pISSN 1226-4512
eISSN 2093-3827

KJPP

Volume 24, Supplement 1, November 2020

The Korean Journal of Physiology & Pharmacology

November 4 (Wed) - November 5 (Thu), 2020
온라인 학술대회

www.kjpp.net

저희 아라시스템은 '생명공학의 발전'이라는 큰 명제아래 중추적인 핵심리더의 역할보다는 그 중심의 기틀을 확립시키고자하는 "Life Science Foundation"의 역활을 충실히 이행하는 중점을 두고 성장해 나가고 있는 회사입니다.

현재 우리나라는 '바이오산업'의 거센 파도속에서 표류중인채, 멀리 빛나는 등대불을 보고 항해하고 있는 실정으로, 한국의 바이오과학기술은 대부분 미국이나 유럽등의 바이오산업에 선두그룹을 내준체로 외국의 유수한 연구소의 수많은 방법론, 패러다임을 따라 실험하면서 그들을 따라가기에 급급한 실정입니 다. 이에 저희 아라시스템은 이미 말씀드린데로, 어깨를 나란히 한다는 말보다는 보다 한걸음 한걸음씩 충실히 연구개발하여 기초생명공학시약 및 과학기자재의 국산화에 초점을 맞추어 고객님들께 다가갈 것을 약속드립니다.

또한 외국제품중 많은 인지도와 높은 Technical Products 을 선별하여 제품을 확보하고있어 보다 저렴하게 공급을 할수있도록 많은 노력을 기울이고 있습니다. More than Quality','More than Low Price', 'More than Service'를 위해서 저희 아라시스템은 언제나 고객여러분의 목표에 충실한 보조자로서 항상 여러분의 옆에서 같이 노력할 것을 약속드립니다.

