-0062279) and supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013006279).
Key Words: Suberoylanlide hydroxamic acid, Apoptosis, Tumor necrosis factor alpha, Lung cancer

P05-35

Hydroquinone intensifies the death of valproic acid-treated SK-LU-1 cells

Bo Ram Han, Bo Ra You, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim, Woo Hyun Park

Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, JeonJu, 561-180, Republic of Korea

Valproic acid (VPA) is an inhibitor of histone deacetylase (HDAC). It has been reported that shows an anti-cancer effect on various cancer cells. Hydroquinone (HQ) as an autophagy inhibitor can regulate many biological events such as apoptosis. In the present study, we evaluated the effect of VPA and HQ on apoptosis and autophagy in lung cancer cells (A549, SK-LU-1, NCI-H460 and Calu-6). VPA inhibited the growth of lung cancer cells. However, lung cancer SK-LU-1 cells were the most resistant to VPA. This drug induced apoptosis, which was accompanied by the loss of mitochondrial membrane potential (MMP; ΔΨm), PARP-1 cleavage and caspase-3 activation in lung cancer cells except SK-LU-1 cells. In addition, VPA led to autophagy, as evidenced by LC3B increase and p62 decrease in lung cancer cells. Interestingly, the levels of LC3B were already increased in VPA-untreated SK-LU-1 cells. Treatment with HQ enhanced cell death and the loss of MMP in VPA-treated SK-LU-1 cells. In conclusion, VPA induced apoptosis and autophagy in lung cancer cells. Autophagy might be a therapeutic target in anticancer drug resistance. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2008-0062279) and supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2013006279).
Key Words: Valproic acid, Apoptosis, Autophagy, Lung cancer

P05-36

Differential Expression of Taste Receptors in Tongue Papillae

Ha-Jung Choi, Soo-Young Ki, Young-Kyung Cho, Ki-Myung Chung, Kyung-Nyun Kim

Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Sciences, Gangneung-Wonju National University, Gangneung, 210-702, Korea

Taste is important to survive and to maintain quality of life of animals. Several species of mammals, including human, can detect sweet, bitter, sour, salty and umami taste. Sweet or umami taste helps animals to find nutrients such as carbohydrates or amino acids, whereas bitter taste prevents animals to intake probable toxic substances. Tongue has 4 kinds of papillae which are filiform, fungiform (FU), foliate (FO) and circumvallate papilla (CV). Tongue papillae except filiform papilla include taste buds. Taste sensitivities of these papillae are different each other. Although the reason of the different taste threshold of the taste papillae is not known yet, it might be due to the differential expression of taste receptors. This study was performed to determine the expression levels of taste receptors in FU, FO and CV. DBA2 mice of 42-60-day-old were used. Messenger RNAs were extracted from the murine epithelial tissues including FU, FO and CV. Cloned DNAs were synthesized by reverse transcription. Quantitative PCRs were performed to determine mRNA expression levels of taste receptors. Expression levels of taste receptors were calculated as relative that to GAPDH. Results of qPCR revealed that the relative expression levels and patterns were different in FU, FO and CV. All three type I taste receptors were expressed in FU, FO and CV. All 35 kinds of type II taste receptors were more expressed in FO and CV than in FU. Expression levels of Tas2r108 and Tas2r137 were highest in all tested papilla. The different physiological taste thresholds in tongue papillae may due to the different expression levels and patterns of taste receptors, at least in part.
Key Words: taste receptor, fungiform papilla, foliate papilla, circumvallate papilla, qPCR

P05-37

Expression of Bitter Taste Receptor Tas2r108 mRNA in Murine submandibular gland

Su-Young Ki, Ha-Jung Choi, Ki-Myung Chung, Young-Kyung Cho, Kyung-Nyun Kim

Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Sciences, Gangneung-Wonju National University, Gangneung, 210-702, Korea

Mammals have 3 pairs of major salivary glands, the parotid, submandibular, and sublingual glands. Saliva secretion of these glands is modulated by taste perception. Salivary glands are composed mainly of acinar and ductal cells. Primary salivas are secreted by acinar cells and are modified during ductal flow. It was recently founded that Tas2r108, out of murine 35 bitter taste receptors, was observed to the highest expression levels in the submandibular gland by qPCR. The objectives of the present study are to investigate whether acinar or ductal cells of the submandibular gland express Tas2r108. In this study, male 42-60 days old DBA2 mice were used. Messenger RNAs were extracted from the submandibular gland for generating digoxigenin (DIG) labeled-cRNA probes. These probes were transcribed in anti-sense and sense orientation using T7 RNA polymerase. To estimate these probes

concentration, dot blot was performed using labeled probes. ISH was performed on murine submandibular gland to detect Tas2r108 mRNA. Anti-sense was visualized by dot blot in order to dilute DIG labeled-cRNA probes. ISH results showed that the anti-sense probes labeled acinar and duct cells in the submandibular gland, whereas no staining was visible in sense controls. Interestingly, the expression levels of Tas2r108 were higher in acinar cells than in ductal cells. These results suggest that Tas2r108 may play a role in primary secretion than in ductal modification of saliva composition in the submandibular gland.
Key Words: bitter taste receptor, Tas2r108 mRNA, submandibular gland, RT-PCR, in situ hybridization

P05-38

A monoclonal antibody against transmembrane proteins of human umbilical vein endothelial cells is a potential inhibitor of endothelium-dependent relaxation in rat aorta

Bong-Woo Park[1], Seung Hyo Jung[1], Donghyen Lee[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Hwan Myung Lee[2], Junghwan Kim[3], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, Konkuk University School of Medicine, Danwol-dong 322, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795; [3]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin 449-714, South Korea

In previous study, we produced antibodies from rat immunized by human umbilical vein endothelial cells (HUVECs). However, unanswered question remains still about vascular function of the antibodies. The current study explored vasoreactivity, especially focused on vascular contractility, of a functional antibody that is expressed on plasma membrane of HUVECs developed in previous study. Among the developed antibodies, A-7 antibody significantly attenuated endothelium-dependent vasorelaxation in response to acetylcholine (ACh) but not to sodium nitroprusside or histamine. In addition, A-7 antibody did not affect norepinephrine -stimulated contraction in both endothelium-intact and -denuded aorta. Immunocytochemical test showed that A-7 alleviated Ach-increased expression of ACh receptor on the plasma membrane of HUVECs. These findings suggest that monoclonal A-7 antibody may act as an inhibitor on endothelium-dependent vasorelaxation to ACh, probably in part through downregulation of Ach receptor expression.
Key Words: Antibody, Plasma membrane protein, Acetylcholine receptor, Vasorelaxation

P05-39(O-3)

TGF-β1-induced apoptosis via Nox4 is mediated by ERK1/2-mTORC1 activation in podocytes

Ranjan Das[1], Shanhua Xu[1,2], Xianglan Quan[1], Tuyet Thi Nguyen[1], Seung-Kuy Cha[1], Seong-Woo Jeong[1], In Deok Kong[1], Kyu-Sang Park[1]

[1]Department of Physiology, [2]Department of Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, Korea

TGF-β, a pleiotropic cytokine, accumulates during kidney injuries and results in chronic renal diseases. We have previously reported that TGF-β1 induces selective upregulation of mitochondrial Nox4 playing critical roles in podocyte apoptosis. Here, we investigated the regulatory mechanism of Nox4 upregulation by mammalian target of rapamycin (mTOR) activation on TGF-β1-induced apoptosis in podocytes. TGF-β1 treatment markedly increased phosphorylation of mTOR and its downstream target p70S6K and 4EBP1. Blocking TGF-β receptor-I by SB431542 completely blunted phosphorylation of mTOR, p70S6K and 4EBP1. simTOR and adenoviral constructs overexpressing wild type (WT), constitutively active (CA) or kinase-dead (KD) were used to deduce the role of mTOR in Nox4 upregulation by TGF-β1. Inhibition of mTORC1 by low dose of rapamycin or siRNA mediated knockdown of p70S6K protected podocytes through attenuation of Nox4 protein expression and subsequent oxidative stress-induced apoptosis by TGF-β1. Pharmacological inhibition of MEK-ERK cascade, but not PI3K-Akt pathway, abolished TGF-β1-induced mTOR activation. Inhibition of neither ERK1/2 nor mTORC1 reduced the TGF-β1-stimulated increase of Nox4 mRNA level, however, significantly inhibited total Nox4 expression, ROS generation and apoptosis induced by TGF-β1. Moreover, siRNA mediated double knockdown of Smad2/3 or siSmad4 completely suppressed ERK-mTOR activation by TGF-β1. Our data suggest that TGF-β1 increases translation of Nox4 protein level through Smad2/3-ERK1/2-mTOR axis and this pathway is independent of transcriptional regulation of Nox4 by Smad2/3. Activation of this pathway plays a crucial role in ROS generation and mitochondrial dysfunction leading to podocyte apoptosis. Therefore, inhibition of ERK1/2-mTOR pathway could be a therapeutic and preventive target against proteinuric and chronic kidney diseases.
Key Words: TGF-β, Smad2/3, mTOR, NADPH Oxidase 4, ERK1/2, podocytes

P05-40

Scoparone inhibits PDGF-BB-induced vascular smooth muscle cells migration via inactivation of mitogen-activated protein kinases signaling pathway

Gyoung Beom Lee[1], Seung Hyo Jung[1], Kang Pa Lee[1], Donghyen Lee[1], Suji Baek[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan Myung Lee[2], Kyung Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

Vascular smooth muscle cells (VSMCs) migration and proliferation are key processes in the neointima formation that occurs in atherosclerosis and restenosis. These events are potently stimulated by platelet-derived growth factor (PDGF), which is induced in various types of cells including platelets, endothelial cells, and macrophages under physiological or pathophysiological conditions. Scoparone (6,7-dimethoxycoumarin) is known to vasorelaxant and inhibit proliferation at high dose in VSMCs. However, the effect of scoparone on VSMCs migration has not been investigated. In the present study, we examined whether scoparone affects migration in VSMCs. Scoparone dose-dependently suppressed PDGF-BB-induced migration in VSMCs. It inhibited the PDGF-BB-increased phosphorylations of mitogen-activated protein kinases (MAPKs), p38 MAPK and extracellular signal-regulated kinase (ERK) 1/2. Moreover, scoparone inhibited PDGF-BB-induced sprout outgrowth in rat aortas. These results indicate that scoparone inhibits migration by suppressing the phosphorylations of p38 MAPK and ERK 1/2 in VSMCs. Therefore, scoparone may be a potential agent for prevention of pathogenesis such as vascular restenosis or atherosclerosis.
Key Words: Scoparone, Vascular smooth muscle cells, Migration, Platelet-derived growth factor, Restenosis, Atherosclerosis

P05-41

Nafamostat mesilate induces protective effects against TNF-α-induced vascular endothelial cell dysfunction by inhibiting reactive oxygen species production

su jeong choi[1], Jung-Bum Park[1], Harsha Nagar[1], Shin Kwang Kang[2], Saet-byel Jung[3], Sungju Jee[4], Byeong Hwa Jeon[1], Cuk-Seong Kim[1]

[1]Department of Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea, [2]Department of Thoracic and Cardiovascular Surgery, [3]Department of Endocrinology, [4]Department of Rehabilitation Medicine

Nafamostat mesilate (NM) is a serine protease inhibitor with anticoagulant and anti-inflammatory effects. NM has been used in Asia for anticoagulation during extracorporeal circulation in patients undergoing continuous renal replacement therapy and extra corporeal membrane oxygenation. Oxidative stress is an independent risk factor for atherosclerotic vascular disease and is associated with vascular endothelial function. We investigated whether NM could inhibit endothelial dysfunction induced by tumor necrosis factor-α (TNF-α). Human umbilical vein endothelial cells (HUVECs) were treated with TNF-α for 24 h. The effects of NM on monocyte adhesion, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) protein expression, p38 mitogen-activated protein kinase (MAPK) activation, and intracellular superoxide production were then examined. NM (0.01–100 μg/mL) did not affect HUVEC viability; however, it inhibited the increases in reactive oxygen species (ROS) production and p66shc expression elicited by TNF-α (3 ng/mL), and it dose dependently prevented the TNF-α-induced upregulation of endothelial VCAM-1 and ICAM-1. In addition, it mitigated TNF-α-induced p38 MAPK phosphorylation and the adhesion of U937 monocytes. These data suggest that NM mitigates TNF-α-induced monocyte adhesion and the expression of endothelial cell adhesion molecules, and that the anti-adhesive effect of NM is mediated through the inhibition of p66shc, ROS production, and p38 MAPK activation.
Key Words: Nafamostat Mesilate, ICAM-1, VCAM-1, Reactive Oxygen Species, p66shc

P05-42

HN1 Promotes Tumorigenicity through Activation of the SREBP-1 and -2 Lipogenic Signaling Pathway in Hepatocellular Carcinoma

Hua Jin, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim

Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death in the world and its overall 5-year survival rate is less than 12%. Although it has been reported that HN1 is expressed in various cancers, the functional significance of HN1 in HCC is not clearly identified. We have studied the importance of HN1 function in hepatocellular carcinoma cell lines, HepG2 and SNU449. HN1 was highly expressed in human HCC cells. Knockdown of HN1 significantly inhibited the proliferation of HCC cells and decreased expression of Poly ADP-ribose polymerase (PARP) and Caspase-9 as well as increased expression of cleaved-caspase-9 and cleaved-PARP with the silencing of HN1 showed the incidence of apoptosis of human HCC cells. The number and size of the HCC colonies were found to be diminished by silencing of HN1. Knockdown of HN1 significantly inhibited the invasion and metastasis of HCC cells. The mRNA and protein levels of vimentin, β-catenin and UPA were significantly decreased in HN1 knockdown HCC cells. To further investigate the effects of HN1 on HCC cells, we performed gene expression profiling. Statistical analyses of gene expression data from HN1 silencing HCC cells revealed that 130 genes were significantly upregulated, while 379 genes were downregulated. Putative gene networks showed that the expressions of SREBP-1 and -2 which regulate the lipogenic signaling pathway were significantly suppressed by HN1 silencing. We confirmed that knockdown of HN1 significantly inhibited the protein levels of SREBP-1 and -2 expression in HCC cells. Using clinical data from HCC patients, gene expression profiling data revealed that overexpression of HN1 was significantly associated with tumor aggressiveness and poor prognosis in patient with HCC. In vivo xenograft animal study, HN1 knockdown significantly inhibited the tumor weight and growth. Tunel assay also showed that HN1 knockdown significantly induced cell apoptosis in xenograft animal models. In conclusion, HN1 promotes the proliferation and invasion of HCC cells in part through the activation of SREBP-1 and -2 lipogenic signaling pathway. Therefore, our results suggest that targeting HN1 may constitute a therapeutic strategy for HCC.
Key Words: HN1, hepatocellular carcinoma cells, metastasis, cell proliferation, SREBP-1, SREBP-2

P05-43

TonEBP/NFAT5 suppresses adipogenesis via modulation of mitotic clonal expansion during early phase of differentiation in 3T3-L1 cells

Soo Jin Kim, Han Na Choi, Hyun-Woo Kim, Jin Bong Park, Byeong Hwa Jeon, Sang Do Lee

Department of Physiology, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

Obesity resulting from excessive accumulation of white adipose tissue is closely related to the chronic diseases such as diabetes, hyperlipidemia, and hypertension. White adipose mass is determined by the number and size of adipocytes. The differentiation of adipocyte can be divided into two broad stages. Determination phase results in the conversion of the stem cell to a preadipocyte. The committed cells undergo terminal differentiation manifested by formation of lipid droplets as well as adipocyte specific protein. TonEBP/NFAT5 belongs to the Rel family of transcription factors and plays important roles in the development and maintenance of kidney. However, recent reports suggest that TonEBP/NFAT5 function is not limited to the renal medulla. Although the functions of TonEBP/NFAT5 during chondrogenesis and myogenesis are reported recently, its role in the adipogenesis is not well known. In this study, we analyzed the role of TonEBP/NFAT5 in adipogenesis using 3T3-L1 cells. Mouse 3T3-L1 cell line is widely used as an in vitro model for studying terminal adipocyte differentiation. TonEBP/NFAT5 protein expression was dramatically reduced during adipocyte differentiation of 3T3-L1 cells. RNAi-mediated knock down of TonEBP/NFAT5 facilitated adipogenesis. Whereas sustained expression of TonEBP/NFAT5 using adenovirus suppressed the formation of lipid droplet and the expression of FABP4, marker for terminally differentiated adipocytes. Also, TonEBP/NFAT5 inhibited the expression of PPARγ, master regulator of terminal adipocytes. Inhibited adipogenesis by TonEBP/NFAT5 regulates in the early phase of the adipocyte differentiation process via modulation of mitotic clonal expansion and insulin signaling pathway. These results suggest that TonEBP/NFAT5 may be an important regulatory factor in the differentiation of adipocytes.
Key Words: TonEBP/NFAT5, adipogenesis, mitotic clonal expansion

P05-44

Sirtuin 6 inhibits proliferation and invasion of hepatocellular carcinoma cells

Hua Jin, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim

Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

Sirtuin6 (SIRT6) has been reported to be dynamic in its chromatin binding in response to stimuli resulting in altering the transcriptional landscape of tumor development. Despite SIRT6 has been link to involve in tumorigenesis, the molecular mechanisms underlying SIRT6 protein downregulation in human cancers remain unknown. In this study we aimed to investigate the functional significance of SIRT6 in hepatocellular carcinoma (HCC). SIRT6 was highly expressed in human HCC cells. Overexpression of SIRT6 significantly inhibited the proliferation of HCC cells whereas knockdown of SIRT6 significantly increased the proliferation of HCC cells. In colony formation assay showed that the colony numbers were diminished by overexpression of SIRT6 and increased expression of cleaved-caspase-9 and cleaved-PARP. On the other hands, knockdown of SIRT6 in HCC cells did not change the apoptotic related protein levels. In addition, overexpression of SIRT6 significantly inhibited the invasion and metastasis of HCC cells whereas knockdown of SIRT6 induced increase the invasion and metastasis abilities of HCC cells with time dependent manner. In addition, overexpression of SIRT6 significantly decreased the protein levels of vimentin, β-catenin, and UPA whereas knockdown of SIRT6 in HCC cells induced increase the protein levels of UPA, vimentin, and twist. P-β-catenin levels was increased by overexpression of SIRT6 and was diminished by knockdown of SIRT6. Together, SIRT6 regulates the proliferation and invasion of HCC cells and may plays as a tumor suppressor.
Key Words: SIRT6, hepatocellular carcinoma cells, metastasis, cell proliferation, β-catenin

P05-45

Ursolic Acid inhibits the growth of human esophageal squamous cell carcinoma cells by inducing autophagy

Navin Ray, Woo Hyun Park, Sung Zoo Kim, Suhn Hee Kim, Soo Mi Kim

Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

Autophagy is a phenomenon that involved in tumorigenesis. It has been reported that autophagy can kill the cells and thus the activation of the nonapoptotic autophagic cell death program is emerging as a potential cancer therapy. Ursolic acid (UA) is a natural phytochemical anticancer agent. It has been shown that UA inhibits tumor growth and has anti-cancerous property in several human cancers. However, the important biological function of UA on human esophageal squamous cell carcinoma (ESCC) has not been explored. Our endeavor from this study is to discover anti-tumorigenic property of UA in ESCC cells. The result of MTT assay showed that UA significant decreased the viability of ESCC (TE-8 and TE-12) cells in a dose-dependent manner. Colony numbers and sizes from the ESCC were found to be diminished with a dose and time dependent manner in treatment of the UA. UA significantly induced apoptosis of ESCC cells. The Poly ADP-ribose polymerase (PARP) and Caspase-3 protein levels were significantly decreased and the cleaved-caspase-3 and cleaved-PARP protein levels were significantly increased with a dose dependent manner. Autophagy was induced by UA in ESCC cells. After exposure to 30μM of UA for 24 hr, there were a large accumulated vacuoles in cytoplasm and displayed punctuated staining of LC3, a marker of autophagosome. Induction of autophagy was confirmed by measuring of LC3 protein levels. UA significantly increased the protein levels of LC3II, a processed form of LC3I by in a dose dependent manner. Taken together, our results indicate that UA induces ESCC cell death by inducing autophagy.
Key Words: ursolic acid, esophageal squamous cancer cells, autophagy, LC3, apoptosis

P05-46

CTHRC1 Stimulates Growth and Metastasis in Esophageal Adenocarcinoma Cells by Activation of the β-catenin/c-Myc Signaling Pathway

Jie Gao[1,2], Kwang Bok Lee[2], Soo Mi Kim[1]

[1]Department of Physiology, [2]Department of Orthopetid Surgery, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Republic of Korea

Despite many attempts to understand the biology of EAC, the biological mechanisms of EAC progression remain elusive. In the present study, we investigated the underlying molecular mechanisms by which CTHRC1 regulates growth and metastasis in EAC cells. Knockdown of CTHRC1 significantly diminished the growth rates of EAC cells (BE3 and OE33) by MTT assay. Apoptotic proteins were significantly increased by knockdown of CTHRC1 in EAC cells. Cleaved-caspase9 and cleaved-PARP were significantly increased after silencing of CTHRC1 in EAC cells. Knockdown of CTHRC1 significantly diminished the metastasis of EAC cells by matrigel invasion assay. The mRNA and protein levels of vimentin, twist, MMP9, and uPA were significantly decreased in CTHRC1 knockdown EAC cells. In addition, knockdown of CTHRC1 in EAC cells significantly reduced levels of β-catenin and c-Myc, but increased p-β-catenin level. Therefore, CTHRC1 regulates the growth and invasion/metastasis of EAC cells through activation of the β-catenin/c-Myc pathway. Our results suggest that targeting CTHRC1 may constitute a potential therapeutic strategy for EAC.
Key Words: esophageal adnocarcinoma cells, CTHRC1, β-catenin/c-Myc, growth, metastasis

P05-47

Effect of Macrophage on Induction of Gefitinib Resistance in EGFR Mutated Non Small Cell Lung Cancer Cells

Subodh Sharma[1], Soo Jin Kim[1], Taehee Kim[1], Young Hwan Kim[2], Ji Yeong Mun[1], Han Na Choi[1], Min Woong Kang[2], Sang Do Lee[1]

[1]Department of Physiology, [2]Department of thoracic surgery, Chungnam National University School of Medicine, Daejeon, 301-747, Korea

It is well known that tumor-associated macrophages (TAMs), which are abundant in the microenvironment of several tumors, including non-small-cell lung cancer (NSCLC), secrete pro-tumorigenic factors that contribute to cancer progression. However, there is no evidence indicating the involvement of macrophage in inducing drug resistance

in case of lung cancer. To see the effect of macrophage in induction of resistance to tyrosine kinase inhibitors (TKI), epidermal growth factor receptor (EGFR) mutated cells (PC9 and HCC827) were co-cultured with macrophage and then treated with TKI (Erlotinib and Gefitinib). PC9 and HCC827 cells co-cultured with macrophage were much more resistant to erlotinib and gefitinib than those cultured alone. Macrophage can induce cancer cells to enhance migration and invasion via secretion of soluble factors. Therefore we prepared THP-1 cells derived macrophage conditioned medium (CM), and then treated to the PC9 cells. Enhanced migration and invasion of PC9 cells were confirmed upon induction with macrophage CM. Gefitinib induced apoptosis, DNA fragmentation, and cleaved of apoptotic protein PARP and caspase-3 were markedly reduced in macrophage CM treated PC9 cells. U937 cells derived macrophage CM also showed inducibility of Gefitinib resistance. Whereas, THP-1 and U937 derived monocyte CM had no effect on Gefitinib resistance indicating these induction of gefitinib resistance is specific to soluble factors secreted from macrophage. From these results, we can conclude that soluble factors secreted from macrophage induce EGFR TKIs resistance as well as migration and invasion in EGFR mutated NSCLC cells.
Key Words: Macrophage, NSCLC, gefitinib

P05-48

Gas6/Mer signaling induces transactivation of LXRα-target gene arginase 2 and vascular endothelial growth factor via STAT1 transcription factor in macrophages

Eunjin Lim, Si Yoon Kim, Youn-Hee Choi, Jihee Lee

Injury Defense Research Center, School of Medicine, Ewha Womans University, Seoul 158-056, Korea

Mer plays a central role in intrinsic inhibition of the inflammatory response by immune cells. Previously, we demonstrated that the Mer signaling increases the transcriptional liver X receptor (LXR) α/β activity to promote the resolution of acute sterile inflammation. Here we aimed to understand the downstream pathway of growth arrest-specific protein 6 (Gas6)/Mer signaling leading to LXR expression and transcriptional activity in mouse bone-marrow derived macrophages (BMDM). We examined the role of signal transducer and activator of transcription1 (STAT1), which acts as an enhancer of LXR expression and LXR-mediated transcription of alternative activation markers, such as arginase 2 (Arg2) and vascular endothelial growth factor (VEGF) in BMDM. Exposure of BMDM to Gas6 enhanced phosphorylation of STAT1. Gas6-induced STAT1 phosphorylation was inhibited in BMDM from Mer-/- mice or by the specific inhibitor of PI3K, or Akt. Gas6-induced LXR mRNA and protein expression was reduced in BMDM from STAT1-/- mice or BMDM in the presence of STAT1 specific inhibitor, fludarabine. Gas6- induced transcriptional activity from the liver X receptor element (LXRE) promoter in RAW 264.7 cells was completely inhibited by fludarabine. Moreover, enhanced mRNA and protein expression of LXR's target genes, such as Arg2, VEGF, ABCG1 and ApoE, by Gas6 was also reversed in BMDM from STAT1-/- mice or BMDM and RAW 264.7 cells pretreated with fludarabine. Our data suggest that Gas6/Mer signaling leads to increased transcriptional LXR activity and its target genes related to lipid and cholesterol metabolism as well as the anti-inflammatory response via STAT1.
Key Words: Mer, LXR, bone marrow-derived macrophages, PI3K/Akt. STAT1

P05-49

Nafamostat mesilate attenuates transient focal ischemia/reperfusion-induced brain injury via the inhibition of endoplasmic reticulum stres

Sun Kwan Kwon, Moonsang Ahn, Hee-Jung Song, Shin Kwang Kang, Saet-byel Jung, Nagar Harsha, Sungju Jee, Jae Young Moon, Kwang-sun Suh, Sang Do Lee, Byeong Hwa Jeon, Dong Woon Kim, Cuk seong Kim

Department of Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea

Nafamostat mesilate (NM), a serine protease inhibitor, has a broad range of clinical applications that include use as an anticoagulant during hemodialysis in cerebral hemorrhage patients, as a hemoperfusion anticoagulant for patients with intravascular coagulation, hemorrhagic lesions, and hemorrhagic tendencies, and for the improvement of acute pancreatitis. However, the effects of NM on acute cerebral ischemia have yet to be investigated. Thus, the present study utilized a rat model in which transient middle cerebral artery occlusion (MCAO) was used to induce ischemic injury to investigate the effects of NM on infarct volume and histological and biological changes. NM (1 mg/kg) was intravenously administered prior to and after the MCAO procedure. Compared to control rats, the administration of NM significantly decreased infarct size and the extent of brain edema after the induction of focal ischemia via MCAO. Additionally, NM treatment attenuated MCAO-induced neuronal degeneration and activation of microglia and astrocytes. NM treatment also inhibited the MCAO-induced expression levels of glucose-regulated protein 78 (GRP78), CATT/EBP homologous protein (CHOP), and p-eukaryotic initiation factor 2α (eIF2α), which are endoplasmic reticulum (ER) stress markers, in the cerebral cortex. The present findings demonstrate that NM exerts neuroprotective effects in the brain following focal ischemia via, at least in part, the inhibition of ER stress.
Key Words: Nafamostat mesilate, cerebral ischemia, MCAO, ER stress

P05-50

Statin pretreatment inhibits LPS-induced EMT via the downregulation of TLR4 and NF-κB in hBECs

Seon Mee Park[1], Yangmi Kim[2]

Department of [1]Gastroenterology, Department of [2]Physiology, College of Medicine, Chungbuk National University, Korea

Epithelial-mesenchymal transition (EMT) of biliary epithelial cells (BECs) plays an important role in biliary fibrosis. Lipopolysaccharide (LPS) promotes EMT in BECs. This study investigated the effects of simvastatin on the LPS-induced EMT in BECs. Following exposure to 1 μg/mL LPS for 5 days, the mRNA and protein levels of E-cadherin decreased, while those of vimentin increased. The TRL4 mRNA levels increased after a 5-day exposure to LPS or TGF-β1 (5 ng/ml). Compared with the BECs treated with LPS alone, co-treatment with simvastatin plus LPS induced a remarkable increase in E-cadherin expression and a slight decrease in vimentin expression. The LPS-induced TLR4 expression was slightly decreased by co-treatment with simvastatin and LPS. In the proliferation analysis, LPS-induced BEC growth was remarkably inhibited by simvastatin (1 μM) treatment. With regard to BEC morphology, compared with BECs pretreated with simvastatin before LPS exposure (preSL), BECs pretreated with LPS (postSL) or co-treated with LPS plus simvastatin (LS) demonstrated additional EMT characteristics (cell morphology changes from a round shape to a

spindle-like morphology). Furthermore, pretreatment of BECs with simvastatin (preSL) inhibited the LPS-induced EMT by downregulating NF-κB phosphorylation. These findings indicate that while LPS and TGF-β1 promote EMT, BECs pretreated with simvastatin (preSL) inhibited LPS-induced EMT by downregulating TLR4 and NF-NF-κB . Conclusions: Our results demonstrate that simvastatin pretreatment (preSL) inhibits the LPS-induced EMT of human BECs. This finding suggests that simvastatin can be considered a new agent to prevent the biliary fibrosis associated with the EMT of BECs.
Key Words: Biliary epithelial cells, Epithelial-mesenchymal transition, Lipopolysaccharide, Toll-like receptor- 4, Simvastatin

P05-51

Regulation of Autophagy by Rapamycin has inhibition cardio-toxicity role in Doxorubicin-Induced Cardiac Progenitor/Stem cells Dysfunction

Ji Hye Park[1,2,3,#], Sang Mo Kwon[1,2,*]

[1]Laboratory of Vascular Medicine and Stem Cell Biology, Department of Physiology, Pusan National University School of Medicine, South Korea, [2]Convergence Stem Cell Research Center, Department of Physiology, Pusan National University School of Medicine, South Korea. [3]Pusan National University Medical Science, Education Center (BK21 Program), Pusan National University School of Medicine, Yangsan 626-870, Korea

Background Although doxorubicin (DOXO) is widely used for chemotherapy against various solid cancer and childhood cancer, but using the DOXO is limited by serious cardiac toxicity through loss of cardiomyocyte. Recently reported researches suggested that endogenous cardiac stem/progenitor cells (eCPC) play important roles in cardiomyocyte homeostasis. Furthermore, most recently reported research suggested that DOXO occurred eCPC depletion. However, these underlying mechanisms have not been fully demonstrated. In addition, autophagy has emerging signaling pathway for regulation of cellular bioactivities, such as proliferation, differentiation and senescence. Thus, in this study, we first examined whether autophagy signaling regulation could rescue to DOXO-mediated eCPC dysfunction. **Method & Results** For this study, c-kit positive eCPCs were isolated from infant-derived cardiac tissues as previously reported. To determined optimal DOXO concentration in eCPC, we used eCPC viability using MTS assay. As shown result, over the 500nM of DOXO treatments were significantly reduced eCPC viability. Next, immunoblotting was used to detect expression of regucalcin(SMP30; calcium regulator protein), mTOR, and LC3 (autophagy maturation related protein). SMP30 and LC3 expressions were time dependently reduced after DOXO treatment in eCPC. However, mTOR expressions were significantly increased after treatment with DOXO in eCPC. Next, we examined whether administered with rapamycin (mTOR inhibitor) could rescue SMP30 and LC3 expression. After treatment with rapamycin, reduced SMP30 and LC3 expressions were significantly increased in DOXO-induced eCPC. Additionally, intracellular Ca2+ levels were analyzed by Fluo-8 assay, and reduced Fluo-8 level in DOXO-treated eCPC groups were significantly reduced fluorescence intensities after treatment with rapamycin. **Conclusion** From the above results, rapamycin could be rescue autophagy formation and SMP30 expression DOXO-treated eCPC through mTOR inhibition and intracellular Ca2+ handling. Thus, rapamycin might be suppressive effects of DOXO-mediated cardiotoxicity through autophagy signaling regulation in eCPC.
Key Words: Endogenous cardiac stem/progenitor cells, autophagy, cardiotoxicity, doxorubicin, rapamycin

P05-52(O-8)

Serum protein Fetuin-B is involved in immune cells and vascular smooth muscle cells-linked atherosclerotic plaque stability

Donghyen Lee[1], Seung Hyo Jung[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Suji Baek[1], Bong-Woo Park[1], Junghwan Kim[2], Hwan-Myung Lee[3], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin 449-714, Korea, [3]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

Coronary artery disease frequently progresses in an abrupt fashion, and the occlusion of vessels develops rapidly, which is largely related to thrombosis resulting from the disruption of atherosclerotic plaque. It is generally known that the disruption of atherosclerotic plaque can be associated with the stability of atherosclerotic plaque that is implicated with responses in various cell types including immune cells and vascular smooth muscle cells (VSMCs). However, influenceable risk factor for plaque rupture-linked plaque stability remains unclear. In this study, we investigated proteins expressed differentially in serum from patients with acute myocardial infarction (AMI) and stable angina using a proteomic analysis and identify the new molecule fetuin-B, which was up-regulated in the AMI group. We thus explored a potential involvement of fetuin-B in atherosclerotic plaque stability. Fetuin-B induced vascular plaque stability-linked responses, including the deposition of lipid and production of cytokines in macrophages, the activation of MMP-2 in monocytes, and the activation of apoptosis and MMP-2 in VSMCs. In addition, in vivo treatment with fetuin-B resulted in the decreased collagen accumulation and VSMC content and the increased number of macrophages in the atherosclerotic plaque. From these findings, we concluded that fetuin-B may play an important role in the development of AMI through regulation of atherosclerotic plaque stability. This study may provide therapeutically beneficial information for patients at high risk of AMI.
Key Words: Acute myocardial infarction, Stable angina, Atherosclerotic plaque, Fetuin-B

P05-53

DJ-1 contributes to sphingophosphorylcholine-induced differentiation of human mesenchymal stem cells into smooth muscle cells

Suji Baek[1], Kang Pa Lee[1], Seung Hyo Jung[1], Gyoung Beom Lee[1], Donghyen Lee[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan-Myung Lee[2], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

Human mesenchymal stem cells (hMSCs) have a self-renewal and a differentiation capacity to diverse cell types such as chondrogenic, adipogenic and myogenic cells. Sphingophosphorylcholine (SPC) is known to induce differentiation of hMSCs into smooth muscle cells (SMCs). In the present study, we investigated correlation between SMC differentiation and its related proteins in SPC-stimulated hMSCs. We analyzed the proteins expressed differentially in SPC-stimulated hMSC using proteomics techniques and found that the oxidized form of DJ-1 protein was predominantly altered in hMSCs in response

to SPC. DJ-1 oxidation in SPC-stimulated hMSCs was validated by immunoblot analysis. Treatment with SPC resulted in expression increment of α-smooth muscle actin (SMA) in hMSCs. The knockdown of DJ-1 significantly enhanced α-SMA expression in hMSCs. Moreover, overexpression of DJ-1 attenuated the increased expression of α-SMA in hMSCs treated with SPC. These results indicate that DJ-1 may be involved in differentiation of hMSCs into SMCs in response to SPC. Therefore, DJ-1 may be a critical protein linked to differentiation of hMSCs into SMCs

Key Words: Human mesenchymal stem cell, Sphingophosphorylcoline, Smooth muscle cell, Differentiation

P05-54

532 nm laser irradiation suppresses restenotic lesion-related responses in PDGF-BB-stimulated vascular smooth muscle cells

Seung Hyo Jung[1], Suji Baek[1], Kang Pa Lee[1], Gyoung Beom Lee[1], Bong-Woo Park[1], Dong Hyeon Lee[1], Hwan-Myung Lee[2], Young Min Bae[1], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

Vascular restenotic lesions after balloon injury are implicated in various responses including apoptosis, migration and proliferation in vascular smooth muscle cells (VSMCs). These cellular events in vascular restenotic lesions are involved in diverse growth factors including plate-derived growth factor (PDGF). Many studies have shown increasing interest in the effects of low-power laser irradiation (LPLI) at various wavelength ranges on components of the cardiovascular system in normal and pathologic states, including vascular endothelium and cardiac muscle cells. However, it remains unclear whether the vascular restenotic lesion-linked processes in VSMCs are affected by LPL irradiation, especially 532 nm pulsed irradiation. In the present study, we explored the effects of LPLI (with diode laser 532 nm pulsed wave) on the cellularity of VSMCs in the presence of PDGF-BB. Proliferation was analyzed by 2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide assay, and migration was measured by the scratch wound healing assay and Boyden chamber assay. Flow cytometry assay and terminal deoxynucleotidyl transferase dUTP nick end labeling analysis were performed to detect apoptosis induction. Protein activation was tested by Western blotting. Proliferation and migration were confirmed by ex vivo aortic sprout assy. LPLI arrested PDGF-BB-induced proliferation in VSMCs. PDGF-BB-increased migration in VSMCs was inhibited by LPLI. LPLI stimulated apoptotic induction in VSMCS in the presence of PDGF-BB. Activations of caspase3 and Bax, as well as p38 MAPK, in VSMCs treated with PDGF-BB were enhanced by exposure to LPLI. In addition, LPLI reduced PDGF-BB-evoked aortic sprout outgrowth. These findings demonstrate that LPLI may induce the inhibition of proliferation and migration and also stimulated the induction of apoptosis in PDGF-BB-treated VSMCs. This study may provide useful information for therapeutic strategy of vascular restenosis.

Key Words: Vascular smooth muscle cells, Low power laser, Migration, Platelet-derived growth factor, Apoptosis

P05-55

Angiotensin II induces migration via APE/Ref-1-mediated transactivation of sphingosine-1-phosphate receptor in vascular smooth muscle cells

Kang Pa Lee[1], Dong-Youb Lee[1], Dong Hyen Lee[1], SeungHyo Jung[1], Suji Baek[1], Yuri Park[1], Hwan Myung Lee[2], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, Seoul 143-701, Korea, [2]Department of Cosmetic Science, College of Natural Sciences, Hoseo University, Asan 336-795, Korea

Angiotensin II (Ang II) acts as a major mediator in migration and proliferation of vascular smooth muscle cells (VSMCs), which are important events in the development of cardiovascular diseases such as atherosclerosis and restenosis. However, whether Ang II affects the actions of apurinic/apyrimidinic endonuclease/redox factor-1 (APE/Ref-1) and sphingosine-1-phosphate (S1P) signaling is not clarified yet. In this study, we investigated the functional role of APE/Ref-1in epigenetic regulation of S1P receptor (S1PR) in response to Ang II in VSMCs. Ang II enhanced S1PR1 expression in rat aortic smooth muscle cells (RASMC) in dose- and time-dependent manners, which was inhibited by treatment with the Ang II receptor (AT) 1 inhibitor and antioxidant. Ang II stimulated the production of H2O2 and the expression of S1PR1 was also elevated by treatment of H2O2. Moreover, Ang II significantly induced the translocation of cytoplasmic APE/Ref-1 into nuclear fraction in RASMCs. The knockdown of APE/Ref-1 with small interference RNA abolished the overexpression of S1PR1 in response to Ang II. H3 histone acetylation and APE/Ref-1 binding at the S1PR1promoter were increased in RASMCs treated with Ang II. In addition, Ang II-induced migration was suppressed by AT1 and S1PR1 inhibitors in RASMCs. These results indicate that Ang II may stimulate the epigenetic regulation of S1PR1 expression via H2O2-mediated APE/Ref-1 translocation, which may consequently be involved in the Ang II-induced VSMC migration.

Key Words: Angiotensin II, Vascular smooth muscle cell, Sphingosine-1-phosphate receptor, APE/Ref-1, Histone modification, Migration

P05-56

Inhibition of Pi transport across plasma and mitochondrial membrane prevents high phosphate-induced vascular calcification

Tuyet Thi Nguyen[1], Shanhua Xu[1,2], Ranjan Das[1], Xianglan Quan[1], Ji-Hee Kim[1], Kyu-Hee Hwang[1,2], Seung-Kuy Cha[1], Seong-Woo Jeong[1], In Deok Kong[1], Kyu-Sang Park[1]

Department of [1]Physiology and [2]Global Medical Science, Yonsei University Wonju College of Medicine, Wonju, Korea

Inorganic phosphate (Pi) is very crucial for cell signaling and energy metabolism. However, hyperphosphatemia in chronic kidney disease (CKD) patients or even high normal range of plasma Pi level in non-CKD individuals is associated with serious cardiovascular complications including medial calcification, left ventricular hypertrophy and positively correlated with cardiovascular events. But the underlying mechanisms of Pi-induced these toxicities have been still debated. Thus, we have investigated whether cellular and mitochondrial Pi uptake followed by reactive oxygen species (ROS) generation act as a critical role in high Pi-induced vascular calcification in a rat aortic smooth muscle cell line, A7r5. Type III Na^+-Pi cotransporters (PiT-1/2), the predominant plasmalemmal Pi transporters expressed in A7r5 cells, were up-regulated both in total protein levels and surface abundance

by high Pi incubation. Using patch clamp technique, we observed that Pi induced a Na^{+}-dependent inwardly rectifying current in A7r5 cells as well as in PiT-1 overexpressed HEK cells. Cellular uptake of Pi elicited cytosolic alkalinization which facilitated Pi transport into mitochondrial matrix. Increased mitochondrial Pi uptake accelerated superoxide generation, upregulation of osteogenic genes and calcific changes in A7r5 cells. Vascular calcification by high Pi was prevented by mitochondrial ROS scavenger. Inhibition of Pi transport by PiT-1/2 knockdown or pharmacologic blocking of mitochondrial Pi transport restored all these pathogenic changes by high Pi. High Pi activated ERK/mTOR signaling, inhibition of which abolished osteogenic gene upregulation and vascular calcification. Taken together, we propose that mitochondrial oxidative stress, Pi transport across plasma and mitochondrial membranes and ERK/mTOR signaling could be therapeutic targets for Pi-induced vascular calcification and cardiovascular morbidities.
Key Words: plasma membrane phosphate transporter PiT-1/2, mitochondria phosphate transport, ERK, mTOR, vascular calcification

P05-57

Cul3-KLHL22 E3 ubiquitin ligase and TWIST target anterior gradient-2 and regulate tumor progression

Seok Yun Jung, Sang-Mo Kwon

Laboratory for Vascular Medicine & Stem Cell Biology, Department of Physiology, School of Medicine, Pusan National University, 49, Busandaehak-ro, Mulgeum-eup, Yangsan-si, Gyeongsangnam-do, Korea

Anterior gradient-2 (AGR2) is a well-known pro-oncogenic/metastatic factor that is overexpressed in multiple types of cancer. In this study, we elucidated novel regulatory mechanisms of AGR2. We found that Twist1, a basic helix-loop-helix transcription factor, enhanced AGR2 promoter activity. Additionally, chromatin immunoprecipitation assays indicated that Twist1 bound directly to the AGR2 promoter through the E-box. Silencing of AGR2 by shRNA inhibited the proliferation, migration, and colony formation of MCF7 cells. These data indicated that Twist1 was critical for AGR2 activity and that AGR2 may be a potential tumor biomarker. Next, we identified a novel Cullin3 (Cul3)-Bric-a-brac/Tramtrack/Broad complex (BTB) adaptor protein, Cul3-Kelch-like protein (KLHL) 22 E3 ligase. KLHL is a BTB domain adaptor protein that assembles with Cul3; the role of KLHL22 in cancer biology is still largely unknown. Our studies showed that KLHL22 bound directly to AGR2 through Kelch domains and ubiquitinated AGR2. Additionally, Cul3-KLHL22 E3 ligase polyubiquitinated AGR2 through lysine 6-linked ubiquitin chains and downregulated the expression of AGR2 and the downstream proteins cathepsin B and D. Importantly, KLHL22 inhibited the migration and colony-forming capacities of MCF7 breast cancer cells. Finally, in a mouse xenograft model of breast cancer, KLHL22 significantly inhibited tumor growth. Taken together, our data suggested that the Cul3-KLHL22-mediated ubiquitination signal inhibited AGR2 expression and breast cancer progression.
Key Words: Anterior gradient-2, Cul3-KLHL22 E3 ligase, tumorigenesis

P05-58

Engineered M13 Phage as a Novel Therapeutics to enhance Endothelial Progenitor Cell-based Neovascularization

Sung Wook Kim, Jun Hee Lee, Sang Mo Kwon

Laboratory for Vascular Medicine&Stem cell biology, Medical Research Institute, Department of Physiology, Pusan National University, Pusan, Korea

Enhancement of transplanted EPC survival, proliferation, differentiation and migration at the ischemic region is significantly important to the EPC therapy. To enhance EPC function, EPC therapy was constantly studied in many different ways. But M13 phage was not in used on EPC therapy. In this study, we fabricated genetically engineered M13 phages with two functional peptides, RGD peptide and SDKP peptide, on their minor and major coat proteins and treated on EPCs to improve the EPC function. Next, Engineered M13 phage treated on EPC to enhance EPC function before transplantation in mouse hind limb ischemia. Consequently, EPC proliferation was increased by stimulating cyclin D expression. In addition, engineered M13 phage enhanced survival, migration and differentiation on EPC via Akt and ERK activation. In vivo study used mouse hind limb ischemia model, engineered M13 phage treated EPC transplantation group was significantly improve blood perfusion. Histological analysis suggested that engineered M13 phage treated EPC proliferation, survival and differentiation was improved in ischemic hind limb by confirming caspase-3, PCNA, CD31 antibodies. Genetically engineered M13 phage is a promising candidate for the development of EPC therapy for hind limb ischemia treating due to its promising structural features and advantages. We showed that the engineered M13 phage were able to support EPC proliferation and differentiation as well as EPC survival in ischemic region. Genetically engineered M13 phage will be a useful platform for EPC therapy in various ischemic diseases.
Key Words: M13 phage, neovascularization, therapy, EPC

P05-59

Tat-biliverdin reductase A protects insulin-producing INS-1 cells from islet amyloid polypeptide (IAPP)-induced apoptosis by alleviating oxidative and endoplasmic reticulum stresses

Su Jin Lee, Hyung Kyung Kang, Won Sik Eum[1], Soo Young Choi[1], Hyeok Yil Kwon

Department of Physiology, College of Medicine, [1]Department of Biomedical Science and Research Institute of Bioscience and Biotechnology, Hallym University, Chunchon 200-702, Korea

Islet amyloid polypeptide (IAPP) is a major component of pancreatic amyloid deposit, which is a characteristic histopathological finding for type 2 diabetes mellitus (T2DM). IAPP in amyloid deposit has been closely associated with the β cell degeneration, and the extent of its deposition correlates negatively with β cell mass in the T2DM. We recently developed a cell-permeable fusion protein, Tat-biliverdin reductase A (Tat-BLVRA) and investigated the anti-inflammatory effects in macrophage cells. In this study, we transduced Tat-BLVRA into INS-1 rat insulinoma cells and examined its protective effect against IAPP-induced cell apoptosis. Tat-BLVRA was successfully delivered into INS-1 cells in time- and dose-dependent manner and was maintained within the cells for at least 48 h. Exposure of cells to IAPP-induced apoptotic cell death determined by MTT assay and Hoechst staining. Pre-treatment with Tat-BLVRA increased the survival of INS-1 cells exposed to IAPP in a dose-dependent manner. Tat-BLVRA markedly decreased IAPP-induced production of reactive oxygen species and malondialdehyde. These protective effects of Tat-BLVRA against IAPP were well correlated with the changes in the levels of signaling mediator molecules of cell apoptosis/survival (Bcl-2, Bax, caspase-3, PARP, JNK, and Akt) and endoplasmic stress (CHOP, ATF-4, BiP, XBP-1, caspase-12). These

results showed that the transduced Tat-BLVRA efficiently prevented IAPP-induced cell apoptosis of INS-1 cells by alleviating oxidative and endoplasmic reticulum stresses. Further, these results suggested that Tat-mediated BLVRA transduction may be a potential therapeutic strategy to prevent β cell loss in patients with T2DM.
Key Words: Type 2 diabetes mellitus, β Cell apoptosis, IAPP, Transduction, Tat-BLVRA

P06-01

Absence of of hypoxic augmentation of vasoconstriction in the femoral artery from eNOS deficient mice

Hae Jin Kim, Hae Young Yoo, Yin Hua Zhang, Sung Joon Kim

Department of Physiology, Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, KOREA

Previously we have reported that hypoxia augments the agonist-induced contraction of skeletal arteries (HVC) from rats. As for the mechanism, hypoxic inhibition of eNOS expressed in the arterial myocytes was suggested. To further prove the novel role of muscular eNOS in skeletal artery, we investigate HVC in the femoral arteries (FAs) from wild type (WT), hetero (+/-), and null (-/-) eNOS knockout mice. Absolute contractile force under 80 mM KCl-induced depolarization (80K) was lower in (-/-) than WT and (+/-) FAs. Phenylephrine (PhE)- or Ang II-induced contraction of FAs normalized to the 80K-contraction was higher in (+/-) and (-/-) than WT. Immunohistochemical assay of FAs demonstrated, in addition to the strong endothelial eNOS, weak but certain expression of eNOS in the medial layer of FAs. The medial expression of eNOS was not observed in (+/-) and (-/-) FAs as well as WT carotid arteries (CAs). Consistently, hypoxia (3 % PO2) largely augmented PhE-induced contraction in WT FAs while not in (+/-), (-/-) FAs. WT CAs did not show the HVC. NOS inhibitor, L-NAME (0.1 mM), also augmented PhE-contraction in endothelium-denuded WT FAs while not in WT CAs. Taken together, the muscular expression of eNOS in skeletal arteries contributes to intrinsic attenuation of alpha-adrenergic vasoconstriction. The counterbalancing effect is effectively alleviated under hypoxia, resulting HVC under the sympathetic stimulation, which might play a role in maintaining the blood pressure under emergency.
Key Words: Hypoxia, eNOS, smooth muscle, skeletal artery, knockout mouse

P06-02

Augmented vascular reactivity and hypoxic pulmonary vasoconstriction in monocrotaline-induced pulmonary arterial hypertension rats

Hae Jin Kim[1,2], Yin Hua Zhang[1], Sung Joon Kim[1,2], Hae Young Yoo[3]

[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea, [3]Chung-Ang University College of Nursing, Seoul 156-756, Korea

Pulmonary arterial hypertension (PAH) is a progressive and eventually lethal disease caused by vascular proliferation, increased vascular resistance and remodeling of pulmonary arteries (PA). Monocrotaline (MCT) is a pyrrolizidine alkaloid phytotoxin which is widely used for developing pulmonary arterial hypertension (PAH-MCT) rat model caused by injury of pulmonary endothelial cells; however, the pulmonary arterial contractility of the MCT-induced PAH rat model has not been fully understood yet. Here we investigate the effects of vasoactive agonists and the response of PA to hypoxia. The hypoxic pulmonary vasoconstriction (HPV) was analyzed using ventilated/perfuse (V/P) lungs. Histological study revealed the vascular remodeling (i.e. medial thickening of PA) and right ventricle hypertrophy 3 weeks after application of MCT to rats. The basal pulmonary arterial pressure (PAP) and the increase of PAP by raised perfusion flow rate (up to 40 ml/min) were significantly higher in PAH-MCT model. Furthermore, high K+ (40 mM KCl) and Angiotensin II-induced PAP were higher in PAH-MCT rats. Different from the loss of HPV in chronic hypoxia-induced PAH model, the hypoxia-induced PAP increase in V/P lungs was not impaired in the PAH-MCT rats. An application of NO synthase inhibitor (L-NAME) induced PAP increase that was higher in PAH-MCT than control rats. The decreased compliance of PA might underlie the higher responses to agonists and contractile conditions. Despite the toxicological effect of MCT on the pulmonary endothelium, the regenerated endothelial function (e.g. NOS) might partly counterbalance the severe contraction and PAP increase in the PAH-MCT rats.
Key Words: Pulmonary arterial hypertension, Hypoxic pulmonary vasoconstriction, Monocrotaline, Vascular reactivity

P06-03

Smooth Muscle Cell Genome Browser: Enabling the Identification of Novel Serum Response Factor Target Genes

Moon Young Lee[1,2], Chanjae Park[1], Robyn M. Berent[1], Paul J. Park[1], Robert Fuchs[1], Hannah Syn[1], Albert Chin[1], Jared Townsend[1], Craig C. Benson[3], Doug Redelman[1], Tsaiwei Shen[4], Jong Kun Park[5], Joseph M. Miano[3], Kenton M. Sanders[1], Seungil Ro[1]

[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, United States of America, [2]Department of Physiology, Wonkwang Digestive Disease Research Institute and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, Jeollabuk-do, Korea, [3]Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York, United States of America, [4]LC Sciences, 2575 West Bellfort Street Suite 270, Houston, Texas, United States of America, [5]Division of Biological Science, Wonkwang University, Iksan, Jeollabuk-do, South Korea

Genome-scale expression data on the absolute numbers of gene isoforms offers essential clues in cellular functions and biological processes. Smooth muscle cells (SMCs) perform a unique contractile function through expression of specific genes controlled by serum response factor (SRF), a transcription factor that binds to DNA sites known as the CArG boxes. To identify SRF-regulated genes specifically expressed in SMCs, we isolated SMC populations from mouse small intestine and colon, obtained their transcriptomes, and constructed an interactive SMC genome and CArGome browser. To our knowledge, this is the first online resource that provides a comprehensive library of all genetic transcripts expressed in primary SMCs. The browser also serves as the first genome-wide map of SRF binding sites. The browser analysis revealed novel SMC-specific transcriptional variants and SRF target genes, which provided new and unique insights into the cellular and biological functions of the cells in gastrointestinal (GI) physiology. The SRF target genes in SMCs, which were discovered in silico, were confirmed by proteomic analysis of SMC-specific Srf knockout mice. Our genome browser offers a new perspective into the alternative expression of genes in the context of SRF binding sites in SMCs and provides a valuable reference for future functional studies.