기타 소모품 및 장비 : Balances, Vernier Calipers, pipet tip, tube외 다수 취급

TEL : 02-6083-6333 FAX : 02-6442-6330 Address : 서울시 광진구 자양2동 672 한솔리베르 1808

MAGIC TREE
www.magictree.kr

MAGICTREE

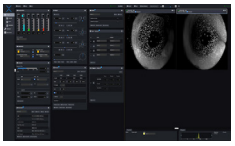
LUXENDO
A BRUKER COMPANY

LIGHT-SHEET EQUIPMENT THAT CAN IMPLEMENT ALL EXPERIMENTS IN VARIOUS FIELDS

MuVi SPIM LS
Multiple View
Live Sample

MuVi SPIM LS
Live Sample
PhotoManipulation

MuVi SPIM CS
Multiple View
Cleared Sample



Lux Control
Acquisition& Processing

InVi SPIM
Inverted View

InVi SPIM PM
PhotoManipulation

InVi SPIM Lattice Pro
Illumination Module

Lux DATA
Storage & Processing

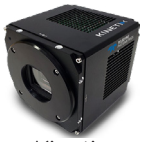
QuVi SPIM
Quantitative View

LCS SPIM
Large Cleared Sample

TruLive3D Imager
Dual Illumination

TELEDYNE PHOTOMETRICS
Everywhereyoulook™
Part of the Teledyne Imaging Group

ADVANCED IMAGING CAMERA TECHNOLOGY

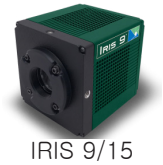


Kinetic

Prime 95B

Prime BSI

Prime Express



IRIS 9/15

Moment

CoolLED
Simply Better Control

FLUORESCENCE LED ILLUMINATION

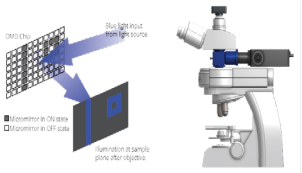
pE-300 series
Lite/White/Ultra

pE-340
Fura

MIGHTEX

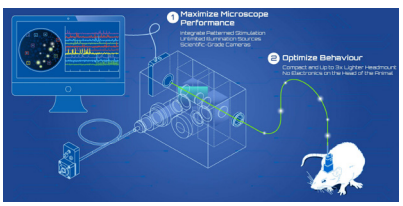
PATTERNED ILLUMINATION & STIMULATION

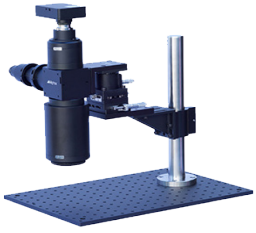
Polygon1000 G & L type



IN VIVO IMAGING & STIMULATION

OASIS Implant





OASIS Macro

OASIS Micro

TEL: 02)355-5963 http://www.magictree.kr

[보건의료전문가용]

LG Fertility

LG #FERTILITY IS GOOD

Follitrope®

IVF-M HP™ | IVF-M HP Multidose™

Ganilever® | IVF-C™

LG Chem

[07795] 서울특별시 강서구 마곡중앙10로 70 LG 사이언스파크 E14 고객상담전화 080-023-5757 | www.lgchem.com

IF_BR_A003_001_AUG20

문의사항 / 교육 및 데모 신청

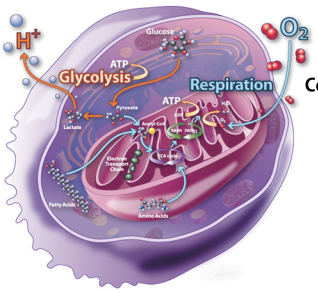
Rhino Bio, Inc.
라이노바이오(주)

대전광역시 유성구 테크노4로 17, C동 614호
Tel. 042-933-1620 | Fax. 042-933-1621
www.rhinobio.co.kr | info@rhinobio.co.kr

Cellular Metabolism Analyzer

Agilent Technologies
Seahorse Bioscience

Extracellular Acidification Rate (ECAR) or Proton Efflux Rate (PER) = Glycolysis



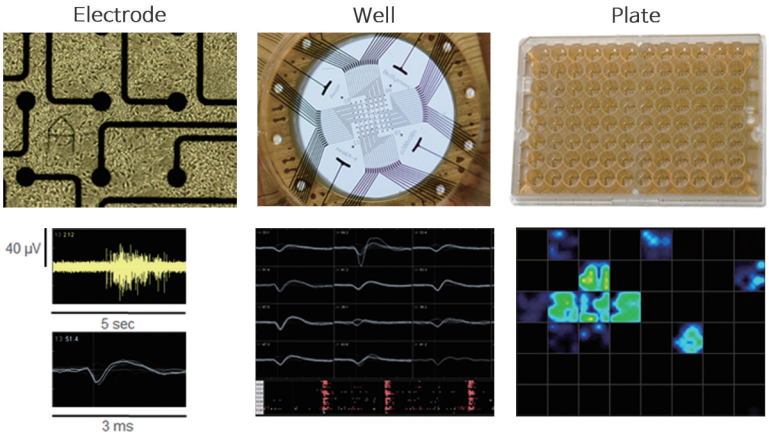
Oxygen Consumption Rate (OCR) = Mitochondrial Respiration

Measurements

- Mitochondrial Function
- Glycolytic Rate
- ATP Production Rate
- Metabolic Phenotype
- Dependency of Cellular Energy Production

Microelectrode Array (MEA)

AXION BIOSYSTEMS



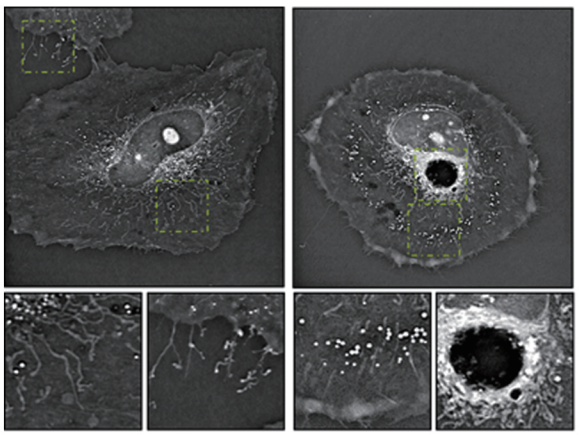
Neuron 및 Cardiomyocyte에서의 Functional Assay!