Key Words: smooth muscle cell, jejunum, colon, serum response factor, transcriptome

P06-04

Serum Response Factor Is Essential for Prenatal Gastrointestinal Smooth Muscle Development and Maintenance of Differentiated Phenotype

Chanjae Park[1], Moon Young Lee[1,2], Paul J Park[1], Se Eun Ha[1], Robyn Berent[1], Robert Fuchs[1], Joseph M Miano[3], Laren S Becker[4], Kenton M Sanders[1], Seungil Ro[1]

[1]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, USA, [2]Department of Physiology, Wonkwang Digestive Disease Research Institute and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, Jeollabuk-do, Korea, [3]Aab Cardiovascular Research Institute, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA, and [4]Gastroenterology and Hepatology, Stanford University School of Medicine, Stanford, California, USA

Background/Aims Smooth muscle cells (SMCs) characteristically express serum response factor (SRF), which regulates their development. The role of SRF in SMC plasticity in the pathophysiological conditions of gastrointestinal (GI) tract is less characterized. **Methods** We generated SMC-specific Srf knockout mice and characterized the prenatally lethal phenotype using ultrasound biomicroscopy and histological analysis. We used small bowel partial obstruction surgeries and primary cell culture using cell-specific enhanced green fluorescent protein (EGFP) mouse lines to study phenotypic and molecular changes of SMCs by immunofluorescence, Western blotting, and quantitative polymerase chain reaction. Finally we examined SRF change in human rectal prolapse tissue by immunofluorescence. **Results** Congenital SMC-specific Srf knockout mice died before birth and displayed severe GI and cardiac defects. Partial obstruction resulted in an overall increase in SRF protein expression. However, individual SMCs appeared to gradually lose SRF in the hypertrophic muscle. Cells expressing low levels of SRF also expressed low levels of platelet-derived growth factor receptor alpha (PDGFR low) and Ki67. SMCs grown in culture recaptured the phenotypic switch from differentiated SMCs to proliferative PDGFR low cells. The immediate and dramatic reduction of Srf and Myh11 mRNA expression confirmed the phenotypic change. Human rectal prolapse tissue also demonstrated significant loss of SRF expression. **Conclusions** SRF expression in SMCs is essential for prenatal development of the GI tract and heart. Following partial obstruction, SMCs down-regulate SRF to transition into proliferative PDGFR low cells that may represent a phenotype responsible for their plasticity. These findings demonstrate that SRF also plays a critical role in the remodeling process following GI injury.
Key Words: Gastrointestinal tract, Platelet-derived growth factor receptor alpha, Rectal prolapse, Serum response factor, Smooth muscle cell

P06-05

Loss of Cdo leads to alteration in N-cadherin and connexin with intercellular coupling defects and cardiomyopathy

Hyun-Ji Kim[1,3,#], Myong-Ho Jeong[2,3,#], Kyu-Sil Choi[3], Young-Hwan Song[4], Gordon F. Tomaselli[5], Jong-Sun Kang[2,3,*], Hana Cho[1,3,*]

[1]Department of Physiology, [2]Department of Molecular Cell Biology, Sungkyunkwan University School of Medicine, Suwon, 440-746, [3]Samsung Biomedical Research Institute, Samsung medical center, Seoul 135-710, [4]Devision of Cardiology, Seoul National University Bundang Hospital, 463-707, Republic of Korea, [5]Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Dysregulation of adherens and gap junctional proteins, N-cadherin and Connexin 43 (Cx43) is associated with cardiomyopathy and fibrosis. A cell surface protein Cdo interacts with N-cadherin/β-catenin mediating cell adhesion signaling in skeletal myogenesis however its role in cardiomyocyte junctions is unknown. Here we report that Cdo is essential in regulation of cardiomyocyte coupling via modulation of N-cadherin and Wnt/β-catenin signaling. Cdo-deficient mice exhibit marked fibrosis and reduced cardiac function with shortened QTc intervals. Cdo-deficient cardiomyocytes exhibit elevated N-cadherin levels and Cx43 accumulation at lateral borders, accompanied by altered gap junction coupling which is rescued by wildtype but not by an N-cadherin binding-deficient Cdo. Additionally, Cdo deficiency also derepresses Wnt/β-catenin signaling which elevates N-cadherin and Collagen I expression. Conversely, Wnt signaling activation in turn inhibits Cdo expression in cardiac cells, suggestive of a feedback loop. Taken together, Cdo deficiency leads to alterations in intercellular coupling, contributing to fibrosis and cardiac remodeling.
Key Words: N-cadherin, Connexin 43, Cardiomyopathy, Cdo

P06-06

Sildenafil is effective to enhance the proliferation of skeletal myoblasts

Mei Huang, Keon Jin Lee, Mi Kyoung Ahn, Eun Hui Lee

Department of Physiology, College of Medicine, The Catholic University of Korea, Banpo-daero 222, Seocho-gu, Seoul 137-701, Republic of Korea

Sildenafil is a specific inhibitor of phosphodiesterase type 5 and clinically used to treat erectile dysfunction and pulmonary artery hypertension because of its vasodilatation effect due to the relaxation of smooth muscle cells. In cardiac muscle, the cardioprotective effects of sildenafil have been reported. The effectiveness of sildenafil on skeletal muscle has been controversial. In skeletal muscle, sildenafil reduces the cellular damage and muscle fatigue. However, sildenafil also induces the atropy of skeletal muscle. The effects of sildenafil on skeletal muscle in cellular levels have not been examined. In the present study, sildenafil effects on skeletal muscle cells was examined using mouse primary skeletal myoblasts and myotubes. Sildenafil was effective to enhance the proliferation of myoblasts and there was no macroscopic change in the shape of myotubes.
Key Words: skeletal muscle, sildenafil

P06-07

Interaction between mitsugumin 29 and TRPC3 participates in regulating calcium transients in skeletal

Jin Seok Woo[1], Ji-Hye Hwang[1], Mei Huang[1], Mi Kyoung Ahn[1], Mi Ri Oh[1], Chung-Hyun Cho[2], Jianjie Ma[3], Eun Hui Lee[1]

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Banpo-daero 222, Seocho-gu, Seoul 137-701, Republic of

Korea , [2]Department of Pharmacology, College of Medicine, Seoul National University, Seoul 110-799, Republic of Korea, [3]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, Columbus, OH 43210, USA

Mitsugumin 29 (MG29) is related to the fatigue and aging processes of skeletal muscle. To examine the roles of MG29 in conjunction with its binding protein, the canonical-type transient receptor potential cation channel 3 (TRPC3), in skeletal muscle, the binding region of MG29 to TRPC3 was studied along with the functional relevance of the binding in mouse primary skeletal myotubes using coimmunoprecipitation assays and Ca2+ imaging experiments. The N-terminus and the IeII loop of MG29 constitute the binding region for TRPC3. The myotubes that expressed the MG29 mutant missing the entire TRPC3-binding region showed a disrupted binding between endogenous MG29 and TRPC3 and a reduction in Ca2+ transients in response to membrane depolarization without affecting ryanodine receptor 1 activity, the resting cytosolic Ca2+ level, and the amount of releasable Ca2+ from the sarcoplasmic reticulum. Among the proteins mediating Ca2+ movements in skeletal muscle, TRPC4 expression was significantly decreased by the MG29 mutant. Therefore, MG29 could be a new factor for regulating Ca2+ transients during skeletal muscle contraction possibly via a correlation with TRPC3 and TRPC4.
Key Words: Mitsugumin 29, skeletal muscle

P06-08

Inhibition of nNOS facilitates myofilament disarray and cardiac hypertrophy in Ang II-induced hypertensive rat

Ji Hyun Jang, Zai Hao Zhao, Sung Joon Kim, Yin Hua Zhang

Department of Physiology, Seoul National University, College of Medicine, Seoul, Republic of Korea

Previous reports using neuronal nitric oxide (nNOS) gene knockout mice suggest that nNOS may be essential in the preservation of left ventricular (LV) structure and contractile function following post infarction-induced heart failure. Recently, we have shown that the protein expression and the activity of nNOS are up-regulated in hypertensive rat heart and exert protective roles against sustained pathologic stimulation. However, underlying cellular mechanisms of nNOS remain unclear. Accordingly, we aim to investigate myocardial phenotypes following chronic nNOS inhibition in sham and angiotension II (Ang II)-induced hypertensive rats (Ang II). Co-infusion of nNOS inhibitor S-Methyl-L-thiocitrulline (SMTC, 28 ng/kg/min) with Ang II (125 ng/kg/min) maintained high systolic and diastolic pressures. However, echocardiographic results showed that SMTC+Ang II significantly increased the diameter of LV septum and posterior wall thickness but reduced end-diastolic dimension compared to sham, sham+SMTC or Ang II. Furthermore, ejection fraction and fractional shortening (systolic function of LV) were increased in SMTC+Ang II, suggesting structural and functional remodeling. Histological staining of longitudinal section of the hearts showed that left ventricular chambers were thickened with excessive interstitial fibrosis and reduced chamber sizes (concentric hypertrophy). In line with the findings, mRNA expressions of ANP and β-MHC (hypertrophic marker genes) were both elevated in LV myocytes from SMTC+Ang II. Transmission electron microscopic images revealed that without changing the length of thick filament, both sarcomere and I-band lengths were significantly elongated in SMTC+Ang II. Our results suggest that nNOS is essential in the maintenance of myocardial structure and function under pressure overload. Importantly, nNOS-regulation of myofilament structure shed light on a new conceptual framework for better understanding of the hypertrophic progression of the hear
Key Words: nNOS, myofilament, hypertrophy

P06-09

Signaling pathway and physiological role of WNK1 in mouse skeletal muscle

Hanul Kim[1,2,3], Ji-Hee Kim[1,2,3], Kyu-Hee Hwang[1,2,3], Kyu-Sang Park[1,2,3], Seong-Woo Jeong[1,2,3], Seung-Kuy Cha[1,2,3], In Deok Kong[1,2,3]

Departments of [1]Physiology and [2]Global Medical Science, and [3]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

WNK (with-no-lysine [K]) kinases are serine/threonine protein kinases with an atypical placement of the catalytic lysine. While WNK1 is highly expressed in skeletal muscle, however, signaling pathways and physiological role of WNK1 in skeletal muscle have been ill-defined. WNK1 and WNK2, not WNK4 are expressed in mouse skeletal muscle. WNK1 is downstream effector of insulin and IGF-1 receptor signaling. Insulin stimulates WNK1 via phosphoinositide-3-kinase-Akt/PKB signaling cascades in differentiated C2C12 mouse skeletal muscle cell-line. The WNK1 expression is significantly decreased in db/db mice, a hyperinsulinemic type II diabetic model compared to that of wild type mice. Furthermore, an expression of downstream effectors of insulin signaling including GLUT4 and is markedly reduced in skeletal muscle of db/db mice. These results suggest that WNK1 function as downstream effector of insulin signaling and regulate glucose transport in skeletal muscle providing new insights for increased susceptibility to insulin resistance and diabetes in skeletal muscle. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A2060764)]
Key Words: WNK1, Skeletal muscle, Insulin

P06-10

Inhibition of Endoplasmic Reticulum Stress Normalizes Augmented Myogenic Responses in Coronary Arteries of the Spontaneously Hypertensive Rats

Soo-Kyoung Choi, Mihwa Lim, Duck-Sun Ahn, Young-Ho Lee

Department of [1]Physiology, College of Medicine, [2]BK 21 Plus Project for Medical Sciences, Yonsei University, C.P.O Box 8044, Seoul,120-752, Korea

Endoplasmic reticulum (ER) stress has been shown to play a critical role in the pathogenesis of cardiovascular complications. However, the role and mechanisms of ER stress in hypertension remain unclear. Thus, we hypothesized that enhanced ER stress contributes to the maintenance of hypertension in spontaneously hypertensive rats (SHRs). We investigated this hypothesis by suppressing ER stress in SHRs and their normotensive controls, Wistar-Kyoto rats (WKYs) with ER stress inhibitor (taurine-conjugated ursodeoxycholic acid, TUDCA). Sixteen-week old male SHRs and WKYs were treated with TUDCA (100 mg/kg/day, IP injection) or PBS (control, 300 μl/day, IP injection) for two weeks. There was a decrease in systolic blood pressure in SHR treated with TUDCA. The pressure-induced myogenic tone was significantly increased,

whereas endothelium-dependent relaxation was significantly attenuated in SHRs compared with WHYs. Interestingly, treatment of ER stress inhibitor normalized myogenic responses and endothelium-dependent relaxation in SHRs. These data were associated with an increase in expression or phosphorylation of ER stress markers (Bip, CHOP, ATF6, XBP-1, IRE1, and eIF2α) in SHRs, which were normalized by TUDCA treatment. Furthermore, phosphorylation of myosin light chain was increased in SHRs, which was reduced by the treatment of TUDCA. In conclusion, ER stress inhibition decreases systolic blood pressure and normalizes myogenic response and endothelium-dependent relaxation in SHRs. Moreover, ER stress inhibition normalizes expression or phosphorylation of ER stress markers, and phosphorylation of myosin light chain in SHRs. Therefore, we suggest that ER stress could be a potential target for hypertension.
Key Words: myogenic tone, endothelium-dependent relaxation, coronary artery, hypertension, ER stress

P06-11(O-6)

Does eNOS-palmitoylation involve in palmitic acid-enhanced cardiac inotropy in rat cardiac myocyte?

Chun Li Jin, Ji Hyun Jang, Yu Na Wu, Zhai Hao Zhao, Sung Joon Kim, Yin Hua Zhang*

Department of Physiology, Seoul National University College of Medicine, Seoul 110-799, Korea

Background and aim: Palmitic acid (PA) is a predominant metabolic substrate for cardiac β-oxidation. Our recent results have shown that PA increases contraction of cardiac myocytes, however, the underlying mechanisms remain unclear. Palmitoylation is an important post-translational modification that affects the translocation and the activity of target proteins in a variety of cell types including cardiomyocytes. Since endothelial nitric oxide synthase (eNOS) is known to be palmitoylated and the activity of eNOS is essential in fatty acid-dependent β-oxidation in muscle, we aim to identify whether eNOS is palmitoylated by PA and the involvement of eNOS-palmitoylation in PA-regulation of cardiomyocyte contraction. **Results:** Our results showed that palmitic acid (PA, 100 μM) significantly increased eNOS palmitoylation but reduced nitric oxide (NO) production 22.12% in LV cardiomyocytes. Pre-treatment of LV myocytes with an inhibitor of palmitoylation, 2-bromopalmitic acid (2BP, 100 μM) significantly reduced eNOS palmitoylation and increased NO production. Inhibition of nNOS with S-methyl-l-thiocitrulline (SMTC, 100 nM, 30min-1hr) or NOS with Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 1 mM, 30min-1hr) reduced NO production further by 12.05%, suggesting that PA reduction of NO are mainly due to eNOS and eNOS palmitoylation is associated with reduction of its activity. Furthermore, PA did not affect total protein expression or the phosphorylation of eNOS (eNOS-Ser^{1177}). Functionally, PA significantly increased myocyte contraction. L-NAME but not SMTC prevented PA-enhanced myocyte contraction. 2BP, on the other hand, failed to affect PA-induced myocyte contraction. **Conclusion:** PA induces eNOS-palmitoylation and attenuates eNOS-dependent NO production. Although eNOS plays a pivotal role in PA-induced myocyte inotropy, our results indicate that eNOS-palmitoylation does not seem to mediate PA-induced cardiac inotropy in normal rat cardiac myocyte.
Key Words: palmitoylation, cardiac myocyte, contraction, eNOS, palmitic acid

P06-12

Beta1-adrenergic receptor antagonist and nitric oxide stimulator, nebivolol, prevents spontaneous contraction induced by metabolic substrates in rat cardiomyocytes

Zai Hao Zhao, Ji Hyun Jang, Jae Hwi Sung, Yin Hua Zhang*

Department of Physiology & Biomedical Sciences, Seoul National University, College of Medicine, Seoul, 110-799 Korea

Nebivolol, a beta1-adrenergic receptor antagonist and nitric oxide (NO) stimulator, has been implicated in the treatment of heart failure, atrial fibrillation and hypertension. Recently, we have established an in vitro model of metabolic syndrome, where metabolic substrates induce delayed after-contractions (DACs) in left ventricular (LV) myocytes from rat heart after beta-adrenergic stimulation with isoprenaline (ISO), a precursor for ventricular arrhythmias. Here, we aim to investigate whether nebivolol affects the occurrence of DACs in this model with supplementation of metabolic substrates in normal Tyrode's (NF). Our results showed that NF increased basal and ISO-stimulation of LV myocyte contraction and induced DACs. The effects of NF were mirrored by increases in the diastolic/peak amplitudes of Ca^{2+} transients ($[Ca^{2+}]_i$) and facilitated time constant of $[Ca^{2+}]^i$ decay (tau). Furthermore, NF significantly reduced the phosphorylation of endothelial nitric oxide synthase at Ser^{1177}, indicating the possibility that impaired eNOS-NO signaling is involved in the greater and abnormal myocyte contraction. Inhibition of constitutive NOS (eNOS and neuronal NOS, nNOS) with Nω-Nitro-L-arginine methyl ester (L-NAME, 1 mM) and S-methyl-L-thiocitrulline (SMTC, 100 nM) maintained greater myocyte contraction or $[Ca^{2+}]^i$ in NF+ISO but significantly increased the occurrence of DACs. Treatment of LV myocytes with nebivolol (100 nM-1 μM) following NF+ISO attenuated myocyte contraction and reduced diastolic/peak amplitudes of $[Ca^{2+}]^i$. Importantly, nebivolol abolished ISO-induced DACs in NF. Similarly, cordycepin (50 μM-100 μM), which was an adenosine mimetic and exerts anti-adrenergic effect, reduced DACs in NF+ISO without changing myocyte contraction. Nebivolol abolishes DACs following beta-adrenergic stimulation with metabolic substrates supplemented. Similar results of nebivolol and cordycepin suggest that NO-signaling in the protective role of nebivolol needs further investigation.
Key Words: nebivolol, fatty acid, arrhythmias, nitric oxide

P06-13

Angiotensin IV protects cardiac reperfusion against via AT4R by inhibiting apoptosis and inflammation

Byung Mun Park[1], Seung Ah Cha[1], Sun Hwa Lee[2], Byung Hyun Park[3], Yuan Kuichang[1], Suhn Hee Kim[1]

Department of [1]Physiology, [2]Internal Medicine, and [3]Biochemistry, Chonbuk National University Medical School, Jeonju, Korea

Angiotensin IV (Ang IV) is formed by aminopeptidase N (APN) from angiotensin III (Ang III) by removing the first N-terminal amino acid. Previously, we reported that Ang III has some cardioprotective effects against global ischemia in Langendorff heart. However, it is not clear whether Ang IV has cardioprotective effects. Here, we investigated the effect of Ang IV on myocardial ischemia-reperfusion (I/R) injury. Before ischemia, Sprague-Dawley rats received Ang IV (1 mg/kg/day) for 3 day. Anesthetized rats were subjected to 45 min of ischemia by ligation of left anterior descending coronary artery followed by reperfusion and rats were sacrificed 1 day after reperfusion. Pretreatment with

Ang IV attenuated I/R-induced increases in plasma creatine kinase (CPK) and lactate dehydrogenase (LDH) concentrations, and infarct size. I/R also caused increases in Bax, caspase-3 and caspase-9 protein levels, and a decrease in Bcl-2 protein level in ventricles, which were attenuated by pretreatment with Ang IV. Co-treatment with AT4R antagonist or inhibitors of downstream signaling pathway including phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and mammalian target of rapamycin (mTOR) attenuated Ang IV-induced changes in CPK and LDH levels, infarct size, and apoptosis-related proteins. Furthermore, I/R increased the expression of TNF-α, MMP-9, VCAM-1, and NF-kB protein levels, which were attenuated by the pretreatment with Ang IV. After co-treatment with AT4R antagonist and inhibitors of downstream signaling pathway, the inflammatory protein levels were increased. Therefore, these results suggest that Ang IV has cardioprotective effect against I/R injury by inhibiting apoptosis and inflammation via AT4R and PI3K-Akt-mTOR pathway. supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No 2008-0062279).
Key Words: Angiotensin IV, ischemia-reperfusion, apoptosis, inflammation

P06-14

Cereblon gene dysfunction improves cardiac performance and mitochondrial energy metabolism in mice

Sujin Noh, Hyoung Kyu Kim, Tae Hee Ko, Seung Hun Jeong, In-Sung Song, Sung Ryul Lee, Hye Jin Heo, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han

National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Department of Health Sciences and Technology, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

Cerebelon (CRBN) is a protein interacting with calcium-actrivated potassium channels. Mutation in this protein causes mild mental retardation in humans. Recent studies have suggested the novel function of the protein as an AMPK inhibitor through a direct interaction with AMPK1a subunit. Disruption in the gene enhances hepatic AMPK activity and halts high-fat diet induced obesity and insulin resistance in mouse model. This study aims to determine the effect of the knockout of CRBN in hearts and its relevance to mitochondria. The body weight, heart rate and heart/body ratio of Control (CRBN+/+) and CRBN KO (CRBN-/-) models (8 weeks) were examined. Echocardiography was used to assess in vivo cardiac functions of animals. To evaluate mitochondrial function, cardiac mitochondria of CRBN+/+ and CRBN-/- were isolated and examined for their ATP contents and ATP production rate, ROS production rate, oxygen consumption rate (OCR) and membrane potential (ΔΨm). Body weight, heart weight and heart/body ratio were not significantly different between CRBN+/+ and CRBN-/- mice. Echocardiography showed enhanced cardiac contractility in CRBN-/- mice as evidenced by increased ejection fraction (%) and fractional shortening (%). Results of the mitochondrial studies showed that basal ATP contents and substrate/ADP-stimulated ATP production rates were significantly higher in CRBN-/- mice than CRBN+/+. Moreover, basal H2O2 level and rotenone-induced ROS production rates were significantly lower in CRBN-/- mice than CRBN+/+. OCR and ΔΨm in both groups were maintained at similar levels. These results suggest CRBN is an important mitochondrial functional regulator which links cytosol to mitochondrial energy metabolic signaling.
Key Words: CRBN, cardiac function, mitochondria

P06-15

DQAsome, a Mitochondria targeting carrrier, shows cardiac toxicity via suppresing cardiac Ca2+ signaling

Hyoung Kyu Kim, Seung Hun Jeong, Tae Hee Ko, Sujin Noh, In-Sung Song, Sung Ryul Lee, Hye Jin Heo, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Joon Sig Choi, Jin Han

Cardiovascualr and Metabolic Disease Center, Colleage of Medicine, Inje University, Busan, Korea

DQAsomes (dequalinium-based liposome-like vesicles) are mitochondria-targeted vesicular pharmaceutical nanocarrier systems. They have been designed for the delivery of DNA and low-molecular weight molecules to mitochondria within living mammalian cells. Although targeting ability of DQAsomes is well known, it had shown lower stability with cell toxicity. To overcome its low-stability and cellular toxicity, we newly developed modified-DQAsomes (DQA0 and DQA80) by using different combination of DOPE and DOTAP molecules. Their mitochondria targeting efficiency, effect on whole heart and isolated cardiac myocyte and effect on mitochondrial function were extensively tested and compared with original DQAsome. As results, all DQAsomes successfully targeted to mitochondria in perfused heart and intraventricular injected hearts. DQAsome and DQA0 showed significant cardiac toxicity which impaired cardiac output, ventricular cell contraction and mitochondrial oxygen consumption rate at doses of 2 or 20 ug/ml. However, DQA80 did not significantly affect the cardiac output, single cardiac myocyte contraction and mitochondrial oxygen consumption. Our results conclude that modified DQAsome-DQA80 significantly improve the targeting efficiency without cardiac and mitochondrial toxicity. DQA80 is the suitable mitochondria targeting carrier which will be used for delivery of DNA and low-molecular weight molecules to mitochondria in clinical application
Key Words: DQAsome, Heart, Calcium, Mitochondria

P06-16

The difference between vascular smooth muscle contraction and relaxation in four different aortic regions and their aortic parameters in rats

Bolor-Erdene Sarankhuu*, Nari Kim*

National Research Laboratory for Mitochondrial Signaling Laboratory, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan 613-735, Korea

Abstract **Objective:** The aortic aneurysm is a common disease with dysfunction of vascular contraction and relaxation. It is mainly found in the abdominal and thoracic aorta but also found in the arch and ascending aortic region to a lesser extent. However, the path-physiological cause of spatial specificity of aortic aneurysm is unclear. To understand this spatial specificity, we tested whether different aortic regions respond differently to contracting and relaxing stimulations. Furthermore, we tested whether there are expressional differences in contractile protein components between the ascending, arch, thoracic and abdominal regions of the aorta. **Methods:** Aortic isometric tension and effect of Phenylephrine (Phe) and acetylcholine (Ach) were measured for the four different aortic regions in Wistar rats. Histological studies of the four different aortic tissues were performed using Hematoxylin-Eosin,Masson'sTrichrome stains for collagen and Verhoeff's stains for elastin.To investigate collagen mRNA and protein expressions, real-time PCR and western blot method was used. **Results:** The Phe-

dose-dependent contraction in aortic strips was significantly different. Theascending aorta showed significantly higher contraction than the three other aortic regions. The Achinduced relaxation in thoracic aortic regions was significantly lower than the three other aortic regions. In the histological study, we observed that the shapeof theascending and archaortic lumens were elliptic, but the abdominal and thoracic aortic lumens were circular. The elliptic areas were divided into thin and thick tunica media.The thickness between thin and thick tunica media area was significantly different in the ascending and arch aorta. The amounts of collagen mRNA and protein expression in the ascending and arch aortic segments are less than in the thoracic and abdominal aortic wall. Verhoeff's stain showed that the lamellar of elastin in the ascending aortic segments was greater than in the thoracic and abdominal aortic tunica media wall. **Conclusion:** These data suggest that the thoracic aorta has lower relaxibility with higher intracellular collagen contents which may relate with higher occurrence of aneurysm in this aortic region.
Key Words: Ascending, Arch, Thoracic, Abdominal, collagen fiber, elastic fiber

P06-17

Role of Formyl Peptide Receptors on Mobilization of Peripheral Blood Stem Cells in Myocardial Ischemia Injury

Soon Chul Heo[1], Yang Woo Kwon[1], Geun Ok Jeong[1], Jung Won Yoon[1], Tae Wook Lee[1], Il Ho Jang[1], Jae Kyung Park[1], Jae Ho Kim[1,2]

[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-870, Gyeongsangnam-do, Republic of Korea, [2]Research Institute of Convergence Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan 626-770, Gyeongsangnam-do, Republic of Korea

Increasing evidence suggests that circulating bone marrow-derived stem cells promote repair of ischemic tissues. However, the role of FPR2 in mobilization of bone marrow-derived stem cells and repair of myocardial infarction has not been explored. This study was undertaken to investigate the role of FPR2 on mobilization of bone marrow stem cells and repair of infarcted heart. WKYMVm, which is a specific agonist for FPR2, was administered in a murine model of acute myocardial infarction and the percentages of subpopulations (CD34+/Flk1+ or Sca1+/Flk1+ cells) of peripheral blood stem cells (PBSCs) in peripheral blood were quantified by fluorescence-activated cell sorter analysis. Intraperitoneal injection of WKYMVm stimulated mobilization of CD34+/Flk1+ or Sca1+/Flk1+ PBSCs with a peak stimulation on day 4. Moreover, WKYMVm administration prevented tissue damage and improved cardiac function. In mice transplanted with bone marrow derived from GFP transgenic mice, WKYMVm stimulated homing of GFP-positive bone marrow cells into infarcted heart and formation of GFP-positive blood vessels. WKYMVm-induced mobilization of PBSCs and repair of infarcted heart were abrogated in FPR2-knockout mice, but not in FPR1-knockout mice. Furthermore, WKYMVm-induced mobilization of PBSCs and repair of infarcted heart were completely abrogated in wild type mice transplanted with bone marrow from FPR2-knockout mice, but not in FPR2 knockout mice transplanted with bone marrow from wild type mice. These results suggest that FPR2 is a new receptor regulating mobilization of PBSCs from bone marrow into peripheral blood and FPR2 activation can be beneficial for therapy of myocardial infarction.
Key Words: mobilization, formyl peptide receptor, stem cells, myocardial infarction

P06-18

Antihypertensive effects of fermented garlic extract through NO-cGMP-PKG pathway in SHR

Byung Mun Park[1], Seung Ah Cha[1], Yuan Kuichang[1], De Gil Kang[1], Suhn Hee Kim[1]

[1]Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea, [2]Department of Physiology, Wonkwang University Oriental Medicine, Iksan, Korea