Maestro EDGE / PRO

실시간 세포 단층촬영 현미경

NANOLIVE
Looking inside life

- Marker-free
- ∞ Live Cell
- Compatible
- 3D in sec
- RI analysis



KJPP The Korean Journal of Physiology & Pharmacology

www.kjpp.net
pISSN 1226-4512, eISSN 2093-3827

Aims and Scope

The Korean Journal of Physiology & Pharmacology (Korean J. Physiol. Pharmacol., KJPP) is the official journal of both the Korean Physiological Society (KPS) and the Korean Society of Pharmacology (KSP). The journal launched in 1997 and is published bi-monthly in English. KJPP publishes original, peer-reviewed, scientific research-based articles that report successful advances in physiology and pharmacology. KJPP welcomes the submission of all original research articles in the field of physiology and pharmacology, especially the new and innovative findings. The scope of researches includes the action mechanism, pharmacological effect, utilization, and interaction of chemicals with biological system as well as the development of new drug targets. Theoretical articles that use computational models for further understanding of the physiological or pharmacological processes are also welcomed. Investigative translational research articles on human disease with an emphasis on physiology or pharmacology are also invited. KJPP does not publish work on the actions of crude biological extracts of either unknown chemical composition (e.g. unpurified and unvalidated) or unknown concentration. Reviews are normally commissioned, but consideration will be given to unsolicited contributions. All papers accepted for publication in KJPP will appear simultaneously in the printed Journal and online.

This Journal is Indexed/Tracked/Covered by

- Science Citation Index Expanded (SCIE), SCOPUS, PubMed, PubMed Central (PMC), EMBASE, KoreaMed, Synapse, KoMCI, BIOSIS Previews, Chemical Abstracts Service (CAS), Crossref, Google Scholar.

Publishers

Eun Joo Baik, President of The Korean Physiological Society (*Ajou University, Korea*)
Hoi Young Lee, President of The Korean Society of Pharmacology (*Konyang University, Korea*)

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: master@koreaphysiology.org

- Pharmacology

280, Gwangpyeong-ro, #1813 Rosedale Officetel, Gangnam-gu, Seoul 06367, Korea
Tel: 82-2-326-0370
E-mail: head@kosphar.org

Subscription

Annual Institutional Subscription Rate: U.S. $80.00. Personal Subscription Rate: U.S. $50.00. 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 24, No. 6, 2020.
Printed by MEDrang Inc. (Tel. 82-2-325-2093, Fax. 82-2-325-2095, E-mail. info@medrang.co.kr)
Subscribing organizations are encouraged to copy and distribute the contents for non-commercial purposes.
This journal was supported by the Korean Federation of Science and Technology Societies (KOFST) Grant funded by the Korean Government.

KJPP The Korean Journal of Physiology & Pharmacology

Editorial Board

2020 대한생리학회 임원명단

고 문 강두희 강복순 김광진 김기순 김기환 김명석 김용근 김 전 김종규
김종환 김중수 나흥식 남숙현 남택상 민병일 박병림 박양생 박재식
박춘식 박형진 배선호 문창현 서덕준 서창국 신홍기 양일석 엄대용
엄융의 연동수 윤평진 이상호 이석강 이승일 이원정 이윤열 이종은
이종흔 이진옥 이중우 조경우 조양혁 홍승길

자문위원 김선희 김종연 박경표 방효원 백은주 서인석 안덕선 홍성근

회 장 백은주

차기회장 김종연

이 사 장 안덕선

총무이사 정승수

기금위원장 서인석

정보이사 김병주

교육이사 염재범

기획이사 조영욱

교육위원 민선식 박규상 방효원 이민구
임인자 임채헌 정한성 한재희

기획위원 김형규 이무열 이은희
임승순 전주홍

국제이사 임채헌

학술이사 박규상

간행이사 강동묵

학술위원 강동묵 곽효범 권상모 김상정
김성준 남주현 배영민 손종우

부편집장 김상정 김성준 안동국 이지희
한 진

오석배 염재범 우선희 우현구
이용석 이은희 임승순 임채헌
진영호 차승규 황선욱

이 사 강다원 강동묵 강봉균 강 엽 공인덕 곽지연 구용숙 권성춘 권혁일
김경년 김나리 김동욱 김명준 김민선 김병주 김보경 김상정 김선희
김성주 김성준 김세훈 김양인 김영미 김용운 김원재 김의용 김재호
김정훈 김종연 김진혁 김창주 김형찬 나창수 류판동 박경표 박규상
박명규 박사훈 박소라 박우현 박원선 박종성 박중진 박지호 박진봉
방효원 배영민 배재훈 배혜란 백은주 서상원 서석효 서인석 송대규
신동민 신형철 심은보 안덕선 안도환 안동국 안승철 양훈모 염재범
오석배 오우택 우선희 우재석 윤신희 윤영욱 이 광 이덕주 이무열
이문영 이배환 이상진 이석호 이성중 이수환 이영만 이영호 이은희
이장헌 이정범 이지희 이호섭 임인자 임중우 임채헌 장성호 장연진
장은화 전병화 전양숙 전제열 정성우 정승수 정승준 정진섭 정창섭
정한성 조영욱 조하나 진영호 천상우 최세영 최윤희 최장규 한 진
한상준 한승호 한인옥 한재희 한호재 한희철 호원경 홍성근

감 사 방효원 이규필

Acknowledgement

Supported by

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

Sponsored by

기억네트워크 연구센터 (서울의대)
말초신경병증연구센터 (동아의대)
미토콘드리아 스트레스 자기방어 연구센터 (연세원주의대)
비만매개질환연구센터 (계명의대)
염증-암 미세환경 연구센터 (이화의대)
치아-치주 복합체 연구센터 (서울치대)

Sponsorship Booths

(주)싸이텍코리아
바이오텍
루사이언스
슈어메디칼
코넥스트

Paper Advertisement

아라시스템
매직트리
LG화학
라이노바이오

Contents

Welcome Message

대한생리학회 회원여러분,

올해 저희는 경험하지 못한 COVID19사태를 맞이하여 일상을 그리워하면서도 연구에 매진하고 계시리라 믿습니다.

봄에 늘 해오던 기초의학학술대회가 취소되면서 회원 간의 만남이 제한되어 함께하고 싶은 마음은 더욱 커지는 것 같습니다. 올해는 처음으로 온라인학회로 개최하게 되었습니다. 오는 11월 4-5일 "Physiology : Connected and Continued"라는 주제로 Environmental physiology, Physiome-based of cardiotoxicity, Membrane protein 등의 새로운 academic session의 추가로 더욱 풍성하고 알찬 학회가 될 것 같습니다. 온라인 교육 환경의 변화로 생리학교육 workshop은 회원들에게 큰 도움이 될 것으로 생각됩니다.

이번 온라인 학술대회는 앞으로 올 미래의 환경에 대비하고 변화와 도전을 슬기롭게 대처해나갈 생리학회 회원들에게는 그 동안의 연구성과를 공유하고 새로운 정보를 교환하는 학회와 더불어 회원님들의 성장과 친목의 새로운 방법을 개척하는 장이 되기를 기대해 봅니다.

그간 학회준비를 위해 헌신해주신 이사님을 비롯한 도움을 주신 모든 분들께 머리숙여 감사드립니다.