Hypertension can cause a variety of complications and serious problem to threaten the lives and health directly. Garlic (Allium sativum L.) has long been used as an antihypertensive and has been reported to have antioxidant, anti-thrombotic, anti-inflammatory and anti-cancer effects. The aim of the present study was to investigate the effect of fermented garlic extract (FGE), which contains high level of stable nitrite, on blood pressure and its mechanism in spontaneously hypertensive rats (SHR). Acute feeding of FGE reduced systolic blood pressure (SBP) showing the peak level at 30 min and recovery to control level within 2 to 3 hr depending on dosage. Chronic feeding of FGE for 2 weeks reduced significantly SBP, right ventricular hypertrophy and BNP mRNA expression. The expressions of endothelial nitric oxide synthase (eNOS) and protein kinase G (PKG) proteins in aortic tissues were significantly increased and aortic cGMP concentration was also increased by chronic feeding of FGE. The anti-hypertensive effect as well as increased eNOS and PKG protein expressions by FGE feeding were attenuated by ODQ, a soluble guanylyl cyclase (sGC) inhibitor. By intake of FGE, the relaxation response of thoracic aorta to acetylcholine and sodium nitroprusside was significantly accentuated in Wistar-Kyoto rats (WKY) and tended to accentuate in SHR without significance. FGE also reduced SBP in WKY. Therefore, FGE with high nitrite have antihypertentive effect through eNOS, sGC, PKG and cGMP pathway in SHR. supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No 2008-0062279).
Key Words: fermented garlic extract, hypertension, nitrite, eNOS, cGMP

P06-19

Echinochrome A inhibits vascular smooth muscle cell phenotype changing

Kyowon Seo, Seunghun Jeong, Jin Han, Nari Kim

Cardiovascular and Metabolic Disease Center (CMDC), National Research Laboratory for Mitochondrial Signaling, Inje University, Busan 614-735, Korea

Phenotype modulation of vascular smooth muscle cells (VSMCs) plays an important role in the pathogenesis of various vascular diseases including atherosclerosis. Platelet-derived growth factor (PDGF) initiates the various biological effects that contribute to VSMC phenotype changing in progression of atherosclerosis. Here we report that echinochrome A (Ech A) which is a naphthoquinoid pigment from sea urchins, has inhibits effect of the VSMC phenotype changing. Increased synthetic states of VSMC by PDGF-BB were initiated with osteopontin (OPN), and under the Ech A treatment, synthetic states of VSMC exhibited were decreased. Increased VSMC proliferation by PDGF-BB was attenuated by Ech A pretreatment in a dose and time dependent manners. Furthermore, under the Ech A treatment, VSMC exhibited decreased phosphorylation of protein kinase B (Akt) and mTOR, Taken together, Ech A treatment may inhibits VSMC's phenotype changing by PDGF-BB via Akt and mTOR signaling pathways. Therefore, Treatment of Ech A may be a potential therapeutic strategy for the prevention

of vascular remodeling in proliferative vascular diseases, including atherosclerosis.
Key Words: VSMC, Echinochrome A, Phenotype, Atherosclerosis

P06-20

The effect of microRNA-34c on angiogenesis capacity of high glucose-insulted mesenchymal stem cells

Yong Sook Kim[1], Youngkeun Ahn[2]

[1]Biomedical Research Institute, Chonnam National University Hospital, Gwangju, South Korea [2]Department of Cardiology, Chonnam National University Hospital, Gwangju, South Korea

Rationale: MicroRNAs (miR) are actively involved in the regulation of the physiological function of stem cells. Angiogenesis of stem cells are affected by diabetic stress, and we studied the role of miR in high glucose-stressed MSCs. **Materials and Methods:** We found miR-34c was induced by high glucose in MSCs. Stem cell factor (SCF) is the targets of miR-34c, and was studied in human bone marrow mesenchymal stem cells (BMCs). In vitro angiogenesis was assessed by Matrigel assay. Myocardial infarction (MI) was induced in nude mouse, and BMCs were injected 7 days later. **Results:** In vitro angiogenesis assay showed that miR-34c impaired tube formation of BMC, and SCF was confirmed as a target of miR-34c. In miR-34c transfected BMCs, the levels of mRNA and protein of SCF were decreased. Additionally, SCF knockdown by siRNA resulted in the blockade of in vitro angiogenesis. Kruppel-like factor 4 (KLF4) was unexpectedly induced by both SCF knockdown and miR-34c overexpression in BMCs. In KLF4-overexpressed BMCs, tube formation was completely blocked, while the level of SCF was not changed. When KLF4 was knockdowned by siRNA, miR-34c failed to inhibit tube formation in BMCs. From these results, miR-34c was suggested to target SCF to inhibit angiogenesis, and KLF4 might be a downstream effector of SCF in BMCs. In order to determine whether the therapeutic potential of BMCs was influenced by miR-34c, mouse MI model was used. BMCs were transfected with miR-control or miR-34c and injected into the myocardium. Cardiac fibrosis was 18.24±4.70% in miR-34c-BMC group and 10.01±0.18% in miR-con-BMC group. Fluorescence immunostaining for vWF revealed decreased blood vessel density in the miR-34c-BMC group as compared with the miR-con-BMC group. Capillary density was also smaller in the miR-34c-BMC group than in the miR-con-BMC group. Collectively, miR-34c negatively regulated the angiogenic potential through inhibition of SCF, and subsequent induction of KLF4 in BMCs. **Conclusion:** Our results show that the angiogenic activity of BMCs is finely regulated by miR-34c-SCF-KLF4 axis which is a potent translational target for optimizing therapeutic activity of autologous BMCs for cardiac repair.
Key Words: Mesenchymal Stem Cells, Angiogenesis, Myocardial infarction, High glucose, MicroRNA-34c

P07-01

Epigallocatechin-3-Gallate Rescues LPS-impaired Adult Hippocampal Neurogenesis through Suppressing the TLR4-NF-κB Signaling Pathway in Mice

Kyung-Joo Seong[1,2], Hyun-Gwan Lee[1,2], Hyun-Mi KO[3], Min Suk Kook[4], Ji-Yeon Jung[1,2], Won-Jae Kim[1,2,**]

[1]Dental Science Research Institute, Medical Research Center for Biomineralization Disorders, [2]Department of Oral Physiology, [3]Department of Oral and Maxillofacial Surgery, School of Dentistry, Chonnam National University, Gwangju 500-757, Republic of Korea, [4]Department of Microbiology, collage of Medicine, Seonam Universtity, Namwon 55724, Republic of Korea

Adult hippocampal dentate granule neurons are generated from neural stem cells (NSCs) in the mammalian brain, and the fate specification of adult NSCs is precisely controlled by the local niches and environment, such as the subventricular zone (SVZ), dentate gyrus (DG), and Toll-like receptors (TLRs). Epigallocatechin-3-gallate (EGCG) is the main polyphenolic flavonoid in green tea that has neuroprotective activities, but there is no clear understanding of the role of EGCG in adult neurogenesis in the DG after neuroinflammation. Here, we investigate the effect and the mechanism of EGCG on adult neurogenesis impaired by lipopolysaccharides (LPS). LPS-induced neuroinflammation inhibited adult neurogenesis by suppressing the proliferation and differentiation of neural stem cells in the DG, which was indicated by the decreased number of Bromodeoxyuridine (BrdU)-, Doublecortin (DCX)- and Neuronal Nuclei (NeuN)-positive cells. In addition, microglia were recruited with activatingTLR4-NF-κB signaling in the adult hippocampus by LPS injection. Treating LPS-injured mice with EGCG restored the proliferation and differentiation of NSCs in the DG, which were decreased by LPS, and EGCG treatment also ameliorated the apoptosis of NSCs. Moreover, pro-inflammatory cytokine production induced by LPS was attenuated by EGCG treatment through modulating the TLR4-NF-κB pathway. These results illustrate that EGCG has a beneficial effect on impaired adult neurogenesis caused by LPS-induced neuroinflammation, and it may be applicable as a therapeutic agent against neurodegenerative disorders caused by inflammation.
Key Words: Epigallocatechin-3-gallate, Neuronal Inflammation, Neural stem cells, Adult Neurogenesis, TLR4, NF-κB signaling

P07-02

The effect of BD1047 in CCL2 mediated microglia activation in zymosan induced hyperalgesia in rats

Young Bae Kwon

Department of pharmacology, Medical School, Chonbuk National University, Jeonju, Korea

Although intrathecal blockage of sigma-1 receptor (Sig-1R) produces a potent anti-nociception through neuronal regulation in several pain models, it is unclear interactions between neuron and microglia under inflammatory condition. Here we are aimed to address anti-nociceptive mechanism of BD1047 (a selective Sig-1R antagonist) through regulation of chemokine CCL2 mediated microglia activation. Intraplantar injection of zymosan in rats elevated spinal microglia activation with phosphorylated p38 (p-p38), and increased CCL2 immunoreactivity in dorsal root ganglion (DRG) but not in spinal neurons and glia. Intrathecal blockage of CCL2 reduced zymosan-induced hyperalgesia (evoked by thermal and mechanical stimuli) accompanying spinal Fos elevation as well as microglia/p-p38 activation. In spinal cord slice patch-clamp, incubation of CCL2 significantly increased inward current and p-p38 expression, which was reversed by pretreatment of microglia inhibitor (minocycline). RT-PCR and immunohistochemical study revealed that Sig-1R was predominantly located in DRG, which was overlapped with CCL2. In DRG primary culture, zymosan dose-dependently increased secretion and synthesis of CCL2 with reversed by BD1047. Oral administration of BD1047 dose-dependently inhibited zymosan-evoked hyperalgesia as well as CCL2 elevation, microglia/p-p38 activation. Taken together, our results indicated that anti-

nociception of Sig-1R antagonist in inflammatory pain was mediated by the blockage of CCL2 induced microglia activation in the spinal level.
Key Words: sigma-1 receptor, zymosan, CCL2, microglia, hyperalgesia

P07-03

Repetitive motor cortex stimulation for the chronic neuropathic pain

Myeounghoon Cha, Bae Hwan Lee

Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea

Electrical stimulation of the motor cortex is used for reducing spontaneous pain behaviors in animal model of neuropathic pain, but the mechanism of action is still unclear. Previous our report show the pain behaviors are associated with abnormal inhibition in the inhibitory nucleus zona incerta and motor cortex stimulation (MCS) produces antinociception by activating the incertothalamic pathway. We hypothesized that the antinociceptive effects of MCS are due to enhanced long term potentiation (LTP) signaling in the anterior cingulate cortex (ACC). To test this hypothesis, we used a rodent model of neuropathic pain and performed 30 min MCS (50 μA, 50 Hz; 300 μs pulses) for continuative 10 days. The behavioral tests were performed before and after MCS. We found that MCS reduced mechanical threshold immediately after MCS and repetitive MCS suppressed pain-like behavior up to 3 weeks. Whether MCS contribute the pain-related LTP or not, the zeta inhibitory peptide (ZIP, inhibits PKMζ) was injected in the ACC. The comparison of ZIP and vehicle injection, ZIP injection with MCS did not show the mechanical threshold changes. These results support our hypothesis and suggest that MCS produces the synaptic change in the ACC area and mediates the LTP. This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Science, ICT & Future Planning (No. 2015021989).
Key Words: motor cortex stimulation, anterior cingulate cortex, neuropathic pain, ZIP, behavioral test

P07-04

Maresin 1 inhibits TRPV1 in temporomandibular joint (TMJ)-related trigeminal nociceptive neurons and TMJ inflammaion-induced synaptic plasticity in the trigeminal nucleus

Sang Taek Im, Jee Eun Lee, Chul-Kyo Park

Department of Physiology, College of Medicine, Gachon University, Incheon 406-799, Republic of Korea

In the trigeminal system, disruption of acute resolution processing may lead to uncontrolled inflammation and chronic pain associated with the temporomandibular joint (TMJ). Currently, there are no effective treatments for TMJ pain. Recently, it has been recognized that maresin 1, an endogenous pro-resolution lipid mediator that is derived from the addition of docosahexaenoic acid to macrophages, is a potent analgesic for somatic inflammatory pain without noticeable side effects in mice and a potent endogenous inhibitor of transient receptor potential vanilloid 1 (TRPV1) in the somatic system. However, the molecular mechanisms underlying the analgesic actions of maresin 1 on TMJ pain are unclear in the trigeminal system. Here, by performing TMJ injection of a retrograde labeling tracer DiI (a fluorescent dye), I showed that maresin 1 potently inhibits capsaicin-induced TRPV1 currents and neuronal activity via G i-coupled G-protein coupled receptors in TMJ-related trigeminal nociceptive neurons. Further, maresin 1 blocked TRPV1 agonist-evoked increases in spontaneous excitatory post-synaptic current (sEPSC) frequency and abolished TMJ inflammation-induced sEPSC increases (frequency and amplitude) in the trigeminal nucleus. These results demonstrate the potent actions of maresin 1 in regulating TRPV1 in TMJ-related trigeminal nociceptive neurons and TMJ inflammation-induced synaptic plasticity in the trigeminal nucleus. Therefore, these new findings suggest that maresin 1 may serve as a novel endogenous inhibitor for treating TMJ-inflammatory pain in the orofacial region.
Key Words: temporomandibular joint, maresin 1, trigeminal ganglion neuron, TRPV1, synaptic plasticity

P07-05

Neuroprotective Effects of Okadaic Acid Following Oxidative Injury in Organotypic Hippocampal Slice Culture

Un Jeng Kim[1,*], Kyung Hee Lee[2], Bae Hwan Lee[1,3,†]

[1]Department of Physiology, Yonsei University College of Medicine, Seoul 03722, Korea, [2]Department of Dental Hygiene, Division of Health Science, Dongseo University, Busan 47011, Korea, [3]Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul 03722, Korea

Oxidative stress produces neurotoxicity often related with various CNS disorders. A phosphatase inhibitor enhances the actions of the signaling kinases. Protein kinases mediated-action shows the neural protection in brain injury. Phosphatase inhibitor, okadaic acid (OA), may enhance the protection effect and benefit to improve neuronal plasticity in post-injury. Thus, we investigated that the protein prophatase inhibitor affects neuroprotective signaling and neuroplastic changes in hippocampus after oxidative injury. Electrophysiological and biochemical assays were used to observe changes in synaptic efficacy following electrical and/or pharmacological manipulation of synaptic function. Neuronal cell death, as assessed by propidium iodide (PI) uptake, was reduced by OA treatment (24 and 48 h) compared with KA treatment. The pattern of DCFH-DA fluorescence in hippocampal slices corresponded well with PI uptake. The phospho-AKT/AKT ratio showed that the level of phospho-AKT was significantly increased in the OA-treated group. Furthermore, the OA-treated group exhibited significantly increased expression of SOD2 compared with the KA-only group. Optical imaging revealed that KA treatment tended to delay the latency of electrical stimulation and decrease the amplitude of optical signals of synaptic activity. These results suggest that OA may protect hippocampal neurons against oxidative stress and the survived neurons may functional to synaptic plasticity changes.
Key Words: hippocampal slice culture, okadaic acid, oxidative injury, optical imaging, synaptic plasticity

P07-06

NDL-PCBs inhibit store-operated Ca2+ entry

Se-Young Choi[1,2], Keimin Lee[1], Yurim Park[3], Seung-Hyun Lee[1], Mi-Hyeong Park[4], Sungkwon Chung[3], Kyong-Tai Kim[2], Su-Hyun Jo[4]

[1]Department of Physiology, Dental Research Institute, Seoul National

University School of Dentistry, Seoul 110-749, Korea, [2]Department of Life Sciences, Division of Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang 790-784, Korea, [3]Department of Physiology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea, [4]Department of Physiology, Institute of Bioscience and Biotechnology, BK21 plus Graduate Program, Kangwon National University College of Medicine, Chuncheon 200-701, Korea

Polychlorinated biphenyls (PCBs) are ubiquitous pollutants which accumulate in the food chain. Recently, several molecular mechanisms by which non-dioxin-like (NDL) PCBs mediate neurodevelopmental and neurobehavioral toxicity have been elucidated. However, although the G-protein coupled receptor (GPCR) is a significant target for neurobehavioral disturbance, our understanding of the effects of PCBs on GPCR signaling remains unclear. In this study, we investigated the effects of NDL-PCBs on GPCR-mediated Ca2+ signaling in PC12 cells. We found that ortho-substituted 2,2′,6-trichlorinated biphenyl (PCB19) caused a rapid decline in the Ca2+ signaling of bradykinin, a typical Gq- and phospholipase C-coupled GPCR, without any effect on its inositol 1,4,5-trisphosphate production. PCB19 reduced thapsigargin-induced sustained cytosolic Ca2+ levels, suggesting that PCB19 inhibits SOCE. The abilities of other NDL-PCBs to inhibit store-operated Ca2+ entry (SOCE) were also examined and found to be of similar potencies to that of PCB19. PCB19 also showed a manner equivalent to that of known SOCE inhibitors. PCB19-mediated SOCE inhibition was confirmed by demonstrating the ability of PCB19 to inhibit the SOCE current. These results imply that one of the molecular mechanism by which NDL-PCBs cause neurobehavioral disturbances involves NDL-PCB-mediated inhibition of SOCE, thereby interfering with GPCR-mediated Ca2+ signaling.
Key Words: Polychlorinated biphenyl, non-dioxin-like, G-protein coupled receptor, Ca2+ signaling, store-operated Ca2+ entry

P07-07

The effect of ultrasound stimulation on neurogenesis

Dough Kim[1,2], Ha-Jeong Kim[1,2,3], Hak Jong Lee[4], Hyung Soo Han[1,2,*]

[1]Department of Physiology, School of Medicine, Kyungpook National University, Daegu 702-422, Republic of Korea, [2]BK21 Plus KNU Biomedical Convergence Program, Department of Biomedical Science, Kyungpook National University, Korea, [3]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea, [4]Department of Radiology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam 463-707, Korea

Adult neurogenesis is a process involving the generation, development and integration of new neurons in the brain, and it is increased under pathological stimulation, such as traumatic brain injuries. In physiological conditions, neural progenitor cells are involved in the formation of long-term memories. It is reported that non-harmful stimulation, such as random noise, increases learning and memory. Ultrasound can promote tissue regeneration via improvement of blood circulation, stimulation of angiogenesis and acceleration of woud healing and enhancement of ultimate mechanical strength. Effects of ultrasound on the proliferation and differentiation of other stem cells have gained attention. However, the effect of neurogenesis is not studied well. In this reports, we hypothesized that ultrasound stimulation causes induced neural stem cells(NCSCs) proliferation, differentiation and astrocyte activation. To examine that, we checked IGF-1 and IGFR expression on NSCs after ultrasound stimulation to 8 weeks old male rat. We also checked that apoptosis and proliferation near subventricular zone(SVZ). IGFR expression, not IGF-1, is increased on NSCs. Apoptosis of neural stem cell (NSC)/neural progenitor cell(NPC) is also decreased. Taken together, we make the conclusion that ultrasound stimulation on brain can cause neurogenesis, therefore, ultrasound treatment can be used as an efficient and cost-effective method to enhance neurogenesis.
Key Words: Neural Stem cell, Neurogenesis, Ultrasound, Astrocyte, IGF-1, IGFR

P07-08(O-7)

Necrotic cells Influence Migration and Proliferation of Glioblastoma cells through NF-κB/IL-8 Signaling

So-Hee Ahn, Hyunju Park, Jiwoo Lim, Yieun Jung, Jihee Lee Kang, Youn-Hee Choi

Department of [1]Physiology, Ewha Womans University School of Medicine, Seoul 911-1, Korea, [2]Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

Glioblastoma multiform (GBM), derived from astrocytes, is the most common adult primary intracranial tumor. GBM has a very poor prognosis fatal with a median survival of less than 14 months. Remarkable property of GBM is diffuse infiltration into normal brain parenchyma, rapid growth and central necrosis. However, the effect of these necrotic tissues on GBM growth and metastasis is poorly understood at present. In this study, we examined the biological significance of the existence of necrotic tissues through exploring the molecular mechanisms underlying signaling network between necrotic tissues and GBM cells. Scratch wound healing assay showed that the migration of GBM cell line CRT-MG was significantly enhanced by treatment of necrotic cells. Co-culture with necrotic cells induced IL-8 secretion in CRT-MG in dose-dependent manner. Immunohistochemical analysis for IL-8 in human GBM tissues, showed that positively stained cells were mainly distributed along necrotic core. Necrotic cells induced NF-κB activation and its DNA binding to IL-8 promoter, leading to enhanced IL-8 production and secretion in GBM cells. Our study demonstrates that when GBM cells are exposed and stimulated by necrotic cell, the migration and proliferation of GBM cells are enhanced and facilitated through NF-κB/IL-8 signaling pathway.
Key Words: Glioblastoma multiform(GBM), necrosis, IL-8

P07-09

Utilizing Ultrasound to Transiently Increase Blood-Brain Barrier Permeability, Modulate of the Tight Junction Proteins, and Alter Cytoskeletal Structure

Mi Jung Bae[1,2], Young Mi Lee[1], Su Yeon Ryu[1,2,4], Yeoun Hee Kim5, Hyung Soo Han[1], Hak Jong Lee[3]

Department of [1]Physiology, [2]BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University School of Medicine, 101 Dongin 2 Ga, Jung Gu, Daegu 700-422, Korea Department of [3]Radiology, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82 Gumi-ro 173 beon-gil, Bundang-gu, Seongnam 463-707, Korea, [4]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-

842, Korea 5Cheil eye research institute, Cheil eye hospital, 1 Ayang-ro, Dong-gu, Daegu 701-820, Korea

The central nervous system is protected by the blood-brain barrier (BBB). The tight junction (TJ) proteins claudin-5 and zonula occludens-1 (ZO-1) as well as the cytoskeletal component F-actin play key roles in maintaining homeostasis of the BBB. Increases in BBB permeability may be beneficial for the delivery of pharmacological substances into the brain. Therefore, here, we assessed the use of ultrasound to induce transient enhancement of BBB permeability. We used fluorescein isothiocyanate (FITC)-dextran 40 to detect changes in the membrane permeability of bEnd.3 cells during ultrasound treatment. Ultrasound increased FITC-dextran 40 uptake into bEnd.3 cells for 2–6 h after treatment; however, normal levels returned after 24 h. An insignificant increase in lactate dehydrogenase (LDH) leakage also occurred 3 and 6 h after ultrasound treatment, whereas at 24 h, LDH leakage was indistinguishable between the control and treatment groups. Expression of claudin-5, ZO-1, and F-actin at the messenger RNA (mRNA) and protein levels was assessed with real-time polymerase chain reaction and western blotting. Ultrasound induced a transient decrease in claudin-5 mRNA and protein expression within 2 h of treatment; however, no significant changes in ZO-1 and F-actin expression were observed. Claudin-5, ZO-1, and F-actin immunofluorescence demonstrated that the cellular structures incorporating these proteins were transiently impaired by ultrasound. In conclusion, our ultrasound technique can temporarily increase BBB permeability without cytotoxicity to exposed cells, and the method can be exploited in the delivery of drugs to the brain with minimal damage.
Key Words: Blood-brain barrier (BBB), Tight junction protein, Ultrasound, Permeability, cytotoxicity.

P07-10

PAMAM dendrimer-conjugated TA attenuates mechanical allodynia by inhibiting spinal cord microglia activation

Hwisung Kim[1], Hyoungsub Lim[1], Hyunjung Min[1], Sunghyoun Choi[2], Jong-sang Park[2], Sung Joong Lee[1,*]

[1]Department of Neuroscience and Physiology, Dental Research Institute, BK21-Plus, School of Dentistry, Seoul National University, Seoul 03080, Republic of Korea, [2]School of Chemistry and Molecular Engineering, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul, 08826, Republic of Korea

Neuropathic pain is a pathological pain with allodynia and hyperalgesia that is caused by sensory neuron damage such as peripheral nerve injury (PNI). The activation of spinal cord microglia is critical for the development and maintenance of neuropathic pain after PNI. Our previous study showed that triamcinolone acetonide (TA) inhibits microglia activation. However, TA has a limitation in clinical application due to its off-target side effects. To obviate this problem, we developed polyamidoamine (PAMAM) dendrimer-conjugated TA (D-TA), which supposedly delivers TA specifically into microglia. PAMAM dendrimer is a sphere-like shape nano-molecule. In this study, we show that PAMAM-dendrimer is selectively delivered into spinal cord microglia. Intratheal D-TA injection inhibited nerve injury-induced spinal cord microglia activation. D-TA administration reduced mRNA expression of proinflammatory cytokines, such as Nox2, IL-1b, TNFa, and IL-6 in spinal cord after PNI. In addition, D-TA administration significantly attenuacted PNI-induced mechanical allodynia. Conclusively, our data demonstrate that D-TA attenuates neuropathic pain after PNI by inhibiting spinal cord microglia activation, suggesting a therapeutic implication for the treatment of neuropathic pain.
Key Words: neropathic pain, Dendrimer, microglia

P07-11

Activation of satellite glia after peripheral nerve injury induces spinal cord microglia activation and neuropathic pain via ganglioside-TLR2 signaling

Hyoung Sub Lim[1,2], Hyun Kyoung Lee[1], Kyung Chul Noh[1], Byung Hyun You[1], Jae Hoon Oh[3], Hyuck Jun Mok[4], Byung Gon Kim[5], Jong-Sang Park[3], Kwang Pyo Kim[4], Sung Joong Lee[1,2,*]

[1]Department of Neuroscience and Physiology, Dental Research Institute, BK21-Plus, School of Dentistry, Seoul National University, Seoul 110-799, Republic of Korea, [2]Interdisciplinary Program in Neuroscience, College of Natural Science, Seoul National University, Seoul 151-747, Republic of Korea, [3]Department of Chemistry, Seoul National University, Seoul 151-747, Republic of Korea, [4]Department of Applied Chemistry, College of Applied Sciences, Kyung Hee University, Yongin 446-701, Republic of Korea, [5]Departments of Brain Science and Neurology, Ajou University School of Medicine, Suwon 443-721, Republic of Korea

Increasing evidence supports the notion that both microglia and satellite glial cell (SGC) activation play important roles in the development of neuropathic pain after peripheral nerve injury, yet neither the activation mechanisms nor their relative contributions to neuropathic pain have been elucidated. To address this issue, we generated SGC-specific Ikkβ conditional knock-out mice in which IKK/NF-κB-dependent proinflammatory SGC activation is abrogated. In these mice, nerve injury-induced proinflammatory gene expression and macrophage infiltration into the DRG were severely compromised. Likewise, nerve injury-induced spinal cord microglia activation and pain hypersensitivity were significantly attenuated in these mice compared to control mice. However, macrophages recruited into the DRG per se have minimal effects on spinal cord microglia activation suggesting a direct causal effect of SGC activation on spinal cord microglia activation. As an underlying mechanism, we found that SGC activation induces St3gal2 expression in sensory neurons and a subsequent increase in ganglioside GT1b in their afferent axons in the dorsal horn. Studies using St3gal2 knock-out mice indicated that aberrant GT1b increase in the spinal cord is required for the nerve injury-induced spinal cord microglia activation and pain hypersensitivity. Finally, GT1b induced pain-mediating gene expression in primary microglia via direct binding to microglial toll-like receptor 2 (TLR2). Taken together, we present a novel mechanism for spinal cord microglia activation in nerve injury-induced neuropathic pain that is dependent on SGC activation, GT1b increase in the dorsal horn, and activation of microglial TLR2.
Key Words: Neuropathic pain, Microglia, Satellite glia, IkB kinase, ganglioside

P07-12

Spinal leptin enhances NMDA receptor-mediated tactile hypersensitivity via the reactive oxygen species-phosphatidylinositol 3-kinase (ROS-PI3K) pathway in neuropathic rats

Se Jung Jung[1], Euichan Lee[1,2], Jae Beom Jun[1,2], Min Kyung Ko[1], Joong Woo Leem[1,2]

Department of [1]Physiology, [2]Brain Research Institute, Yonsei University College of Medicine, Seoul 120-752, Korea

Recent studies have shown that leptin (an adipocytokine) played an important role in influencing neuropathic pain. It was revealed that leptin enhanced NMDA-induced spinal neuronal excitation. We have previously displayed that upregulation of phosphatidylinositol (3,4,5)-triphosphate (PIP3) levels via reactive oxygen species (ROS) in the dorsal horn was involved in tactile hyperalgesia seen in neuropathic rats. In the present study, we investigated whether leptin aggravated NMDA receptor-mediated neuropathic pain behavior and, if so, whether this leptin-induced effect was mediated through the ROS-phosphatidylinositol 3-kinase (PI3K) pathway. Tactile hyperalgesia of the hind paw, evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was induced using naive rats either by lumbar 5 spinal nerve ligation (L5 SNL) or by intrathecal (i.t.) administration of leptin or glutamate. The L5 SNL-induced tactile hyperalgesia was attenuated by i.t. administration of leptin antagonist, NMDA antagonist MK-801, ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN), or PI3K inhibitor LY294002. When intrathecally administered in naive rats, both leptin and glutamate induced tactile hyperalgesia. Leptin and glutamate administered together induced more severe tactile hyperalgesia than glutamate alone. Leptin-induced tactile hyperalgesia was attenuated by MK801. Both leptin-induced and glutamate-induced tactile hyperalgesia were attenuated by either ROS scavenger PBN or LY294002. The results suggested that spinal leptin enhanced NMDA receptor-mediated tactile hyperalgesia, via the ROS-PI3K pathway, in the neuropathic state. The research was supported by a grant from the Korea Health technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A120254).
Key Words: leptin, NMDA receptors, reactive oxygen species, phosphatidylinositol-4,5-bisphosphate 3- kinase, neuropathic pain

P07-13

Spinal D-serine induces increase in GluN1 phosphorylation and nociception via nNOS activation in mice: involvement of sigma-1 receptors

Sheu-Ran Choi[1], Ji-Young Moon[2], Soon-Gu Kwon[1], Hoon-Seong Choi[1], Mi-Ji Lee[1], Ho-Jae Han[1], Jang-Hern Lee[1]

[1]Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea, [2]KM Fundamental Research Division, Korea Institute of Oriental Medicine, Daejeon 305-811, Republic of Korea

We have recently demonstrated that increased D-serine in the spinal cord by sigma-1 receptor (Sig-1R) activation mediates neuropathic pain in mice. Here we examine the role of D-serine on the enhancement of NMDA receptor function and nociception via increase in phosphorylation of GluN1 subunit (pGluN1) and nNOS activation. NMDA-induced pain behaviors were assessed for a 10-min period after intrathecal (i.t.) NMDA administration. Neuropathic pain was produced by chronic constriction injury (CCI) of sciatic nerve in mice. Mechanical allodynia (MA) and thermal hyperalgesia (TH) were evalutated in CCI-mice. Western blotting and NADPH-diaphorase staining were performed to assess the changes in pGluN1, GluN1 expression and nNOS activation in the spinal cord. I.t. administration of the D-serine degrading enzyme, DAAO attenuated the facilitation of NMDA-induced nociception occured by the Sig-1R agonist, PRE084. Exogenous D-serine facilitates NMDA-induced nociception with a dose-dependent manner and increases in PKC-dependent pGluN1 expression, which were attenuated by preteatment with the nNOS inhibitor, 7-nitroindazole. In CCI-mice, i.t. administration with exogenous D-serine during the induction phase of neuropathic pain restored MA and pGluN1 expression suppressed by the Sig-1R antagonist, BD1047. The serine racemase inhibitor, LSOS or DAAO treatment increased the ratio of phosphorylated nNOS to nNOS expression and decreased the number of NADPH-diaphorase-positive neurons in the spinal cord dorsal horn of CCI-mice. This treatment also attenuated CCI-induced MA and pGluN1 expression, which were restored by the NO donor, SIN-1. I.t. administration with 7-nitroindazole dose-dependently attenuated CCI-induced MA and pGluN1 expression. By contrast, D-serine and nNOS signaling had no effect on CCI-induced TH and GluN1 expression. Collectively, spinal D-serine modulates nNOS activation and NO-induced increase in PKC-dependent pGluN1 expression, and ultimately contribute to the Sig-1R-induced pain facilitation and the peripheral nerve injury-induced induction of chronic neuropathic pain.
Key Words: D-serine, Sigma-1 receptor, neuronal NOS, GluN1 phosphorylation. neuropathic pain

P07-14

Astrocyte gap junction contribute to development of mirror-image mechanical allodynia in peripheral inflammatory rat: Suppressive effect of spinal interleukin-1β on connexin 43 expression

Hoon-Seong Choi, Sheu-Ran Choi, Soon-Gu Kwon, Mi-Ji Lee, Ho-Jae Han, Jang-Hern Lee*

Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Mirror-image pain (MIP) is abnormal pathologic phenomenon which damage on one side of the body can also result in pain from the contralateral unaffected side. Though MIP is known to be mediated by intercellular interactions in CNS, the exact mechanisms underlying the development and modulation of MIP are still unknown. We recently demonstrated that peripheral inflammation induces spinal IL-1β expression which inhibits the astrocyte activation and mediates delayed development of MIP. Here we examined the contribution of spinal IL-1β to astrocyte gap junction and development of MIP. After carrageenan (CA) injection into hindpaw of rats, mechanical allodynia (MA) was evaluated at each time point. Immunohistochemistry and Western blot assay were used to determine the changes of GFAP or connexin (Cx) subtypes expression in the spinal dorsal horn. i.t. injection of carbenoxolone (CBX; a gap junction decoupler) or Gap26 (Cx43 mimetic peptide) at days 0-3 post-CA injection blocked the development of contralateral MA. The Cx43 and GFAP expression was upregulated after CA injection, which was reversed by i.t. CBX administration. Notably, i.t. injection of interleukin-1 receptor antagonist (IL-1ra) at days 0-3 post-CA injection significantly advanced the appearance of contralateral MA and increased Cx43 and GFAP expression compared to that of control rats. However, i.t. CBX or Gap26 restored IL-1ra-induced contralateral MA. Furthermore, IL-1ra-induced increased expression level of Cx43 and GFAP was reversed by i.t. injection of recombinant IL-1β. These results demonstrated that spinal astrocyte gap junction plays a major role for development of contralateral MA during the early phase of peripheral inflammation. Also, blocking of spinal IL-1β induced the Cx43 expression and astrocyte activation which was ultimately facilitated development of contralateral MA in peripheral inflammatory pain model, suggesting that the relationship between spinal IL-1β and astrocyte gap junction plays an important role in the regulation of induction time of MIP.
Key Words: Mirror-image pain, Astrocyte, Gap junction, connexin 43, Interleukin-1β

P07-15

Effects of TCDD exposure on the gonadotropin releasing hormone neurons in mice

Pravin Bhattarai[1], Janardhan Prasad Bhattarai[1], Dong Hyu Cho[2], Seong Kyu Han[1]

[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, [2]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine, Chonbuk National University

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent toxic environmental pollutant which has adverse effects on reproductive development. Till date little is known about the impact of dioxin in GnRH neuronal development and its role in reproductive physiology at hypothalamic level. Thus, in this present study, using whole cell electrophysiology we investigated the effect of various neurotransmitter agonists on gonadotropin releasing hormone (GnRH) neurons in TCDD (10 μg/kg; single dose) exposed prenatal and juvenile mice. GnRH neurons from juvenile offspring of TCDD injected pregnant female mouse on gestational day 15 showed no significant difference in GABAA and kainate receptor mediated neurotransmissions. Single dose of TCDD in juvenile mice showed significant decrease in inward current response of GABAA and NMDA receptor mediated and increase in kainate receptor mediated neurotransmission when examined in peri-pubertal age with respect to control. On the other hand, a single dose of TCDD injected on juvenile period showed no significance difference in GABAA receptor mediated responses on adult female over estrous cycle. Whereas, in diestrus and proestrus phase, there was a significant increase in kainate receptor mediated neurotransmission in adult female. These results support that TCDD is a potent environmental pollutant which may directly affect reproductive physiology at hypothalamic level. This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1C1A1A02036793)
Key Words: GnRH neurons, TCDD, Patch clamp

P07-16

Action of calcitriol on NMDA and kainate receptor-mediated actions in juvenile GnRH neurons

Pravin Bhattarai[1], Janardhan P. Bhattarai[1], Min Sun Kim[2], Dong Hyu Cho[3], Seong Kyu Han[1]

[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, [2]Department of Pediatrics & Research Institute of Clinical Medicine, School of Medicine, [3]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine, Chonbuk National University

Vitamin D is a versatile signaling molecule which plays a critical role in calcium homeostasis. There are a number of studies showing the genomic action of vitamin D in control of reproduction. However, the quick non-genomic action of vitamin D at hypothalamic level has not been well understood. So, to investigate the effect of vitamin D on juvenile GnRH neurons, excitatory neurotransmitters NMDA (30-μM) and kainate (10-μM) were applied in the absence or the presence of vitamin D3 10-nM (VitaD3). The NMDA-mediated responses were decreased by VitaD3 in intact and in presence of TTX, a sodium channel blocker with a mean relative inward current being 0.56±0.07 and (0.66±0.07) ($p<0.05$) respectively. In addition, VitaD3 decreased the frequency of GABAergic spontaneous postsynaptic currents and spontaneous postsynaptic currents induced by NMDA application with a mean relative frequency of 0.595 ± 0.07 and 0.56±0.09 respectively. Further, VitaD3 decreased the kainate-induced inward currents with a relative inward current 0.69±0.10 ($n=5$; $p<0.05$). These results demonstrate that VitaD3 has non-genomic action and partially inhibits the NMDA and the kainite receptor-mediated actions on GnRH neurons suggesting that VitaD3 may regulate HPG axis at the time of pubertal development. This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1C1A1A02036793)
Key Words: GnRH neurons, Vitamin D, Patch clamp

P07-17

Effect of BPA over pre- and post- natal development of Gonadotropin Releasing Hormone Neurons

Janardhan P. Bhattarai, Seong Kyu Han

Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

Over the course of development, various hormones shape the brain for a time specific physiological events like puberty and reproduction. Further, in present modern age, the manmade pollutants are disrupting proper reproductive development. Taking these scenario in account, it is well established that bisphenol-A (BPA), a monomer of polycarbonate plastic is an endocrine disruptor and is a potential hazard for reproductive physiology. So in this study, using patch clamp technique we examined the agonists of various neurotransmitter receptors on GnRH neurons from the pre- and post- natal BPA exposed mice. In the first set of experiment, neonatal offspring of the BPA (125 mg/Kg) exposed female pregnant mice were examined. Interestingly, pups from BPA exposed mothers showed increased response to GABA (100 μM), kainate (10 μM) and AMPA (10 μM) mediated responses than their respective controls. In contrary, GnRH neurons from BPA injected juvenile mice showed decreased GABAA and kainate receptor mediated responses than their control counter parts. Further in another set of experiment gramicidin perforated mode was used to examine the effect of BPA exposure on various neurotransmitter receptor mediated responses on adult GnRH neurons. There were decreased GABA (100 μM), baclofen (10 μM), kainate (10 μM) and AMPA (10 μM) mediated responses in BPA exposed mice than in control counter parts. These results suggest that BPA exposure may directly affect GnRH neurons which is the central regulator of hypothalamic pituitary gonadal axis. This research was supported by Basic Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2058356) and (2014R1A1A2054241)
Key Words: Endocrine disrupting chemicals, Bisphenol-A, GnRH neurons, patch-clamp, GABAergic

P07-18

Loss of Tumor Suppressor PML Promotes Cell Cycle Progression and Proliferation By Enhancing STAT-3 Activity

Jiwoo Lim, Hyunju Park, So-Hee Ahn, Youn-Hee Choi

Department of Physiology, Ewha Womans University School of

Medicine, Seoul 911-1, Korea Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

The promyelocytic leukemia protein PML is involved in many cellular processes such as cell cycle progression, DNA damage, transcriptional regulation, tumor suppression and apoptosis, and PML expression has been known to be down-regulated or abolished in different types of human cancers with a frequent correlation with tumor development. Herein, we examined the relationship of STAT-3 activation and the status of PML protein expression in brain tumors. We observed that cell proliferation and cell cycle progression were increased in pml deficient cells, compared with wild type cells. Interestingly, we found enhanced phosphorylation of STAT-3 and increased expression of STAT-3 downstream genes were detected in pml deficient cells. PML protein expression in NIH3T3 cells suppressed transcriptional activity of STAT-3 in a dose-dependent manner. Conversely, inhibition of PML resulted in enhanced STAT-3 transcriptional activity. These data suggest a regulatory function for PML in tumor progression by inhibiting STAT-3 activity. Further study is in progress to define the molecular mechanisms underlying the suppressive activity of PML in STAT-3 activation, by performing reporter assay.
Key Words: PML, STAT-3

P07-19

Necrotic cells Influence Glioblastoma progression through regulating MCP-1 and MIP-3α expression

Yieun Jung, So-Hee Ahn, Hyunju Park, Jihee Lee Kang, Youn-Hee Choi

Department of Physiology, Ewha Womans University School of Medicine, Seoul 911-1, Korea, Tissue Injury Defense Research Center, Ewha Womans University School of Medicine, Seoul 911-1, Korea

Glioblastoma multiform (GBM), a grade IV astrocytoma, is most lethal and common adult primary intracranial tumor. Among the histological properties of GBM, necrosis has been implicated to be a strong predictor of poor prognosis. However, the effect of tumor necrosis on GBM progression is poorly understood at present. In this study, We examined the effect of tumor necrosis on glioblastoma cells through exploring gene trascrtiption and chemokine expression, such as Monocyte Chemoattractant Protein-1 (MCP-1/CCL2) and Macrophage Inflammatory Protein-3 alpha (MIP-3α/CCL20). Chemokine array and ELISA assay showed that CRT-MG human gliomablastoma cells secreted several chemokines including MCP-1 and MIP-3α in response to necrotic cells. Expression levels of mRNA of MCP-1 and MIP-3α were increased by treatment of necrotic cells in CRT-MG, well correlated with corresponding protein levels. Further study is in progress to determine the mechanism by which necrotic cells induce chemokine expression such as MCP-1 and MIP-3α in GBM cells.
Key Words: Glioblastoma multiform, Necrosis, MCP-1, MIP-3α GBM

P07-20(O-14)

Generation and regulation of pacemaker activity by TRPC3 channels in nigral dopamine neurons

Ki Bum Um, Myoung Kyu Park

Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

Dopamine neurons in the substantia nigra pars compacta (SNc) are slow pacemakers that generate spontaneous action potentials regularly. This regular pacemaking activity regulates the release of basal dopamine to the projection area, such as striatum. Although spontaneous action potentials are essential for maintenance of background dopamine levels and proper functioning of basal ganglia, including the striatum, it is not known what channels are responsible for pacemaking in the midbrain dopamine neurons. Here we report that TRPC3 channels are responsible for pacemaking in the midbrain dopamine neurons. A specific TRPC3 channel blocker, Pyr10, stopped spontaneous firing and following Ca2+ oscillations in dopamine neurons. However, in TRPC3 knockout mice, dopamine neurons showed normal spontaneous firing rate and Pyr10 did not affect spontaneous firing and Ca2+ oscillations at all, suggesting that spontaneous firing in the TRPC3 KO mice is driven by other channels. When TRPC3 channels were blocked by pyr10 with TTX present, neurons hyperpolarized to average of -52 mV and then these inhibition of pacemaking were recovered by somatic injection current, suggesting a TRPC3 conductance are constitutively active and lead tonic depolarization. Our results suggest that TRPC3 channels are essential for pacemaking and determination of tonic firing rate in midbrain SNc dopamine neurons.
Key Words: dopamine neuron, pacemaking, TRPC3

P07-21

Noradrenergic Regulation of Cerebellar Output during Arousal

Seung-Eon Roh[1,2], Seung-Ha Kim[1], Chang-Eop Kim[1], Yong-Gyu Kim[1,2], Sun Kwang Kim[3], Sang Jeong Kim[1,2]

[1]Department of Physiology and [2]Department of Biomedical Science, College of Medicine, Seoul National University, [3]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, South Korea

Locus coeruleus (LC)-norepinephrine (NE) system exerts prominent effects on neurons and astrocytes in many brain regions, including the cerebellum, thereby contributing to arousal. Also, the interaction between neurons and glia has been proposed to play significant roles in cerebellar circuit regulation and cerebellum-dependent motor behavior. However, the possible NE-driven interaction between neurons and glia in the cerebellum and its physiological roles during arousal and motor response remains to be understood. To address this, we characterized the activity patterns of neurons and astrocytes in cerebellar cortex during arousal. We performed two-photon microscopy to measure calcium activities of Purkinje cell (PC) and Bergmann glia (BG) using viral expression of genetically encoded calcium indicator GCaMP6 and a calcium dye OGB-1/AM. The simultaneous video monitoring of behavior was also applied. Upon arousal stimuli, anesthetized and awake mice showed alpha1-adrenergic receptor (alpha1-AR) dependent BG calcium response along with locomotion initiation on the disk treadmill. Also, the BG calcium response coincided with phasic suppression of PC dendrite calcium spike activity. Taken together, we suggest that noradrenergic signaling may mediate the neuroglia interaction in the cerebellum, in which PC dendrite calcium activity is suppressed possibly by BG calcium-induced inhibitory gliotransmisstion via activation of alpha1-AR during arousal.
Key Words: Cerebellum, Arousal, Locus Coeruleus, Bergmann glia, Purkinje Cell

P07-22

Treatment With Diluted Bee Venom Reduces Both Spinal Inflammatory Responses And Central Neuropathic Pain Behaviors After Spinal Cord Injury In Rats

Ji-Young Moon, Suk-Yun Kang, Seong Jin Cho, O Sang Kwon, Sun Hee Yeon, Kwang-Ho Choi, Jang-Hern Lee[1], Yeonhee Ryu

KM Fundamental Research Division, Korea Institute of Oriental Medicine, Daejeon, Republic of Korea, [1]Deptment of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Chemical acupuncture with diluted bee venom (DBV) has been traditionally used in eastern medicine to treat several inflammatory diseases or chronic pain conditions. We have previously shown that DBV had a potent anti-inflammatory and anti-nociceptive efficacy in several rodent pain models. In the present study, we investigated whether the treatment of DBV into Zusanli (ST36) acupoint suppressed intraspinal inflammatory responses as well as allodynic and hyperalgesic behaviors in the spinal cord injury (SCI) model of rats. SCI was induced by T13 spinal cord hemisection after laminectomy. SCI surgery produced acute migration of the neutrophils and the dramatic increment of myeloperoxidase (MPO) activity in the spinal cord lesions at 24 hours following hemisection. In addition, the mechanical allodynic and thermal hyperalgesic behaviors were developed in the bilateral hind paws throughout the 28 days of experiment. Subcutaneous injection (0.25 mg/kg) of DBV was applied into Zusanli acupoint twice a day for five days. DBV treatment significantly suppressed neutrophils infiltration and the MPO activity at 24 hours after hemisection. Moreover, mechanical allodynia and thermal hyperalgesia were relieved throughout the experimental period. DBV injection also showed the facilitated motor function recovery as indicated by the Basso-Beattie-Bresnahan rating score. Finally, spinal glial fibrillary acidic protein (GFAP) expression, a marker for astroglial activation, was also suppressed by DBV injection. These results demonstrated that the repetitive application of DBV into acupoint not only enhanced functional recovery but also reduced acute-inflammatory response and neuropathic pain behavior after SCI. This study suggests that DBV acupuncture can be a potential clinical therapy for management of SCI.
Key Words: Bee venom, Spinal cord injury, Neuropathic pain, Spinal inflammation, Glial fibrillary acidic protein

P07-23

Immunosuppressive effect of estrogen ameliorates pruritic atopic dermatitis in the pubertal female rats

Jeahee Lee[1], Hye Young Kim[1], Taeho Han[1], Seung Keun Back[2,*] and Heung Sik Na[1,*]

[1]Neuroscience Research Institute and Department of Physiology, Korea University College of Medicine, 126-1 Anam-dong 5 Ga, Seongbuk-Gu, Seoul, 136-705, Korea, [2]Department of Pharmaceutics & Biotechnology, College of Medical Engineering, Konyang University, Chungnam 320-711, Korea

Atopic dermatitis (AD) that is a chronically relapsing inflammatory skin disease accompanied with chronic pruritus greatly affects the quality of life of afflicted children and their families. Nevertheless, its underlying mechanisms or appropriate remedies remain to be elucidated. Many earlier studies have shown that endogenous estrogens are important modulators of immune system and its function. Gender difference of AD, a preponderance or severity of an adolescent boy compared to girl, has long been recognized. Previously, we developed a rat model of atopic dermatitis produced by subcutaneous injection of capsaicin within 48 hours after birth. The model showed the cardinal signs of AD from 3rd to 12th postnatal week (PW). In the present study using this model, we investigated the effect of estrogen on the severity of AD during puberty. To meet this aim, we first compared dermatitis, scratching behaviors and serum levels of IgE among the following 4 groups of AD rats; 1) Male group, 2) Female group, 3) Female-OVX (OVX) group that was subjected to ovariectomy at the 3rd PW and 4) Female-OVX-E2 (E2) group that was subjected to ovariectomy followed by an implantation of silastic tubing containing 17β-estradiol (180 μg/μl) under dorsal neck skin at the 3rd PW. The Male and OVX groups showed more severe pruritic dermatitis with higher level of serum IgE, compared to both the Female and E2 groups in which IL-4 and NGF mRNA expression of the lesional skin was relatively reduced but expression of TGF-β and filaggrin mRNA was markedly increased. Thus, our results indicate that estrogen, at least during pubertal female, ameliorates the signs of AD by the modulation of the skin immunity and epidermal barrier functions.
Key Words: Atopic dermatitis, Dermatitis, Estradiol, Ovariectomy, IgE, NGF, Filaggrin

P07-24

Spontaneous firing system of substantia nigra dopamine neurons: proximal dendrites as an accelerator and the soma as a counteract balancer

Jinyoung Jang, Myoung Kyu Park

Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Suwon, Korea

Pacemaker activity of highly polarized neurons requires proper spatial organization of excitable elements throughout the somatodendritic compartment. In spontaneously firing dopamine neurons, the pacemaker mechanism composed of intrinsic excitability of the somatodendritic compartment is not clearly understood yet. Here we demonstrate that the dynamic complementary interaction between the stably-oscillating soma and the stochastically-behaving proximal dendritic compartments determines pacemaker activity of the dopamine neuron. Ca2+ spikes occurring in the proximal dendritic compartment having a fast Ca2+ dynamics were highly stochastic and its excitability was higher than the soma. But the tight electrical-coupling with the soma makes dendritic Ca2+ fluctuations synchronized with the stable somatic Ca2+ oscillations. Local perturbation experiments suggest that the stochastic proximal dendritic regions drive pacemaking activities, but that the electrically-coupled large-compartment soma counteracts the accelerating activities of the proximal dendrites and balances them. This accelerator-counteract balancer model could explain real pacemaker activity and dynamic aspects of glutamate-induced firing diversity in the dopamine neurons.
Key Words: dopamine neuron, pacemaker mechanism, Ca2+ oscillation, somatodendritic balance

P07-25(O-5)

Novel function of histone demethylase of JHDM in spatial learning and memory

Hye-Jin Kim, Seon-Young Kim, Myoung-Hwan Kim, Sang Jeong Kim, Yang-Sook Chun

Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

Dynamic changes in histone modification play a role in regulating the gene expression program linked to memory formation. Among them, very little is known about the role of Jumonji-containing histone demethylases which erase methyl group of H3K4, H3K9, H3K27, H3K36, and H4K20- associated with memory formation. Here, we documented the physiological role of JHDM (H3K9 demethylase) in learning and memory. Surprisingly, we found that the JHDM overexpressed transgenic mice displayed enhancement of spatial-memory formation and contextual fear conditioning compared to wild-type mice. Conversely, JHDM-deficient mice derived by Lenti-viral vector in hippocampus showed the impairment in hippocampus-dependent memory function. We also observed that overexpression and knockdown of JHDM leads to changes in expression of neuronal target genes involved in memory formation. Taken together, our results suggest that JHDM has an important role of memory formation in the hippocampus. These findings suggest that JHDM might be a suitable therapeutic avenue for neurodegenerative diseases associated with learning and memory impairment.

Key Words: Jumonji-histone demethylase(JHDM), learning and memory, hippocampus

P07-26

Hypotaurine action mediated by α-homomeric & αβ-hetromeric glycine receptors in medullary dorsal horn neurons

Sun Mi Oh, Seong Kyu Han, Soo Joung Park

Department of Oral Physiology & Institute of Oral Bioscience, School of Dentistry, Chonbuk National University, Jeonju, Korea

Hypotaurine, the immediate precursor of taurine in mammalian tissues is biosynthesized from cysteine in astrocytes and play as an indigenous neurotransmitter. The medullary dorsal horn plays a key site in the processing of orofacial nociceptive information to higher brain areas for orofacial pain perception. Patch clamp technique was used to examine the direct effects of hypotaurine and the receptor types involved in the actions. Under the condition of high chloride pipette solution, hypotaurine induced inward currents at different concentrations (300 μM, 1 mM and 3mM) were almost blocked by strychnine (2 μM), a glycine receptor antagonist, but not affected by gabazine (3 μM), a synaptic GABAA receptor blocker, suggesting the involvement of glycine receptors. Further, to figure out the type of glycine receptors activated by hypotaurine, a low concentration of picrotoxin which blocks α-homomeric glycine receptors was applied prior to hypotaurine and glycine. Hypotaurine (300 μM and 1 mM) and glycine (100 μM) induced inward currents were partially blocked by picrotoxin (50 μM). Similarly hypotaurine- and glycine-induced inward currents were also partially blocked by bicuculline (10μM), which serves as α2 homomeric glycine receptor blocker. Overall, these results indicate that hypotaurine affects SG neuronal activities by α-homomeric and αβ-heteromeric glycine receptors. This research was supported by Basic Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2015R1D1A3A01018700).

Key Words: Hypotaurine, orofacial pain, patch clamp technique, glycine receptors

P07-27

Role of Neuregulin-2 in synaptogenesis in newborn granule cells

Kyu-Hee Lee, Hyun-Su Lee, Che Ho Yang, Won-kyung Ho, Suk-Ho Lee

Department of Physiology, Seoul National University College of Medicine, 103 Daehak-ro, Jongno-gu, Seoul, Korea

Neuregulin-2 (NRG2) was identified as a member of proteins containing EGF-like domain. NRG2 is expressed in a few restricted regions in the brain including the hippocampal dentate gyrus (DG) and subventricular zone, where neurogenesis persists during adulthood. Little is understood about the role of NRG2 in developments of newborn neurons. To study the role of NRG2 in synaptogenesis, we infected the newborn granule cells (GCs) in the ex vivo culture of hippocampus with retrovirus encoding microRNA against NRG2 (miNRG2). The miNRG2 was designed to be expressed under the control of Tet-On expression system. We recorded feed-forward GABAergic or glutamatergic postsynaptic current (GPSC or EPSC) evoked by stimulation of inner molecular layer of the DG. Depletion of endogenous NRG2 by treatment of doxycycline (dox) from 4 dpi (day-post-injection) suppressed GPSC amplitude. In contrast, dox-treatment from 7 dpi displayed no significant effect on GPSCs, suggesting NRG2 is essential in GABAergic synapse formation but not in its maintenance. Next, we studied the role of NRG2 in glutamatergic synapse formation by treating dox from 7 dpi. Whereas such dox treatment had no effect on GPSC, it lowered both amplitudes of AMPA- and NMDA-EPSCs, and abolished the normal increase in the ratio of AMPA- to NMDA-EPSC. In parallel, dendritic arborization of newborn GCs was reduced. These effects of knockdown were rescued by simultaneous overexpression of the intracellular domain of NRG2. Consistently, pharmacological inhibition of ErbB4, the receptor of Nrg2, suppressed the development of GABAergic synapses, but not glutamatergic synapses. These results suggest that the NRG2-mediated forward and reverse signalings participate in GABAergic and glutamatergic synaptogenesis, respectively.

Key Words: hippocampus, neurogenesis, synaptogenesis, neuregulin

P07-28

Portal hypertension is associated with the impairment of arterial baroreflex and hypoexcitability of aortic baroreceptor neurons in cirrhotic rats

Choong-Ku Lee, Jae-Won Lee, Seong-Woo Jeong

Department of Physiology, Yonsei University Wonju College of Medicine, Wonju 220-701, Korea

Portal hypertension (PH) is a frequent clinical syndrome that is characterized by an increased portal venous pressure, and is most commonly caused by chronic liver disease. Clinical studies have suggested that portal hypertension causes cardiovascular autonomic dysfunction (CAD) including decreased baroreflex sensitivity (BRS) and heart rate variability. In the present study, we examined the time courses of PH and CAD progresses in cirrhotic rats, and whether portal hypertension causes impairment of arterial baroreflex and functional plasticity of the aortic baroreceptor (AB) neurons like liver cirrhosis. In this regard, we produced rats with either PH or liver cirrhosis by a partial portal vein ligation and intraperitoneal injection of thioacetamide (TAA), respectively. Time courses of baroreflex dysfunction and PH development were similar in TAA-induced cirrhotic rats. One week after

surgery, the portal venous pressure was significantly increased in PH rats compared with sham-operated rats. As assessed by measurement of the heart rate changes during phenylephrine-induced baroreceptor activation, BRS was significantly decreased in PH rats. Under the current clamp mode of the patch-clamp technique, cell excitability was recorded in Di-I labeled AB neurons. The number of action potential discharge in A- and C-type AB neurons was significantly reduced due to increased rheobase and threshold potential in PH rats compared with sham-operated rats. Real-time PCR and western blotting experiments revealed that NaV1.7, NaV1.8, and NaV1.9 transcripts and proteins were significantly down-regulated in the nodose ganglion neurons from PH rats compared with sham-operated rats. Consistent with these molecular data, TTX-sensitive NaV currents as well as both TTX-sensitive and TTX-resistant NaV currents were significantly decreased in A- and C-type AB neurons, respectively, from PH rats compared with sham-operated rats. Taken together, these data suggest that PH may cause the impairment of arterial baroreflex in cirrhotic rats. Moreover, the cellular mechanisms underlying the PH-induced hypoexitability of AB neurons include down-regulation of voltage-gated sodium channels. This research was supported by Basic Science research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2013424).
Key Words: liver cirrhosis, portal hypertension, arterial baroreflex, baroreceptor, sodium channel

P07-29

Intraplantar injection of DHEAS or PREGS enhance P2X mediated mechanical allodynia via sigma-1 receptors in rats

Soon-Gu Kwon, Sheu-Ran Choi, Hoon-Seong Choi, MiJi Lee, Jang-Hern Lee

Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

The role of peripheral neurosteroids in nociception and their related mechanisms have not been thoroughly investigated. Since there is emerging evidence that neurosteroids affect P2X mediated membrane current in cellular system, we have determined to investigate the possible modulatory role of dehydroepiandrosterone sulphate (DHEAS) and pregnenolone sulfate (PREGS) on the P2X mediated pain at the peripheral level. We performed concomitant intraplantar injection of DHEAS or PREGS with αβmeATP and observed paw withdrawal frequency to the innocuous mechanical stimulation in rats. Neurosteroid itself did not produced any detectable changes paw withdrawal frequency. Otherwise, when DHEAS or PREGS co-injected with sub-effective dose of αβmeATP, they dose dependently developed αβmeATP induced mechanical allodynia which was almost totally prevented by TNP-ATP (a P2X antagonist). These results demonstrated that DHEAS and/or PREGS potentiated the activity of P2X receptors which result in the enhancement of αβmeATP induced mechanical allodynia. Subsequently, we investigated the possible action of GABAA, NMDA and sigma-1 receptors, representative neurosteroid's target receptors, in DHEAS or PREGS + αβmeATP induced mechanical allodynia. As a result, pre-treatment of BD-1047 (a specific sigma-1 antagonist) effectively prevented the facilitatory effects induced by neurosteroids, but muscimol (a GABAA agonist) or MK-801 (a NMDA antagonist) did not affect such potentiation of mechanical allodynia. Overall, we addressed that peripheral DHEAS and/or PREGS potentiated P2X induced mechanical allodynia, and this action was mediated by sigma-1 but not by GABAA nor NMDA receptors.

Key Words: neurosteroid, sigma-1, P2X, mechanical allodynia

P07-30

Investigation of leak channels important for pacemaking in the nigral dopamine neurons

Su Yun Hahn, Myoung Kyu Park

Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

Dopamine neurons in the midbrain are slow pacemakers that generate spontaneous firing autonomously and regularly. However, it is still not known what ion channels are responsible for pacemaking in dopamine neurons. So far, unidentified Na+-permeable leak channels appear to play a major role in driving pacemaker activity. Since resting membrane potentials of midbrain dopamine neurons are maintained between -55-45 mV far from the equilibrium potential of K+(Ek), there could be a conductance responsible for persistent depolarization of membrane potential. Therefore, we have investigated background Na+-permeable ion channels responsible for maintaining membrane potential depolarized in nigral dopamine neurons, using Ca2+ measurement and patch-clamp techniques. Since extracellular Ca2+ and Na+ can play a key role in determining resting leak conductances, we examined whether extracellular Ca2+ and Na+ influence membrane potentials, firing rates, and inward currents in dopamine neurons. Lowering [Ca2+]e from 2.0 to 0.5mM increased the Na+ leak inward current and heavily affected spontaneous firing rates. A nonselective cation channel blocker for TRPC channels, SKF96365, did not completely block background Na+ conductances. Despite usage of TTX and Cs+ which block Nav and Kv channels, the component of Na+ leak conductances survived. RT-PRC showed the presence of mRNA for NALCN1, a background Na2+ leak channel, in dopamine neurons. These results suggest that there could be a TTX- and Cs+-resistant Na+ leak channel controlled by [Ca2+]e and that NALCN could play an important role in the pacemaking of the midbrain dopamine neurons.
Key Words: nigral dopamine neurons, pacemaking, Ca2+, leak channel, NALCN,

P07-31

LTP of dendritic spines in nigral dopamine neurons : possible link of reward and burst

Min Jung Kim, Miae Jang, Myoung Kyu Park

Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA

During reinforcement learning, glutamatergic inputs to the midbrain dopamine neurons generate bursts that encode initially reward itself but later cues that inform upcoming reward. However, little is known about the cellular mechanism for bursts. Very recently, we found that dopamine neurons in the substantia nigra pars compacta(SNc) have two morphologically and functionally distinct types of glutamatergic synapses, spine synapses and shaft synapses on the same dendrite. Thus we have explored how these glutamate synapses generate burst firing in nigral dopamine neurons, using high-resolution two-photon confocal microscopy and wholl-cell voltage-clamp recordings in the TH-eGFP mouse midbrain slices. By consecutive focal glutamate uncaging pulses on the tip of spine heads or dendritic shafts, similar to physiological release of neurotransmitter glutamate, we found that

stimulation above a certain level increased diameter of spine heads and then increased firing frequency, which was in accord with the defined frequency of bursts But it failed to increase firing frequency unless spine swelling entailed. After spine enlargement, stimulation with lower pulses evoked burst firing, suggesting that spine enlargement is enough to generate burst firing. These results suggest that spine swelling is able to generate burst firing in dopamine neurons. Therefore, this synaptic plasticity of glutamatergic spine synapses could be a possible link between reward and burst in midbrain dopamine neurons.
Key Words: Dopamine neuron, burst, dendritic spine, substantia nigra, two-photon confocal imaging

P07-32

Electroacupuncture alleviates mechanical allodynia via spinal opioidergic and alpha2-adrenergic mechanisms in oxaliplatin- or vincristine-induced neuropathy mice model

Jung-Wan Choi[1], Suk-Yun Kang[1], Kwon O Sang[1], Yeon Sun Hee[1], Yeon-Hee Ryu[1], Hyun-Woo Kim[2]

[1]Korea Institute of Oriental Medicine, Daejeon, 305-811, South Korea
[2]Department of Physiology and Institute of Brain Research, Chungnam National University School of Medicine, Daejeon, 301-747, South Korea

This study investigated whether and how electroacupuncture (EA) determines the antinociceptive effect and related neuronal mechanism in the chemotherapy-induced neuropathic pain in mice. Oxaliplatin (OXA, 10 mg/kg) was intraperitoneally injected on days 0 and 6, and vincristine (VCR, 0.1 mg/kg) was intraperitoneally administered once a day for 7 consecutive days to induce neuropathic pain. EA stimulation (2 Hz, 1-2 mA, 30 min) was applied at the ST36 acupoint bilaterally once in every 2 days. Repeated EA stimulation significantly attenuated OXA- or VCR-induced mechanical allodynia. When compared with gabapentin (GAB, 50 mg/kg, intraperitoneal treatment), EA stimulation appeared at similar results. In a separate set of experiment, the antinociceptive effect of a single EA stimulation 8 days after OXA or VCR treatment was reduced by intrathecal pretreatment with naloxone (NAL, opioid receptor antagonist, 40 ug/kg), idazoxan (IDA, alpha2-adrenoceptor antagonist, 200 ug/kg). Moreover, EA remarkably suppressed the OXA- or VCR-enhanced phosphorylation of NMDA receptor NR1 and NR2B subunit in spinal dorsal horn and intrathecal pretreatment of NAL or IDA blocked the effect of EA. In conclusion, EA stimulation at the ST36 acupoint significantly diminished OXA- or VCR-induced neuropathic pain in mice via the mediation of spinal opioid receptor or alpha2-adrenoceptors.
Key Words: Neuropathic pain, Electroacupuncture, Opioidergic system, Adrenergic system, NMDA receptor

P07-33(O-13)

Agonist-independent activity of mGluR1 underlies homeostatic control of intrinsic excitability via IH in cerebellar Purkinje cells

Hyun Geun Shim[1,2], Sung-Soo Jang[1,3], Dong Cheol Jang[1,4], Joo Min Park[5], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Science, Seoul National University College of Medicine, [3]Neuroscience Research Institute, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul, 110-799, Korea, [4]Department of Brain and Cognitive Sciences, College of Science, Seoul National University, Kwanak-gu, Seoul, 151-742, Korea, [5]Center for Cognition and Sociality, Institute for Basic Science, Daejeon, 305-811, Korea

Homeostatic plasticity, non-Hebbian form of neural plasticity, is triggered to stabilize neural network when long-lasting changes of neural activity occur. Albeit that homeostatic regulation of the intrinsic excitability is a fascinating model for both pathological condition and physiological plasticity, cellular mechanism of homeostatic regulation is still unclear. Given that type 1 metabotropic glutamate receptor (mGluR1) can monitor neuronal activity which results in plastic changes, the receptor could be one candidate for tuning the neuronal activity in an activity-dependent manner. Here we show that agonist-independent action of mGluR1 induces homeostatic intrinsic plasticity via upregulation of the hyperpolarization-activated current (IH). First, we observed that activity-deprivation by chronic treatment of tetrodotoxin (TTX) for 2 days decreased evoked firing rates from cerebellar Purkinje cells (PCs). Interestingly, co-treatment of mGluR inverse-agonist under activity-deprived condition, not neutral antagonist, prevented downregulation of excitability, suggesting that homeostatic intrinsic plasticity required agonist-independent action of mGluR1. Next, we observed elevated IH components, including voltage sag and current density by activity-deprivation hence it was suggested that upregulated IH results in lowered excitability. Indeed, homeostatic upregulation of IH was also rescued by blockade of constitutive mGluR activity with activity-deprived condition. Taken together, this study suggests that mGluR1 is a key player for homeostatic control via modulation of IH.
Key Words: Homeostatic plasticity, Intrinsic excitabiltiy, mGluR1a, cerebellar Purkinje cells, hyperpolarization-activated current

P07-34

Distinct responses of vagal and splanchnic nerves innervating liver to 5-HT receptor agonists in Guinea pigs

Yong Seok Yang*, Jae Jun Han, kyung Min Choi, Hong Soon Lim, Min-Goo Lee

Department of Physiology, Korea University College of Medicine, Seoul 136-705

Background: The primary visceral sensory information from intra-abdominal organs is transmitted to the spinal cord and medulla oblongata via splanchnic nerve and vagus nerve, respectively. However, there has been little study for distribution and function of dual sensory innervation system in intraabdominal organs. **Purpose:** We aimed at elucidating the distribution of 5-HT receptor subtype, the responsiveness to 5-HT and electrophysiological property of 5-HT response in splanchnic and vagal nerves innervating liver. **Method:** Guinea pigs(250g) were used. Dye(DiI) was injected in liver under anesthesis then one week later neural cells were extracted from vagal and dorsal root ganglia. RT-PCR and intracellular calcium imaging technique were used to check 5-HT receptor subtypes and 5-HT response in DiI labeled vagal and DRG neurons. We performed gramicidin perforated conventional patch clamp technique to find various electrophysiological characteristics and 5-HT2 & 5-HT3 response in DiI labeled neurons. **Result:** RT-PCR technique showed that vagal (nodose and jugular) and DRG neural cells had mRNAs for 5-HT subtype 2,3 receptors among seven subtypes of 5-HT receptors. Intracellular calcium imaging revealed that dominantly nodose cells showed response to 5-HT. This response was mediated by 5-HT2 &

5-HT3 receptors. In the study of electrophysiological properties nodose neuron showed inward current induced by 5-HT and 5-HT3 selective agonist but not by 5-HT2 selective agonist. **Summary:** (1) Vagal and DRG neurons had mRNAs for 5-HT subtype 2,3 receptors in the RT-PCR experiment. (2) Nodose vagal neurons responded to 5-HT and these responses are mediated by 5-HT2 & 5-HT3 receptors in intracellular calcium imaging experiment. (3) Nodose vagal neurons responded to 5-HT and 5-HT3 selective agonist but not 5-HT2 selective agonist in patch clamp recording.
Key Words: liver, 5-HT, vagus nerve, splanchnic nerve

P07-35

Primary afferents temporally encode the noxious stimulus for pain signaling

K.W. CHO[1,*], J.Z. LIM[2], S.-P. KIM[3], D.P. JANG[1], S.J. JUNG[2]

[1]Department of Biomedical Engineering, Hanyang University, Seoul, Korea, [2]Department of Biomedical Sciences, Hanyang University, Seoul, Korea, [3]School of Design and Human Engineering, Ulsan National Institute of Science and Technology, Ulsan, Korea

Even though an activation of a primary afferent C-fiber is conceptually assumed to initiate a pain response, there are studies reporting no pain response under the activation of the afferents. One of the strong explanations is the neural network that a fiber's activation is considered as a single input to central nervous system. However, the network process is not enough to interpret all the complex sensations in animal. Moreover, most primary C-fibers contain various chemoreceptors responding to each agonists. For example, TRPV1 receptors and TRPA1 are expressed commonly on a same fiber's membrane. Thus, chemical specific processing might take an account to such a modified pain sensation. In this study, the responses and its characteristics of primary afferents for different chemicals have been investigated on ex vivo single fiber recording setup. The time stamps of the evoked action potentials recorded from the skin-nerve preparation from the mice were recorded. A mouse was sacrificed before each experiment, and their hind paw skin including the saphenous nerve was extracted with surgical methods. After the identification of a single fiber on the recording setup, a chemical was applied. As a result, we obtained the response for a single chemicals and the firing pattern of each showed different patterns. In every trial, the chemicals were applied on the different receptive field of the skin to prevent a counter effect from the other.
Key Word: PAIN

P07-36

Three distinct cerebellum-dependent eye movement learning in pcp2-cre mice

Dong Cheol Jang[1,2], Sang Jeong Kim[1,2]

[1]Department of Brain and Cognitive Science, College of Science , [2]Department of Physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea

Vestibule-Ocular reflex (VOR), which known as cerebellum-dependent eye movement, is a compensatory eye movement to stabilise an image on retina. To deal with dynamic changes of surroundings, this reflex can adapt to the situation and we call it as "VOR learning". There are three well-known VOR learning protocols, such as Gain-up, Gain-down and Phase reversal learning, and these three distinct protocols based on different synaptic mechanism. To investigate whether and how one molecule act in this eye movement learning, we prepared Purkinje neuron specific cre-recombinase expression mice (pcp2-cre mice). Since Purkinje neuron can only provide information to outside of cerebellar cortex, modifying this Purkinje neuron could directly affect to synaptic or intrinsic property of the neuron and finally, VOR learning also can be affected. In this study, we applied these three protocols to pcp2-cre mice. Briefly, This mice had normal range of basal ocular motor performance and they are able to learn every learning protocols. Therefore, we can conclude the idea that this cre-recombinase knock-in mice does not have any defect in not only basal ocular motor performance but also learning of cerebellum-dependent eye movement.
Key Words: Vestibulo-ocular reflex, cerebellum, purkinje neuron

P07-37

Dysregulation of metabotropic glutamate receptor 5 in periaqueductal gray perpetuate chronic neuropathic pain

Geehoon Chung[1,2], Hyun Geun Shim[2,3], Chae Young Kim[2,3], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences, [3]Department of Biomedical Sciences, Seoul National University College of Medicine

Mechanism of neuropathic pain manifestation is still not fully understood. Not all individual with nerve injury end up with intractable pain, also not all nerve injury-induced pain turn into chronic. Given that inherent capability to regulate painful sensation is diverse among individuals, involvement of endogenous pain modulatory system has been suggested. Among related brain areas, periaqueductal gray (PAG) is one of the most important area in terms of endogenous pain modulation. PAG integrates ascending and descending pain modulatory signals from numerous sites and sends signal to rostral ventromedial medulla (RVM), thus modulates pain signals coming from spinal cord. We focused on the role of metabotropic glutamate receptor 5 (mGluR5) in the PAG in that mGluR5 is involved in various pathological change of the nervous system. It is well known that mGluR5 also contributes to the diverse variety of physiological functions through modulation of neuronal activity. Given the essential role of PAG in pain modulation, it is assumable that alteration of mGluR5 activity in the PAG would result in pain modulatory dysfunction and affect manifestation of neuropathic pain. In this study, we measured brain glucose metabolism from neuropathic pain rats and control rats in vivo using flurodeoxyglucose (FDG) – positron emission tomography (PET) to compare whole-brain activity of awake, resting state animals. Consistent with our predictions, reduced activity was observed from PAG and RVM of neuropathic pain rats, which was rescued by administration of the group I mGluR agonist into PAG. This treatment induced powerful analgesic effect as well, which was sufficient to cancel out neuropathic pain symptom. Based on the results from PET study, we further hypothesized that mGluR5 in PAG is persistently active to regulate nociception in normal state. Indeed, we could find that mGluR5 persistently regulates neuronal activity in PAG to control pain transmission, and decline of this activity is responsible for maintenance of neuropathic pain. Our findings provide previously unknown mechanism through which pain become chronic, emphasizing an important role of mGluR5 in pain modulatory function.
Key Words: Neuropathic pain, Periaqueductal gray, Metabotropic glutamate receptor, Brain imaging

P07-38

Upregulation of prelimbic metabotropic glutamate receptor 5 in chronic neuropathic pain state

Chae Young Kim[1,2], Geehoon Chung[1,3], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, [3]Department of Brain & Cognitive Sciences, Seoul National University College of Natural Sciences

In this study, we focused on the change of mGluR5 in the brain following chronic neuropathic pain. Metabotropic glutamate receptor 5 (mGluR5) is widely expressed on brain regions and exerts various cellular effects related to neuronal plasticity. Considering its importance, however, change of mGluR5 in brain and its functional role in pathophysiology of chronic pain is barely known. Based on other mGluR studies about non-pain neuronal diseases, we hypothesized that there would be an expressional change of mGluR5 in pain-related brain regions under chronic neuropathic pain state. We compared expression level of mGluR5 in the brain between chronic neuropathic pain model animals and sham control in vivo using positron emission tomography (PET) with mGluR5 radiotracer [11C]-ABP688. These images showed that expression of mGluR5 in prelimbic cortex is increased in neuropathic pain rats compared to control. To identify its functional role in pain signaling, mGluR5 antagonist MPEP was injected into the prelimbic cortex of neuropathic pain rats and behavioral change was measured. We could find that blocking mGluR5 in prelimbic cortex evoked analgesic effect sufficient to cancel out neuropathic pain symptoms. MPEP-treated neuropathic pain rats showed significantly increased pain threshold to mechanical stimuli, and they preferred to MPEP-conditioned chamber in conditioned place preference experiment. Throughout this process from PET imaging to behavior test, we show that mGluR5 of prelimbic cortex is increased in neuropathic pain state, and this prelimbic mGluR5 is strongly related to pain perception.
Key Words: Neuropathic pain, Prelimbic cortex, Metabotropic glutamate receptor 5, Brain imaging

P07-39

Changes in Field Potentials Following Transcranial Direct Current Stimulation on the Motor Cortex of Rats in Vivo

Ho Koo[1], Yong-Il Shin[2], Yu Fan[3], Sang Hu Han[1], Min Sun Kim[1]

[1]Department of Physiology, Wonkwang University School of Medicine, Iksan, Korea, [2]Department of Rehabilitation Medicine, Pusan National University School of Medicine, Research Institute for Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Pusan, Korea, [3]Department of Meridian & Acupoint, College of Korean Medicine, Wonkwang University, Iksan, Korea

Transcranial direct current stimulation (tDCS) is a noninvasive cortical neuromodulatory tool with therapeutic applications and cognitive enhancing. A number of electrophysiological data of human have shown that tDCS applied to the motor cortex can modulate cortical excitability. Especially, tDCS with sufficient duration and intensity of stimulation enables to induce long-term effects like plasticity. Recently, an in vitro study demonstrated that slow frequency synaptic activation during tDCS was required to induce long-lasting potentiation like plasticity. However, the mechanism how tDCS modulate cortical excitability on the cortex for a long time is still highly unclear. Here, we investigated changes of field potentials, evoked by stimulating contralateral corpus callosum, on the motor cortex of rats before and after anodal tDCS under urethane anaesthesia. Field potentials in the motor cortex were gradually increased for more than one hour after anodal tDCS with or without repeatedly low frequency synaptic activation unlike a previous in vitro study. To elicit these long-lasting effects, the sufficient duration of stimulation (more 20 minutes) was required rather than the high intensity of stimulation. We propose that anodal tDCS with the enough duration of stimulation regardless of repetitive low-frequency synaptic activation may contribute to induce long-term potentiation like plasticity in relation to learning as well as the neuromodulation of the motor cortex.
Key Words: tDCS, neuromodulation, electrophysiology, motor cortex

P07-40

Characterizing the function of Negr1, a newly-identified obesity-related gene, in the nervous system

Kyungchul Noh, Hyunkyoung Lee, Soo-Jeong Kim, Tae-Yong Choi, Se-Young Choi, Sung Joong Lee

Department of Neuroscience and Dental Research Institute, School of Dentistry, Seoul National University, Seoul, 03080, Republic of Korea

Neuronal growth regulator 1 (Negr1) is newly identified obesity-related gene, a member of the immunoglobulin superfamily of GPI-anchored cell adhesion molecule. This gene is recently known to affect synapse formation and dendritic process of both hippocampal and cortical neuron, and categorized as a candidate gene for obesity and psychiatric disorder. Although there are several evidences suggesting the role of Negr1 as a neuronal regulation, in vivo functions of this gene are still unknown. To elucidate the function of Negr1 in the nervous system, we have generated Negr1 knockout mice (KO) and characterized the neurological phenotype by behavioral studies such as anxiety/depression, spatial memory, and recognition. Our data showed that there was no difference in recognition function between wild type and Negr1 KO mice, however, elevated anxiety- and depression-like behavior was observed in the KO group. In electrophysiological studies, we found that hippocampal LTP was severely compromised in the Negr1 KO mice. In our effort to elucidate the underlying mechanisms, we found Lipocalin 2 (Lcn2) gene was decreased in the hippocampus of Negr1 KO mice compared to wild type control mice. In addition, hippocampal synaptic density was increased in hippocampal CA3 and dentate gyrus in Negr1 KO mice. Taken together, our data suggest that Negr1 is involved in mood regulation putatively by regulating synaptic spine density via Lcn2.
Key Words: Negr1, Lcn2, depression, spine density

P07-41

The of roles of GABA on the motivational driving force for the pain attenuation following spinal cord injury in rats

Moon Yi Ko[1], Jun Yeon Lee[1], Su Phil Kim[2], Hee Young Kim[2], Chae Ha Yang[2], Young S. Gwak[2]

[1]Department of Aroma Application Industry, Daegu Hanny University, Kyungsan si, Kyungsanbukdo, 38610, Korea, [2]Department of Physiology, Daegu Haany University, Daegu 42158, Korea

The of roles of GABA on the motivational driving force for the pain attenuation following spinal cord injury in rats GABA is the major inhibitory neurotransmitter in the central nervous system includes sensory and psychiatric pathophysiology. To investigate the roles of GABA at both sensory and psychiatric abnormality, the present study tested GABA-mediated neuropathic pain and reward mechanism following spinal cord injury in rats. SCI was produced by T10 clip compression injury (35g, 1 min) in ages with 180-225 g male SD rats. To test the roles of GABA, paw withdrawal response, in vivo extracellular single cell recording, HPLC-microdialysis and immunohistochemistry were performed. Prior to injury, the baseline paw withdrawal threshold and ultrasound vocalization was recorded, respectively. Post injury days 40, SCI groups showed significantly decreased paw withdrawal threshold at both hindpaws and increased ultrasound vocalization compared to before injury, respectively (*$p<0.05$). In vivo extracellular electrophysiology at the ventral tegmental area (VTA), GABA neuron activity (characterized by less than 1 ms action potential duration and > 5 Hz frequency) showed increased firing rates (13.6 ± 1.7 spikes/sec) compared to sham control groups (7.3 ± 1.1 spikes/sec). In immunohistochemistry, glutamic acid decarboxylase (GAD) 67 showed increased expression compared to sham control groups (*$p<0.05$) at the VTA. In HPLC-microdialysis at the VTA, the concentration of glutamate (608.3±0 nM) and GABA (98.8±50.5 nM) in SCI groups showed increases compared to sham controls (353±0 nM and 4±1.3 nM), respectively. . In addition, the intrathecal administration of GABA receptor agonists at the spinal cord resulted in the attenuation of neuropathic pain and neuronal hyperexcitability that suggests the decreased tone of spinal GABAergic tone following SCI. Taken together, the present study suggests that chronic neuropathic pain reveals the increased activity of VTA GABA neurons that result in the suppression of dopaminergic neurons and motivational driving for the pain attenuation. This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIP)(NRF-2015R1A5A7037508) and NRF-2014R1A1A4A01004179.
Key Words: Pain, GABA, VTA,Spinal cord injury

P07-42

Carvacrol inhibits mGluR1-evoked slow currents in cerebellum

Da Eun Jeon, Sang Jeong Kim

Department of Physiology, College of Medicine, Seoul National University

Terpenes are organic compounds produced by plants and insects. Studies shown that many terpenes affect the central nervous system through TRP channels. Transient Receptor Potential (TRP) channels detect environmental and biological signals and changes, such as temperature, pressure, tastes and smells. Different tissues contain different types of TRP channels; TRPM7 is expressed in most tissues, TRPA1 is expressed in many brain and heart tissues but not in kidney, many TRPVs are expressed in skin and TRPCs are expressed in many brain tissues. Among TRP channel systems, TRP system in the cerebellum is well-known; mGluR1-evoked slow current is mediated by TRPC1 and TRPC3. In this paper, several terpenes have been tested for the effects on mGluR1-mediated slow EPSC in the murine cerebellum. These terpenes were known to permeate blood-brain barrier (BBB). Among terpenes tested, limonene and pinene did not affect both slow and fast EPSC, linalool and eugenol inhibited both slow and fast EPSC, and carvacrol inhibited slow EPSC selectively. Further studies will be done on how carvacrol inhibit mGluR1-mediated slow EPSC.
Key Words: Cerebellum, TRPC, Terpene, Carvacrol, Slow EPSC

P07-43

Anti-inflammatory role of cytoplasmic Ref-1 in cultured astrocytes

Hyang-Joo Lee, Hyun-Sill Cho, Sudip Pandit, Yoon-Hyung Pai, Byeong Hwa Jeon, Jin Bong Park

Department of Physiology, Brain Research Institute, School of Medicine, Chungnam National University, 6 Munhwa-Ro, Jung-gu, Daejeon, 301-131, Republic of Korea

Redox factor-1 (Ref-1) is a ubiquitous protein that is a redox-sensitive regulator of multiple transcription factors as well as an apurinic/apyrimidic endonuclase in the base excision repair pathway. In recent years, the extra-nuclear role of Ref-1 against oxidative stress has been known. Astrocytes seem to be initial and crucial part in the pathological process of the neuro-inflammation, such as in epilepsy. In the present study, we found that Ref-1 increased in hippocampal astrocytes from kainite-injected epileptic rat. To know the functional role(s) of increased Ref-1 in astrocytes, we further assessed the anti-inflammatory action of Ref-1 in cultured astrocytes. Lipopolysaccharide (LPS) treatment increased iNOS expression in the astrocytes, which was significantly decreased by adenoviral infection of Ref-1 cDNA. Ref-1 overexpression also significantly inhibited LPS-induced tumor necrosis factor-α (TNF-α) release. In contrast Ref-1 small interference RNS (siRNA) enhanced LPS-induced iNOS expression. Down-regulation of Ref-1 increased LPS-induced TNF-α release. Nuclear localization signal (ΔNLS) deletion mutation in astrocytes has increased cytoplasmic Ref-1, this effect is similar with wild type. This result supports that endogenous cytoplasmic Ref-1 in astrocytes has anti-inflammatory action. But, both overexpression and knockdown of Ref-1 did not affect the expression of IkB degradation and phosphorylation of NF-kB in LPS-treated astrocytes Overall these data suggest that biological functions of Ref-1, endogenous astrocyte Ref-1 might serve an anti-inflammatory action in astrocytes, which would involve in inflammatory neuro-disease such as epilepsy.
Key Words: Redox factor-1 (Ref-1), Inflammation, astrocytes

P07-44

Clonidine, an alpha-2 adrenoceptor agonist relieves mechanical allodynia in oxaliplatin-induced neuropathic mice; potentiation by spinal p38 MAPK inhibition without motor dysfunction and hypotension

Ji-Hee Yeo[1], Seo-Yeon Yoon[2], Sol-Ji Kim[1], Jang-Hern Lee[3], Alvin J. Beitz[4], Dae-Hyun Roh[1,*]

[1]Department of Oral Physiology and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea, [2]Pain Cognitive Function Research Center, Department of Brain and Cognitive Sciences College of Natural Sciences, Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea, [3]Department of Veterinary Physiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, [4]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, St Paul, MN, USA

Cancer chemotherapy with platinum-based antineoplastic agents including oxaliplatin frequently results in a debilitating and painful peripheral neuropathy. We evaluated the antinociceptive effects of

the alpha-2 adrenoceptor agonist, clonidine on oxaliplatin-induced neuropathic pain. Specifically, we determined if (1) the intraperitoneal (i.p.) injection of clonidine reduces mechanical allodynia in mice with an oxaliplatin-induced neuropathy, and (2) concurrent inhibition of p38 mitogen-activated protein kinase (MAPK) activity by the p38 MAPK inhibitor SB203580 enhances clonidine's anti-allodynic effect. Clonidine (0.01-0.1 mg kg-1 i.p.), with or without SB203580 (1-10 nmol, intrathecal) was administered two weeks after oxaliplatin injection (10 mg kg-1, i.p.) to mice. Mechanical withdrawal threshold, motor coordination and blood pressure were measured. Post-mortem expression of p38 MAPK and ERK as well as their phosphorylated forms (p-p38 and p-ERK) were quantified in the spinal cord dorsal horn of treated and control mice. Clonidine dose-dependently reduced oxaliplatin-induced mechanical allodynia and spinal p-p38 MAPK expression, but not p-ERK. At 0.1 mg kg-1, clonidine also impaired motor coordination and decreased blood pressure. A 10 nmol dose of SB203580 alone significantly reduced mechanical allodynia, while a sub-effective dose (3 nmol) potentiated the anti-allodynic effect of 0.03 mg kg-1 clonidine. Coadministration of SB203580 and 0.03 mg kg-1 clonidine decreased allodynia similar to that of 0.10 mg kg-1 clonidine, but without significant motor or vascular effects. These findings demonstrate that clonidine treatment reduces oxaliplatin-induced mechanical allodynia. The concurrent administration of SB203580 reduces the dosage requirements for clonidine, thereby alleviating allodynia without producing undesirable motor or cardiovascular effects.

Key Words: clonidine, mechanical allodynia, oxaliplatin, p38 mitogen-activated protein kinases

P07-45

Role of capsaicin-sensitive primary afferents in the development of hypersensitivity in a new mouse model for nitroglycerin-induced chronic migraine

Sol-Ji Kim[1], Seo-Yeon Yoon[2], Ji-Hee Yeo[1], Dae-Hyun Roh[1,*]

[1]Department of Oral Physiology and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea, [2]Pain Cognitive Function Research Center, Department of Brain and Cognitive Sciences College of Natural Sciences, Dental Research Institute and Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Republic of Korea

Despite the relatively high prevalence of migraine or headache, the pathophysiological mechanisms triggering headache, especially in relation to chronic migraine are unknown. Since nitric oxide (NO) is well known as a causative factor in the pathogenesis of migraine, we were to establish a new mouse model of chronic migraine using nitroglycerin (NTG), a donor of NO. NTG (10 mg/kg) was repetitively administrated every other day for 9 days. Repetitive administration of NTG produced acute mechanical allodynia and thermal hyperalgesia in the hind paws 2 hours after each injection from the second injection day (day 3) of NTG (Post-treatment responses). In contrast, the cold allodynia significantly occurred in the facial region with similar time course. In addition, the NTG-treated mice appeared a progressive and long-lasting decrease of basal thresholds (Basal responses). These chronic basal pain responses also persisted for 10 days after cessation of NTG administration. This NTG-induced peripheral hypersensitivity in facial region and hind paws was reduced by concomitant treatment of sumatriptan (0.6 mg/kg), a medication used for the treatment of migraine headaches. We next examined whether the depletion of capsaicin sensitive primary afferents (CSPAs) with resiniferatoxin (RTX) modified the development of peripheral hypersensitivity in migraine. Interestingly, RTX (0.02 mg/kg) pretreatment partially prevented the induction of mechanical allodynia in hind paw, whereas it did not affect the development of cold allodynia in facial region. These findings demonstrated that repetitive NTG administration resulted in acute and long-lasting pain responses in both hind paws and facial region. In addition, the NTG-induced mechanical hypersensitivity in hind paws was partially mediated by the CSPAs. Therefore, the development of peripheral hypersensitivity in migraine patients could be dependent on the site, the modality and the primary afferent types.

Key Words: migraine, nitroglycerin, hypersensitivity, resiniferatoxin, capsaicin-sensitive primary afferent

P08-01

Altered rhythmic behaviors in Alzheimer's disease model flies by dim light exposure at night

Manivannan Subramanian[1], Mari Kim[2], Eunil Lee[2], Joong-Jean Park[1]

[1]Department of Physiology, [2]Department of Preventive Medicine, Korea University College of Medicine, 73 Inchon-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

Accumulation of Amyloid β (Aβ) peptides in the brain is the hallmark for the progression of Alzheimer's disease (AD) in humans. The AD is related during aging with aggregation of Aβ42 fibrils in the brain which may arises due to improper cleavage of Amyloid Precursor Protein (APP) by beta-secretase enzyme and other mechanisms. Disruption of circadian rhythmicity with altered sleep-wake cycle, dysregulation of locomotion and increased memory defects have been shown in AD patients. In Drosophila, we show that over-expression of Aβ42 peptide in neurons gave rise to increased locomotor defects and neurodegeneration with aging. Here we have characterized that neuronal over-expression of Aβ42 peptide showed decreased lifespan due to neuronal Aβ toxicity. The AD flies when exposed to dim light at night, shows disruption of circadian rhythmicity and sleep-wake cycle with increased locomotor defects. Hence using fly model system, we hereby report that dim light at night may have an adverse effect on circadian rhythmicity with disturbed sleep-wake behavior in wild type flies and these phenotypes are more severely pronounced in AD flies which becomes sick under dim light exposure. [This work was supported by Future Environmental R&D Grant funded by the Korea Environmental Industry and Technology Institute (No. RE201206020)]

Key Words: APP, circadian rhythmicity, neurodegeneration, Drosophila

P09-01

Signals governing the trafficking of PKD1L1 and PKD2L1 to primary cilia

Kotdaji Ha[1], Insuk So[1]

Department of Physiology, Seoul National University, College of Medicine, Seoul, Republic of Korea[1]

Primary cilia are solitary organelles that extend from the basal body of the apical surface into the extra cellular matrix of most eukaryotic cells. Dysfunctions of primary cilia underlie a multitude of human disorders, including autosomal dominant polycystin kidney disease (ADPKD), yet membrane targeting to the cilium remains poorly understood. Several proteins that contain targeting sequences that serve as a type of cellular zip code to direct the proteins to the cilium have previously reported. Among the aforementioned motifs, arguably the best understood

mechanistically is VxPx motif found in polycystin 2, CNGB1b, and rhodopsin that is necessary for targeting of these proteins to renal cilia, olfactory cilia, and photoreceptor outer segments, respectively. The motif, V765TPD, is detected in PKD2L1 which are reported to play an important role in flow sensing mechanism and calcium regulation in cilia. To confirm the detected ciliary targeting sequence (CTS), hPKD2L1 was overexpressed in mIMCD-3, and localized to cilia. However, we found that Δ V765TPD hPKD2L1 protein does not traffic into cilia, which indicates that this motif is responsible for ciliary localization. We also investigated electrophysiological characteristics of hPKD2L1 and Δ V765TPD mutant. In whole-cell patch clamp of HEK293 cells transiently transfected with hPKD2L1 and the mutant, each produced outward currents, 134.1 ± 48.7 pA/pF and 158.4 ± 27.4 pA/pF, respectively. Since patients with PKD show abnormal sensory cilia function, the aim of our current study is to search for CTS in PKD2L1 and the candidate domain responsible for ciliary localization in PKD1L1 and their unknown role in calcium regulation mechanism in cilia. Here, we try to identify a novel ciliary trafficking determinant in PKD1L1 and PKD2L1 that furthers our understanding of how proteins are selectively targeted to the cilium and their functional role in calcium regulation.
Key Words: PKD1L1, PKD2L1, Cilia, CTS, PKD

P09-02

Klotho ameliorates proteinuria through protecting podocyte injury

Ji-Hee Kim[1], Kyu-Hee Hwang[1], Seong-Woo Jeong[12], In Deok Kong[12], Kyu-Sang Park[12], Seung-Kuy Cha[1-3¶]

Departments of [1]Physiology and Global Medical Science, [2]Institute of Lifestyle Medicine and [3]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

Klotho is an anti-aging protein predominantly produced in the kidney, the extracellular domain of which is secreted into the systemic circulation. Klotho is known to protect organs including the kidney. Whether and how Klotho directly protects the glomerular filter remains largely elusive. Here, we report that Klotho exerts protective role against podocyte injury leading albuminuria in diabetic nephropathy and chronic kidney diseases (CKD). Klotho was expressed in podocytes of mouse and human kidney. Type II diabetic db/db mice showed typical diabetic nephropathy features such as albuminuria and disruption of podocyte silt-diaphragm whose features were rescued by administration of purified Klotho protein. Heterozygous klotho-deficient CKD mice have aggravated albuminuria compared to that in wild-type CKD mice with a similar degree of hypertension and reduced clearance function. Disrupting the integrity of glomerular filter by saline infusion-mediated extracellular fluid volume expansion increased urinary Klotho excretion. These results reveal a novel function of Klotho in protecting the glomerular filter and offer a potential new therapeutic strategy for treatment of proteinuria. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)]
Key Words: Klotho, podocyte, proteinuria, diabetic nephropathy (DN), chronic kidney disease (CKD)

P09-03

Klotho inhibits tumor progression by IGF-1 receptor activation in human clear cell renal cell carcinoma

Ji-Hee Kim[1,3], Kyu-Hee Hwang[1,3], Minseob Eom[2,3], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Kyu-Sang Park[1,3,4], Seung-Kuy Cha[1,3-5,¶]

Departments of [1]Physiology, [2]Pathology, [3]Global Medical Science, [4]Institute of Lifestyle Medicine and [5]Nuclear Receptor Research Consortium, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

Klotho is an anti-aging hormone as emerging role in tumor suppressor. Growth factor receptor (GFRs) signaling regulates cell growth, proliferation, metabolism, and survival in all kind of malignancy, including clear cell renal cell carcinoma (ccRCC). Klotho is known as a regulator of IGF-1 receptor signaling, which has been implicated in the development of highly invasive metastatic ccRCC. However, the correlation of Klotho and IGF-1 receptor in ccRCC has not been examined. Here, we hypothesize that Klotho and IGF-1 receptor expression may correlate with the clinico-pathological parameters of ccRCC. Klotho expression was significantly correlated with clinical outcomes including tumor necrosis, Furman nuclear grade, cystic change, pathologic T stage, and TNM stage. IGF-1 receptor was highly expressed in tumor tissues compared to that in normal adjacent mesenchyme. Reduction of Klotho expression in tissues with high Fuhrman nuclear grade and large tumor size indicates that Klotho suppresses tumorigenesis of ccRCC. Moreover, higher expression of Klotho showed in small size of tumor, absent of cystic change and tumor necrosis, pathologic T stage 1 and TNM stage I. Functionally, Klotho blunted IGF-1-stimulated migration and proliferation in Caki1, a ccRCC cell line. In conclusion, the expression of Klotho is decreased as the grade of cancer progression, which affects clinico-pathological parameters. These results reveal that Klotho is a new prognostic marker for ccRCC and may open new avenues for the development of feasible carcinostatis substance. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)]
Key Words: Klotho, IGF-1, IGF-1R, renal cell carcinoma

P09-04

Orai1 expression is closely related with favorable prognostic factors in clear cell renal cell carcinoma

Kyu-Hee Hwang[1,3,4], Ji-Hee Kim[1,3,4], Sayamaa Lkhagvadorj[2,3], Minseob Eom[2,3], Kyu-Sang Park[1,3,4], Seong-Woo Jeong[1,3,4], In Deok Kong[1,3,4], Seung-Kuy Cha[1,3,4]

Departments of [1]Physiology, [2]Pathology and [3]Global Medical Science, and [4]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

Store-operated calcium (Ca2+) entry (SOCE) is a major Ca2+ entry mechanism in non-excitable cells, including epithelial cells, which are the most common origin of cancer. We previously described Orai1 and STIM1 as the molecular components of SOCE that are related in cancer hallmark of clear cell renal cell carcinoma (ccRCC). However, the clinical relevance of Orai1 and STIM1 expression in ccRCC remains elusive. Here, we report the expression of Orai1 and STIM1 in ccRCC, and compare their expression with the patient's outcome and clinico-pathological parameters. Immunohistochemical staining of Orai1 and STIM1 was

performed with 126 formalin fixed paraffin embedded ccRCC tissues and protein expression was analyzed by western blot on available fresh tissues. The results were compared to generally well-established clinico-pathologic prognostic factors in ccRCC with patient survival. Although Orai1 is plasma membrane protein, Orai1 was mainly expressed in ccRCC nuclei, while STIM1 showed the cytosol expressing pattern in staining. Furthermore, Orai1 expression level was inversely correlated with ccRCC tumor grade, whereas STIM1 expression level was not associated with tumor grade. Higher Orai1 expression was significantly associated with lower Fuhrman nuclear grade, pathologic T stage, and TNM stage with favorable prognosis. Unlike Orai1 expression, the expression level of STIM1 did not correlate with ccRCC grade and clinical outcomes. These results suggest that Orai1 is an appealing prognostic marker and therapeutic target for ccRCC. [This research was supported by Basic Science Research program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)]
Key Words: Orai1, STIM1, Clear cell renal cell carcinoma, Tumorigenesis, Prognosis

P10-01

Improved application of the electrophoretic tissue clearing technology, CLARITY, to intact solid organs

Hyunsu Lee, Incheol Seo, Shin Kim, Jae-Hyung Park

Department of Physiology, Keimyung University School of Medicine, 1095 Dalgubeoldaero, Dalseo-Gu, Daegu, 704-701, Korea

Mapping of tissue structure at the cellular, circuit, and organ-wide scale is important for understanding physiological and biological functions. A bio-electrochemical technique known as CLARITY used for three-dimensional anatomical and phenotypical mapping within transparent intact brain has been recently developed. In the present study, we aimed to improve the original CLARITY procedure and developed specific CLARITY protocols for various intact organs including pancreas. Adult mice were perfused transcardially with hydrogel solution with a mixture of 4% PFA, 4% acrylamide, 0.05% bisacrylamide, 0.25% VA044 in PBS. Organs were extracted and incubated in the same solution at 37°C to initiate polymerization. Hydrogel-embedded organs were placed in an electrophoretic tissue clearing (ETC) chamber. While 4% SDS solution was circulated through the chamber, 250-280 mA was applied across the organs at 42°C for 1-2 weeks. We determined the optimal conditions for reducing bubble formation, discoloration, and depositing of black particles on the surface of tissue, which allowed production of clearer organ images. We also determined the appropriate replacement cycles of clearing solution for each type of organ, and convincingly demonstrated that 250-280 mA is the ideal range of electrical current for tissue clearing. We then acquired each type of cleared organs including brain, pancreas, liver, lung, kidney, and intestine. Additionally, we determined the images of axon fibers of hippocampal region, the Purkinje layer of cerebellum, and vessels and cellular nuclei of pancreas. CLARITY is an innovative biochemical technology for the structural and molecular analysis of various types of tissue. We developed improved CLARITY methods for clearing of the brain, pancreas, lung, intestine, liver, and kidney, and identified the appropriate experimental conditions for clearing of each specific tissue type. These optimized methods will be useful for the application of CLARITY to various types of organs.
Key Words: CLARITY, Brain, Nervous system, Electrophoretic tissue clearing, Purkinje layer

P10-02

The Effect of Bio-active Materials Coated Fabric on Rat Skeletal Muscular Mitochondria

Donghee Lee, Young-Won Kim, Misuk Yang, Hyemi Bae, Inja Lim, Hyoweon Bang, Jae-Hong Ko

Department of Physiology, College of Medicine Chung-Ang University, Seoul 156-756

The bio-active materials coated fabric (BMCF) was coated with bio-active materials containing over 30 kinds of minerals. The clothing made of this fabric has layer of bio-active energy which reacts with far infrared rays from human body and it is penetration into human body. To observe the effects, the fabric (10 and 30%) was worn to old-aged rat then the oxygen consumption efficiency and copy numbers of mitochondria, and mRNA expression of apoptosis- and mitophagy-related genes were verified. By wearing the BMCF, the oxidative respiration significantly increased when using the 30% materials coated fabric. The mitochondrial DNA copy number significantly decreased and subsequently recovered in a dose-dependent manner. The respiratory control ratio to mitochondrial DNA copy number showed a dose-dependent increment. As times passed, Bax, caspase 9, PGC-1α and β-actin increased, and Bcl-2 decreased in a dose-dependent manner on mRNA expressions. However, the BMCF can be seen to have had no effect on Fas receptor. PINK1 expression did not change considerably and was inclined to decrease in control group, but the expression was down-regulated then subsequently increased with the use of the BMCF in a dose-dependent manner. Caspase 3 increased and subsequently decreased in a dose-dependent manner. These results suggest that the BMCF invigorates mitophagy and improves mitochondrial oxidative respiration in skeletal muscle, and in early stage of apoptosis induced by the BMCF is not related to extrinsic death-receptor mediated but mitochondria-mediated signaling pathway.
Key Words: Apoptosis, Bio-active materials coated fabric, Mitochondria, Mitophagy, Oxidative respiration

P10-03

Identification of Primo-Vascular System in Abdominal Subcutaneous Tissue Layer of Rats

Chae Jeong Lim, So Yeong Lee, Pan Dong Ryu

Laboratory of Veterinary Pharmacology, College of VeterinaryMedicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea University, Seoul 151-742, Republic of Korea

The primo-vascular system (PVS) is a novel vascular network that was first reported in the 1960s by Kim, who claimed that the tissue corresponded with the acupuncture meridians. The PVS tissue has been identified in various sites, such as internal organs, brain ventricles, and blood and lymphatic vessels in several animal species. However, the PVS in subcutaneous tissue has not been well identified. In this study, we examined the putative PVS on the surface of abdominal subcutaneous tissue in rats. Hemacolor staining was conducted for the identification of the subcutaneous PVS (sc-PVS). Hemacolor solution 1 (methyl alchol) was first applied to the hypodermis region of interest for 5 s, and then Solutions 2 (eosin) and 3 (methylene blue) were applied to the same region one by one. Then, Solution 3 had been applied for 5 s; the region was washed out with a 0.9% saline solution and further examined under a stereomicroscope, scanning electron microscope (SEM), and transmission electron microscope (TEM). Hemacolor staining

revealed dark blue threadlike structures consisting of nodes and vessels, which were frequently observed bundled with blood vessels. The structure was filled with various immune cells including mast cells and leucocytes. In the structure, there were inner spaces (20–60 μm) with low cellularity. SEM and TEM revealed a bundle structure and typical cytology common with the well-established organ surface PVS, which were different from those of the lymphatic vessel. Among several sc-PVS identified on the rat abdominal space, the most outstanding was the sc-PVS aligned along the ventral midline. The distribution pattern of nodes and vessels in the sc-PVS closely resembled that of the conception vessel meridian and its acupoints. In conclusion, our results newly revealed that the PVS is present in the abdominal subcutaneous tissue layer and indicate that the sc-PVS tissues are closely correlated with acupuncture meridians. Our findings will help to characterize the PVS in the other superficial tissues and its physiological roles.
Key Words: subcutaneous primo-vascular system, hypodermis, Hemacolor staining, electron microscopy, acupuncture meridian

P10-04

Toll-like Receptor 2 is Dispensable for an Immediate-early Microglial Reaction to Two-photon Laser-induced Cortical Injury In vivo

Heera Yoon[1], Yong Ho Jang[2], Sang Jeong Kim[3,*], Sung Joong Lee[2,*], Sun Kwang Kim[1,*]

[1]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul 130-701, [2]Department of Oral Physiology and Neuroscience, School of Dentistry, Seoul National University, Seoul 110-749, [3]Department of Physiology, School of Medicine, Seoul National University, Seoul 110-799, Korea

Microglia, the resident macrophages in the central nervous system, can rapidly respond to pathological insults. Toll-like receptor 2 (TLR2) is a pattern recognition receptor that plays a fundamental role in pathogen recognition and activation of innate immunity. Although many previous studies have suggested that TLR2 contributes to microglial activation and subsequent pathogenesis following brain tissue injury, it is still unclear whether TLR2 has a role in microglia dynamics in the resting state or in immediate-early reaction to the injury in vivo. By using in vivo two-photon microscopy imaging and Cx3cr1GFP/+ mouse line, we first monitored the motility of microglial processes (i.e. the rate of extension and retraction) in the somatosensory cortex of living TLR2-KO and WT mice; Microglial processes in TLR2-KO mice show the similar motility to that of WT mice. We further found that microglia rapidly extend their processes to the site of local tissue injury induced by a two-photon laser ablation and that such microglial response to the brain injury was similar between WT and TLR2-KO mice. These results indicate that there are no differences in the behavior of microglial processes between TLR2-KO mice and WT mice when microglia is in the resting state or encounters local injury. Thus, TLR2 might not be essential for immediate-early microglial response to brain tissue injury in vivo.
Key Words: Brain injury, In vivo two-photon microscopy imaging, Microglia, Toll-like receptor 2

P10-05

MLN4924 can promote U373MG cell migration via src dependent phosphorylation of caveolin-1

Sung Yeon Park, Yang-Sook Chun

Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

MLN4924, recently arising as a promising anticancer agent, is a blocker of NEDD8-activating enzyme, which has significantly been reported to suppress proliferation by inducing DNA damage response, cell cycle arrest, autophagy, apoptosis, and senescence and also migration, and motility of cancer cells. Here, we report that MLN4924 can unexpectedly promote migration in U373MG cells by augmenting the level of Cav1 phosphorylation on Y14, depending on Src activity.
Key Words: caveolin, cell migration, MLN4924

P10-06

Study investigated the effects of Oligonol supplementation on sudomotor activity during heat load in human subjects

Jeong Beom Lee, Sun Jong Kang, Sang Eun Im, Jae Young Heo, Hyun Soo Kim, Sang Mook Kim, Hyun Kyu Kang, Jung Ho Kim, Sung Woon Kim

Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea

Oligonol is a low-molecular weight polyphenol that possesses antioxidant and anti-inflammatory properties. However, nothing is known regarding the impact of Oligonol on sudomotor activity. This study investigated the effect of Oligonol supplementation on sweating response under heat stress in human subjects. Initially, we conducted a placebo-controlled, cross-over trial where participants took a daily dose of Oligonol 200 mg or placebo for one week. After a 4 week washout period, the subjects were switched to the other study arm. As a heat load, half-body immersion into hot water (42 ± 0.5°C for 30 min) was performed in an automated climate chamber. Fibroblast growth factor 21 (FGF-21), orexin, irisin tympanic and skin temperatures were measured. Sudomotor activity, including onset time, sweat rate (SR) and volume (SV), active sweat gland density (ASGD), and sweat gland output (SGO), was tested in four or eight areas of skin. When compared with placebo, Oligonol attenuated increases in tympanic and skin temperatures after the heat load. There was an increasing trend in local sweat onset time, but there was a decrease in FGF-21, orexin, irisin, local SR, SV, ASGD, and SGO for Oligonol compared to placebo. The mean ASGD was significantly higher in the Oligonol group than in the placebo group for 10, 20, and 30 min. This study demonstrates that Oligonol appears to be worthy of consideration as a natural supplement to support more economical use of body fluids against heat stress
Key Words: Oligonol, sudomotor activity, heat load

P10-07

PSA-NCAM-Negative Neural Crest Cells Emerging During Neural Induction of Pluripotent Stem Cells Cause Mesodermal Tumors and Unwanted Grafts

Dongjin R. Lee[1], Jeong-Eun Yoo[1], Jae Souk Lee[1], Sanghyun Park[1], Junwon Lee[1], Chul-Yong Park[1], Eunhyun Ji[1], Han-Soo Kim[2], Dong-Youn Hwang[3], Dae-Sung Kim[4,*], Dong-Wook Kim[1,*]

[1]Department of Physiology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea, [2]Cell Therapy Center and Department of Laboratory Medicine, Yonsei

University College of Medicine, Seoul 120-752, Korea, [3]Department of Biomedical Science, College of Life Science, CHA University, Seongnam, Gyeonggi-do 463-840, Korea, [4]Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea

Tumorigenic potential of human pluripotent stem cells (hPSCs) is an important issue in clinical applications. Despite many efforts, PSC-derived neural precursor cells (NPCs) have repeatedly induced tumors in animal models even though pluripotent cells were not detected. We found that polysialic acid-neural cell adhesion molecule (PSA-NCAM)-negative cells among the early NPCs caused tumors, whereas PSA-NCAM+ cells were nontumorigenic. Molecular profiling, global gene analysis, and multilineage differentiation of PSA-NCAM- cells confirm that they are multipotent neural crest stem cells (NCSCs) that could differentiate into both ectodermal and mesodermal lineages. Transplantation of PSA-NCAM- cells in a gradient manner mixed with PSA-NCAM+ cells proportionally increased mesodermal tumor formation and unwanted grafts such as PERIPHERIN+ cells or pigmented cells in the rat brain. Therefore, we suggest that NCSCs are a critical target for tumor prevention in hPSC-derived NPCs and removal of PSA-NCAM- cells eliminates the tumorigenic potential originating from NCSCs after transplantation.

Key Words: Neural precursor cells(NPCs), Neural crest stem cells(NCSCs), PSA-NCAM, Cell Therapy

P10-08

The role of TRPM7 in the progression of human renal cell carcinoma (RCC)

Soon Hee Kim[1], Su Yeon Ryu[1,2,3], Jae Sik Park[1], Eun Kyoung Yang[1]

Department of [1]Physiology, Kyungpook National University, School of Medicine, Daegu 700-422, [2]BK21 Plus KNU Biomedical Convergence Program, School of medicine Kyungpook National University, Daegu 700-842, [3]Tumor Heterogeneity and Network(THEN) Research Center, School of medicine Kyungpook National University, Daegu 700-842, Korea

Purpose: Transient receptor potential melastatin 7 (TRPM7) is a non-selective cationic channel containing a functional kinase domain. In normal tissues, it is distributed widely. Recent studies have shown that TRPM7 is involved in the regulation of cellular growth, proliferation, differentiation, and migration in various human cancers. However, the role of TRPM7 in pathogenesis of human RCC remains uncertain. Thus, the present study examined the effects of silencing of TRPM7 on the proliferation, migration, and invasion of human RCC cells. **Materials & Methods:** ACHN cells were transfected with 100 nM TRPM7 siRNA using Lipofectamine RNAiMAX. Transfected RCC cells were cultured in Eagle's minimum essential media supplemented with 10% fetal bovine serum. The effect of TRPM7 siRNA on cell viability was determined by WST-1 assay. Cell motility and invasiveness were analyzed using in-vitro wound healing assay and trans-well assay, respectively. All measurements were performed in triplicate at 24 hours after TRPM7 knockdown. Additionally, the protein levels of MMP2, MMP9, TIMP1, and TIMP2 were measured by western blot analysis to verify the influence of TRPM7 siRNAs on the expression of matrix metalloproteinases (MMP) and their inhibitors (TIMP). **Results:** Suppression of TRPM7 had no effects on proliferation of RCC cells compared with negative control. Cell movement was decreased about 50% by silencing RNA targeting TRPM7. Invasion of RCC cells were also suppressed markedly by TRPM7 RNA interference. Silencing of TRPM7 induced upregulation of MMP2 and MMP9 protein expressions. In contrast, the protein levels of TIMP1 and TIMP2 were decreased by silencing RNA of TRPM7. **Conclusion:** TRPM7 knockdown inhibits RCCC cell migration and invasion. In addition, it induces expressive imbalance of MMPs/TIMPs. These results suggest that TRPM7 may have a role in the RCC progression.

Key Words: TRPM7, siRNA, RCC, MMP. TIMP

P10-09

A novel function of JHDM in Hepatic steatosis

Jung-Yup Song[1], Kyung-Hwa Lee[1], Yang-Sook Chun[1,2,3]

[1]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea, [2]Departments of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Korea, [3]Departments of Physiology, Seoul National University College of Medicine, Seoul 110-799, Korea

Histone modification is one of major type of epigenetic regulation that possesses a big part of modulating biological event. JHDM, a Jmjc histone demethylase is known as demethylase of the Histone H3K9 while it binds to H3K4me3 with PHD domain. JHDM also reported that working with some of metabolism-related transcription factors. In this study, we identified the role of JHDM in the progression of non-alcoholic hepatic steatosis. Overexpression of JHDM attenuated hepatic steatosis and insulin resistance in mice fed with high fat diet. The expression of lipogenic genes were decreased in TG mice liver. In contrast, treatment of LXR agonist in JHDM knock down HepG2 cell line showed that further induction of adipogenic genes. In addition, we found that JHDM physically interact with SREBP1c. We suggest that this interaction interrupts the demethylase activity of JHDM, this causes the inhibition of lipogenic event in Liver.

Key Words: Epigenetic regulation, JHDM, hepatic steatosis

P10-10

KSP inhibitor SB743921 induces death of multiple myeloma cells via inhibition of the NF-kB signaling pathway

Bayalagmaa Nyamaa, In Sung Song, Yu Jeong Jeong, Hyoung Kyu Kim, Naru Kim, Jin Han

Department of Physiology, College of Medicine, Inje University, Busan, Korea

SB743921 is a potent inhibitor of the spindle protein kinesin and is being investigated in ongoing clinical trials for the treatment of myeloma. However, little is known about the molecular events underlying the induction of cell death by SB743921 alone or in combination treatment, in multiple myeloma (MM). Here, we report that SB743921 induces mitochondria-mediated cell death via inhibition of the NF-κB signaling pathway, but does not cause cell cycle arrest in KMS20 MM cells. SB743921-mediated inhibition of the NF-κB pathway results in reduced expression of SOD2 and Mcl-1, leading to mitochondrial dysfunction. Moreover, we found that combination treatment with SB743921 and bortezomib induces death in bortezomib-resistant KMS20 cells. Taken together, these data suggest that treatment with SB743921 alone or in combination with bortezomib offers excellent translational potential and promises to be a novel MM therapy.

Key Words: SB743921, NF-kB, multiple Myeloma, combination therapy, superoxide dismutase 2, Mcl-1

P10-11

The Effect of Low-Intensity Ultrasound in Resolution of Synovitis

Jee-In Chung, A Young Kim, Sumit Barua, Soo Yeon Lee, Eun Joo Baik

Department of Physiology, Department of Biomedical Science, Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

Background: Low-intensity ultrasound (LIUS) can be a feasible therapy for arthritic joints with reducing pain and functional improvement. Neutrophils are first line actors in host defense that recruit macrophages. Dead neutrophils are removed during resolution of inflammation. Delayed neutrophil clearance can lead to extended inflammation or even chronic autoimmune disease. Neutrophil extracellular traps (NETs) in arthritic tissue are involved in the pathogenesis of arthritis; however, the functional role of NETs has not been clarified. **Objectives:** The aims of this study were to investigate the effect of LIUS on synovial inflammation and its resolution via neutrophil clearance. **Methods:** Synovitis was induced by intra-articular injection of complete Freund's adjuvant (CFA) into the left knee joint of male Sprague-Dawley rats. LIUS (1 MHz, 200 mW/cm2) was applied for 10 minutes daily. The neutrophil clearance was assessed with the expression of myeloperoxidase (MPO). In addition, the TUNEL staining and NETs formation in the synovium were observed. In neutrophils and macrophages cultures from peripheral blood, the effect of NETs clearance by LIUS was investigated. **Results:** In CFA-induced synovitis, MPO-positive neutrophils exhibited a peak after 2-3 days, filling the inflammatory core. Monocytes and macrophages in the periphery later infiltrated the core, and were reduced thereafter. LIUS revealed reduced synovial hyperplasia and earlier MPO clearance. Neutrophils in the core of the inflamed synovium exhibited NET formation, which increased by LIUS. LIUS also induced NETs in peripheral polymorphonuclear cells in an intensity-dependent manner and potentiated phorbol myristate acetate (PMA)-induced NETosis. PMA-induced NETs were cleared by macrophages; the clearance was enhanced by LIUS. **Conclusion:** NETs act in inflammatory synovitis, and LIUS enhanced the NETs and resulted in neutrophil clearance by enhancing the phagocytosis of macrophages, that might be an underlying factor for therapeutic effect of LIUS in arthritic synovium. Acknowledgements This study was supported by a Korea Research Foundation (KRF) grant from the Korean government (MEST) (2009-0076242); a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (2012R1A2A2A01011417); the Chronic Inflammatory Disease Research Center (NRF-2012R1A5A204183); and a grant from the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Korea (A091120).
Key Words: arthritis, low-intensity ultrasound, neutrophil clearance, neutrophil extracellular trap

P10-12

Role of CXCR2 in Acetylated Pro-Gly-Pro(Ac-PGP)-induced Vascular Regeneration in Murine Hind limb Ischemia Model

Yang Woo Kwon, Soon Chul Heo, Jung Won Yoon, Tae Wook Lee, Ba Reun Kim, Geun Ok Jeong, Jae Ho Kim

Medical Research Center for Ischemic Tissue Regeneration & Medical Research Institute, Department of Physiology, School of Medicine, Pusan National University, Yang san 626-870, Republic of Korea

Many therapeutic approaches to treat ischemic diseases using endothelial progenitor cells (EPCs) have been developed. EPCs can integrate into blood vessels and stimulate neovascularization of the ischemic limbs and hearts. Therefore, mobilization and recruitment of bone marrow-derived endothelial progenitor cells are critical for ischemia-induced neovascularization. The exact signaling, however, involved in the homing of EPCs to sites of endothelial injury remains to be understood. Chemokine receptor 2 (CXCR2), a receptor of interleukin 8 (IL-8), mediates neutrophil migration to the site of inflammation. The angiogenic effects of IL-8 in intestinal vascular endothelial cells are mediated by this receptor. Our hypothesis is that CXCR2 is involved in the regulating growth and survival of endothelial cell and EPCs through the mechanism similar to IL-8-regulated angiogenesis. We explored the role of CXCR2 in angiogenesis and tissue regeneration by using Acetylated Pro-Gly-Pro (Ac-PGP), which is the endogenous degradation product of extracellular collagen and binds to CXCR2. Ac-PGP stimulated chemotactic migration, tube formation ability of human EPCs in vitro. The blockade of CXCR2 abrogated Ac-PGP-induced migration and tube formation of EPCs. Intramuscular injection of Ac-PGP into the ischemic hindlimb resulted in the attenuation of the severe limb loss and the stimulation of blood perfusion and angiogenesis in the ischemic limb. CXCR2 knockout mice showed the attenuation in Ac-PGP-induced in vivo neovascularization and ischemic limb salvage. These results suggest that Ac-PGP has therapeutic effects by stimulating neovascularization through CXCR2-dependent mechanism.
Key Words: Ac-PGP, CXCR2, EPC, neovascularization, ischemia

P10-13

Wnt signaling pathway augments Endothelial Progenitor Cells commitment and its angiogenic potential through SDF1-CXCR4 axis

Yeon Ju Kim, Sang Mo Kwon

Laboratory for vascular medicine&Stem cell Biology, Medical Research Institute, Department of Physiology, Pusan National University, Korea

Endothelial progenitor cell(EPC) has considered as a potential therapeutic strategy for vascular regeneration in ischemic tissue. However, use of EPCs for cell-based therapy is hindered due to attenuated cellular yield and biological activity. In this study, we investigated the role of Wnt pathway that is a powerful regulator of cell proliferation and differentiation. In EPC colony forming assays, we demonstrated that GSK3β inhibitor, CHIR99021 enhanced the number of small, large colony and formed more round shape colony than control. We then examined the role of Wnt pathway in EPC bioactivity and found that proliferation, migration and invasion capacity of EPCs are drastically increased in Wnt pathway activated cells. Interestingly, we also found significant enhancement of CXCR4 expression when Wnt pathway is activated. Consequently, our results suggest that Wnt pathway plays a role in EPC commitment and its angiogenic potential and these effects may be attributed to enhancement of CXCR4 directly regulated by Wnt signal. Thus, regulation of Wnt pathway in EPCs can be an effective strategy for angiogenic therapy.
Key Words: Endothelial Progenitor cell, Wnt pathway, CXCR4

P10-14(O-9)

The Sulfated Polysaccharide Fucoidan Rescues Senescence of Endothelial Colony Forming Cells for Ischemic Repair

Jun Hee Lee[1], Takayuki Asahara[2], Sang-Mo Kwon[1]

[1]Laboratory for Vascular Medicine and Stem Cell Biology, Medical Research Institute, Department of Physiology, School of Medicine, Pusan National University, Yangsan 626-870, Korea, [2]Department Regenerative Medicine Science, Tokai University School of Medicine, Isehara, Kanagawa, 259-1193, Japan

The efficacy of cell therapy utilizing endothelial colony-forming cells (ECFCs) in the treatment of ischemia is limited by the replicative senescence of isolated ECFCs in vitro. Such senescence must therefore be overcome in order for such cell therapies to be clinically applicable. This study aimed to investigate the potential of sulfated polysaccharide fucoidan to rescue ECFCs from cellular senescence and to improve in vivo vascular repair by ECFCs. Fucoidan-preconditioning of senescent ECFCs was shown by flow cytometry to restore the expression of functional ECFC surface markers (CD34, c-Kit, VEGFR2, and CXCR4) and stimulate the in vitro tube formation capacity of ECFCs. Fucoidan also promoted the expression of cell cycle-associated proteins (cyclin E, Cdk2, cyclin D1, and Cdk4) in senescent ECFCs, significantly reversed cellular senescence, and increased the proliferation of ECFCs via the FAK, Akt, and ERK signaling pathways. Fucoidan was found to enhance the survival, proliferation, incorporation, and endothelial differentiation of senescent ECFCs transplanted in ischemic tissues in a murine hindlimb ischemia model. Moreover, ECFC-induced functional recovery and limb salvage was markedly improved by fucoidan pretreatment of ECFCs. To our knowledge, the findings of our study are the first to demonstrate that fucoidan enhances the neovasculogenic potential of ECFCs by rescuing them from replicative cellular senescence. Pretreatment of ECFCs with fucoidan may thus provide a novel strategy for the application of senescent stem cells to therapeutic neovascularization.
Key Words: endothelial colony-forming cells, replicative cellular senescence, fucoidan, vascular repair

P10-15

Novel angiogenic peptide stimulates mouse hindlimb ischemia repair

TaeWook Lee, YangWoo Kwon, SoonChul Heo, Ilho Jang, JaeHo Kim

Physiology, Pusan national university medical school, Yangsan 626-870, South Korea

Ischemia is a major disease which can plague modern living. We searched for peptide drugs to treat ischemia and identified SR-0379 as a potential candidate. In previous studies, SR-0379 significantly stimulated wound healing in rats and had angiogenic property and anti-microbial ability. In our study, we first observed that migration and tube-forming ability of (EPCs) were enhanced by SR-0379 treatment in vitro. However, SR-0379 did not affect cell survival significantly. To determine whether SR-0379 can promote the recovery from hindlimb ischemia and increase repair, we injected SR-0379 into the muscle of ischemia-induced hindlimb of mice. Repair in ischemic injury mouse model was significantly enhanced in peptide injected hindlimb compared to sham-injected hindlimb. In addition, we confirmed the increased expression of CD31 and SMA-α by immunostaining in SR-0379-injected ischemic hindlimb. These results suggest that SR-0379 can be a novel drug candidate for treating ischemic disease.
Key Words: Hindlimb ischemia, SR-0379, Angiogenic peptide, EPC

P10-16

Caffeine links dopamine, serotonin and prolactin release during thermal stress in human

Tae Wook Kim[1,2,*], Jeong Beom Lee[2], Sun Jong Kang[2], Sang Eun Im[2], Jae Young Heo[2], Hyun Soo Kim[2], Sang Mook Kim[2], Hyun Kyu Kang[2], Jung Ho Kim[2], Sung Woon Kim[2]

[1]Department of Health Care, Graduate School, Soonchunhyang University, 646 Eupnae-ri, Shinchang-myeon, Asan 336-745, [2]Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea

The aim of this study was to investigate the serum serotonin (5-HT), prolactin (PRL) and plasma dopamine (DA) levels in humans with and without caffeine ingestion during and after thermal stress (half immersion in 42°C hot water). Eleven male volunteers participated in the randomized experiment (CON, n=15, 200 mL of tap water vs. CAFF, n=15, 3 mg·kg-1 and 200 mL tap water). After 60 min, thermal stress was conducted for 30 min. Blood samples were collected and assessed for 5-HT, DA and PRL with and without caffeine during and after thermal stress. 5-HT was significantly lower in the CAFF group compared to the CON group after thermal stress for 30 min (Post) ($p < 0.05$) and also after 60 min of resting ($p < 0.01$). DA and PRL were significantly higher in the CAFF group than in the CON group at the Post time point ($p < 0.001$). In conclusion, 3 mg·kg-1 caffeine ingestion prior to thermal stress can alter central serotonergic and dopaminergic activity, which may contribute to reduced central fatigue and subsequently, to reduced general fatigue. PRL responses during thermal stress were also significantly related to caffeine ingestion in this study. However, the inhibitory effects of DA on PRL by caffeine remain to be elucidated.
Key Words: Caffeine, Dopamine, Prolactin, Serotonin, thermal stress

P10-17

Improved sweat gland function during active heating in physically trained human

Jeong Beom Lee[1,*], Tae Wook Kim[1,2], Sun Jong Kang[1], Sang Eun Im[1], Jae Young Heo[1], Hyun Soo Kim[1], Sang Mook Kim[1], Hyun Kyu Kang[1], Jung Ho Kim[1], Sung Woon Kim[1], Sang Woo Jang[2]

[1]Department of Physiology, College of Medicine, Soonchunhyang University, 366-1 Ssangyong-dong, Cheonan 331-946 Republic of Korea, [2]Department of Health Care, Graduate School, Soonchunhyang University, 646 Eupnae-ri, Shinchang-myeon, Asan 336-745

Relatively few studies on the peripheral sweating mechanisms of in physically trained human have been conducted. The purpose of this study was to compare the sweating capacities of physically trained human against untrained subjects (controls). Thirty-five healthy male volunteers participated; 15 untrained subjects and 20 physically trained human. Active heat generation was performed for 30 min (running at 60% VO_2max) in a climate chamber (temperature, 25 ± 0.5°C; relative humidity, 60±3%, termed active heating). Sweating data (local sweat onset time, local sweat volume, activated sweat glands, sweat output per gland, whole body sweat loss volume) were measured by the capacitance hygrometer-ventilated capsule method and starch-iodide paper. Mean body temperature was calculated from tympanic and skin temperatures. Local sweat onset time was shorter for tennis athletes ($p < 0.001$). Local sweat volume, activated sweat glands of the torso and limbs, sweat output per gland and whole body sweat loss volume were

significantly higher for physically trained human than control subjects after active heating ($p < 0.001$). Tympanic and mean body temperatures were lower among physically trained human than controls ($p < 0.05$). These results indicate that physically trained human had increased regulatory capacity of their sweat gland function.
Key Words: Active heating, Activated sweat glands, Physically trained human, Sweating function, Sweat onset time, Sweat output

P10-18

Assessment of eye irritation potential of hair dye chemicals using human conjunctival keratinocytes

Ju Hyun Lim[1], Jeong Bum Bae[2], Hae-Rahn Bae[1]

[1]Department of Physiology, [2]Department of Ophthalmology, College of Medicine, Dong-A University, Busan 602-714, Korea

The harmful effects of hair dyes on human heath have been widely studied in the field of dermatology and hemato-oncology. Hair dye-induced toxic conjunctivitis is frequently encountered in the Ophthalmology Clinic, but researches on the hazardous potential to eye are lacking. We investigated the irritation potential of hair dye chemicals to primary cultured human conjunctival epithelial cells. Five common ingredients in hair dyes were selected based on the priority chemicals listed on the labels of 10 commercially available hair dyes. Human conjunctival epithelial cells were primarily cultured using the explant techniques from the conjunctival tissues obtained during conjunctivochalasis surgery.
Key Words: Human conjunctival keratinocytes, Hair dye, Paraphenylenediamine, Cytotoxicity, Toxic conjunctivitis

P10-19

Effect of Samultang on HO-1 Mediated Vascular protection in HUVECs

Eun Sik Choi[1,2], Yun Jung Lee[1,2], Jung Joo Yun[1,2], Min Chol Kho[1,2], Ji Hun Park[1,2], Xian Jun Jin[1,2,3], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]

[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

Samultang (四物湯, SMT) is a well known herbal prescription treating overall haematological disorders defined as blood deficiency, blood stasis and blood loss in traditional Korean medicine. SMT is recorded in several formulary including 'Treasured Mirror of Eastern Medicine' (東醫寶鑑, Donguibogam) and consists of 4 herbs: Angelicae Radix, Cnidii Rhizmoa, Rhemanniae Radix Preparata and Paeonia Radix. Major cause of atherosclerosis and other vascular diseases is inflammation and vascular endothelium is emphasized as central spot of vascular inflammatory process. Thus, we investigated the effects of SMT water extracts on vascular inflammation in HUVECs (Human Umbilical Vein Endothelial Cells). Expression of CAMs (cell adhesion molecules) such as VCAM-1 (vascular cell adhesion molecule-1), ICAM-1 (intracellular adhesion molecule-1), E-selectin (endothelial-selectin) and HL-60 monocyte adhesion were induced by TNF-α stimulation. However, SMT pretreatment (10-50 μg/ml) inhibited CAMs expression and monocyte adhesion significantly. TNF-α stimulation led HUVECs to produce ROS (reactive oxygen species) and translocate NF-κB (nuclear factor-κB) into nucleus, but SMT suppressed ROS production and NF-κB nuclear localization significantly. Furthermore, activation of NF-κB was also suppressed by SMT in dose dependent manner. HO-1 (heme oxygenase-1) protein level was significantly upregulated by SMT treatment (10-50 μg/ml) as HO-1 inducer CoPP (cobalt protoporphyrin) did. Besides, nuclear translocation of Nrf-2 (nuclear factor erythroid 2-related factor 2), which transcripts antioxidative genes including HO-1, was increased by SMT dose dependently. Intracellular NO (nitric oxide) and nitrite products were also increased by SMT treatment. Taken together, these results suggest that SMT might exerts vascular protective effects by suppressing vascular inflammatory process triggered from damaging stimulus including TNF-α and by promoting synthesis of beneficial bioactive substances including HO-1.
Key Words: Samultang (SMT), vascular inflammation, cell adhesion molecules (CAMs), heme oxygenase-1 (HO-1), nitric oxide (NO)

P10-20

Inhibitory Mechanism of Samchuleum on Renal Fibrosis

Jung Joo Yoon[1,2], Yun Jung Lee[1,2], Byung Hyuk Han[1,2,3], Seung Namgung[1,2,3], Min Chol Kho[1,2], Ji Hun Park[1,2], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]

[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, 2Hanbang Body-fluid Research Center, 3Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

Samchuleum (SCE), originally recorded in an ancient Korea medicinal book named "Donguibogam" (東醫寶鑑), is a well-known blended traditional herbal formula. SCE is composed of nine dried herbs: Rehmannia glutinosa, Paeonia japonica, Cnidium officinale Makino, Angelica gigas, Panax ginseng, Atractylodes japonica, Pinellia ternate, Citrus Aurantium, Glycyrrhiza uralensis. Diabetic nephropathy (DN) is associated with morbidity and mortality of diabetic patients. Mesangial cell proliferation is known as the major pathologic features such as glomerulosclerosis and renal fibrosis. Thus, this study investigated the inhibitory effect of SCE (1-50 μg/ml) on high glucose (HG)-stimulated rat mesangial cells (RMC) proliferation and fibrosis. Thymidine incorporation under HG was significantly accelerated, which was inhibited by SCE in a dose dependent manner. Pre-treatment of SCE induced down-regulation of cyclins/CDKs and up-regulation of CDK inhibitor, p21waf1/cip1 and p27kip1 expression. In addition, SCE significantly suppressed the HG-induced ROS production. Thus, SCE consequently inhibited HG-induced mesangial cell proliferation through the inhibition of ROS signaling pathway. HG enhanced expression of fibrosis biomarkers such as collagen IV and CTGF, which was markedly attenuated by SCE. Moreover, SCE inhibits HG-induced fibronectin mRNA expression. SCE decreased TGF-β1 and p-Smad2/Smad4 expression, whereas increased Smad7 expression under HG. Thus, SCE promoted ECM degradation through disturbing TGF-β–SMAD signaling. In conclusion, these results suggested that SCE has a protective effect on renal proliferation and fibrosis. These results suggest that SCE might be effective in the treatment of renal dysfunction leading to DN.
Key Words: Samchuleum (SCE), Mesangial cell, High glucose (HG), CDKs, Collagen IV, TGF-β1

P10-21

Inhibitory Effect of Hwagryunhaedoktang on TNF-α-induced vascular inflammation in human umbilical vein endothelial cells

Byung Hyuk Han[1,2,3], Yun Jung Lee[1,2], Eun Sik Choi[1,2], Seung Namgung[1,2,3], Xian Jun Jin[1,2,3], Ho Sub Lee[1,2,3,*], Dae Gill Kang[1,2,3,*]

[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

Hwangryunhaedoktang (HHT) originally recorded in an ancient Korea medicinal book named "Donguibogam" and has been used for the treatment of inflammatory hemorrhage relevance to vascular inflammation which can cause an atherosclerosis. This study was designed to demonstrate whether HHT has an inhibitory effect on vascular inflammation induced by TNF-α in human umbilical vein endothelial cells (HUVECs). Pretreatment with HHT decreased the adhesion of HL-60 cells to TNF-α-induced HUVEC. HHT suppressed TNF-α-induced expression level of cell adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial cell selectin (E-selectin), as well as dose dependently inhibited TNF-α-induced matrix metalloproteinase-2/-9 activity. HHT significantly decreased TNF-α-induced intracellular reactive oxygen species (ROS) production. Western blot and immunofluorescence analysis showed that HHT inhibited the translocation of p65 NF-κB to the nucleus. Phosphorylation of IκB-α also was inhibited in cytoplasm by pre-incubating with HHT, on the other hand increased expression of IκB-α. In addition, HHT increased the Nrf2 protein expression in nucleus of HUVECs. Protein expression of HO-1 was induced by HHT dose-dependently. In that case of co-treated with SnPP, HO-1 inhibitor, and CoPP, HO-1 inducer, protein expression of HO-1 was reduced and increased at each case. These data showed that treatment with HHT regulate the ROS/NF-κB signaling pathway. Furthermore HO-1 induction was increased by single processing of HHT in dose-dependent manner. Therefore a traditional herbal formula HHT might be potential therapeutic agent of atherosclerosis.
Key Words: Hwangryunhaedoktang(HHT), HUVEC, TNF-α, Vascular inflammation, HO-1

P10-22

Study on the mechanism of vascular relaxation by mantidis ootheca

Hye Yoom Kim[1,2], Yun Jung Lee[1,2], You Mee Ahn[1,2], Rui Tan[1,2], So Heun Lee[1,2,3], Han Sol Lee[1,2,3], Dae Gill Kang[1,2,3,*], Ho Sub Lee[1,2,3,*]

[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, [3]Brain Korea (BK)21 plus team, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 540-749, Republic of Korea

The aim of the present study was to define the effects of aqueous extract of mantidis ootheca (AMO) on the vascular tension and its responsible mechanisms in rat thoracic aortic rings. The eggs of a mantis are enclosed in a foamy pouch. The extracts of AMO induced dose-dependent relaxation of phenylephrine-precontracted aorta, which was abolished by removal of functional endothelium. Endothelium-denudation abolished the AMO-induced vasorelaxation. Pretreatment of the endothelium-intact aortic rings with NG-nitro-L-arginine methylester (L-NAME) and 1H-[1,2,4]-oxadiazolo-[4,3-alpha]-quinoxalin-1-one (ODQ) inhibited the AMO-induced vasorelaxation. Similarly, wortmannin and LY-294002, an inhibitors of the phosphatidylinositol 3-kinase (PI3K), an upstream signaling molecule of eNOS, attenuated the AMO-induced vasorelaxation. Furthermore, K+ channel inhibition with tetraethylammonium, 4-aminopyridine and glibenclamide had inhibitory effects on the AMO-induced vasorelaxation. AMO-induced vascular relaxations were also markedly attenuated by addition of inhibitory muscarinic receptor, atropine and methoctramine. Taken together, the present study suggests that AMO relaxes vascular smooth muscle via endothelium-dependent activation of NO-cGMP signaling through the PI3K/Akt-, possible involvement of K+ channel.
Key Words: mantidis ootheca, vasorelaxation, thoracic aorta, NO-cGMP signaling

INDEX

Authors Index

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

Key Word Index

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

Etc