대한생리학회 회 장 **백은주**
대한생리학회 이사장 **안덕선**

Schedule (일정표)

▶ 11월 4일 수요일

Time	Contents
14:00–16:00	**Special Session – Environmental Physiology**

▶ 11월 5일 목요일

Time	Contents		
	CH 1	CH 2	CH 3
09:00–09:10	Opening Remarks		
09:10–11:10	**Symposium 1:** TRP Channels	**Symposium 2:** Neuron–glia interaction	**Symposium 3:** Metabolism
11:10–12:10	**Poster Session – A**		
12:10–13:00	Lunch		
13:00–15:00	**Symposium 4:** Pathophysiology of vascular contractility and microcirculation	**Symposium 5:** Physiome–based assessment of cardiac toxicity of new drug	**Symposium 6:** Stem cell physiology
15:00–16:00	**Poster Session – B**		
16:00–18:00	**Symposium 7:** The Physiology of Ageing	**Symposium 8:** Biophysics and Physiology of the Membrane Proteins	**Symposium 9:** Workshop–Simulation–based physiologic education
18:00–18:30	**General Assembly**		
18:30–18:40	Closing Remark		

Contents
2020 Yudang Academic Award

▶ Special Session (11월 4일 수요일)

Contents	
Environmental Physiology (14:00–16:00)	**Organizer / Session chair: *이정범 (순천향의대)***
1. Necessity of environmental physiology research according to environmental change	*이정범 (순천향의대)*
2. Effect of thermotherapy on biological profile–focusing on circulating irisin and lipid profile	*박태환 (순천향의대)*
3. Korean women divers, 'Haenyeo' : aging, thermoregulatory responses and cold adaptation	*이주영 (서울대)*
4. A Social History of Environmental Physiology: The Sea Women and Physiological Adaptation Research during the Cold War	*현재환 (부산대)*

▶ Symposium (11월 5일 목요일)

Contents	
Symposium 1: TRP Channels (09:10–11:10)	**Organizer / Session chair: *서인석 (서울의대)***
1. The structure–functional relationships of Trpc4/5 channels	*서인석 (서울의대)*
2. TRPC6 in fibrosis	*차승규 (원주의대)*
3. Lysosome rupture by obinutuzumab binding is mediated by TRPML2 inhibition	*김주영 (연세의대)*
4. Autophagy regulation by the intracellular Ca^{2+} channel TRPML3	*김현진 (성균관의대)*

Contents	
Symposium 2: Neuron–glia interaction (09:10–11:10)	**Organizer / Session chair: *이용석 (서울의대)***
1. Cerebellar glial activity and pain	*김상정 (서울의대)*
2. Role of astrocytic ion channels in cognitive functions	*이창준 (IBS)*
3. Astrocyte–neuron interaction promotes synaptic reorganization after brain injury	*석경호 (경북의대)*
4. Astrocytic synapse pruning regulates hippocampal synaptic plasticity and memory	*박형주 (KBRI)*

Contents	
Symposium 3: Metabolism (09:10–11:10)	**Organizer / Session chair: *임승순 (계명의대)***
1. Lysosomal adaptation to metabolic stress	*이명식 (연세대)*
2. Role of CRTC2 in lipid homeostasis	*구승회 (고려대)*
3. The Connection of SREBP–1c and H_2S Signaling in Lipid Metabolism	*전태일 (전남대)*
4. FXR transcriptional network: a promising therapeutic strategy for NASH	*황성순 (연세대)*

Contents	
Symposium 4: Pathophysiology of vascular contractility and microcirculation (13:00–15:00)	**Organizer / Session chair: *김성준 (서울의대)***
1. Introduction & Differential NO/sGC pathways between pulmonary and systemic arteries	*김성준 (서울의대)*
2. Involvement of autophagy in vascular dysfunction of ang II –induced hypertensive mice	*최수경 (연세의대)*
3. Na^+ permeability changes and reactivity in hypertensive arterial smooth muscle	*배영민 (건국의대)*
4. Oxidative stress and nonselective cation channels in arteries and their contractile regulation	*박상웅 (을지의대)*
5. Mechanisms of connexin–related lymphedema	*Michael J. Davis (U. Missouri, USA)*

Contents	
Symposium 5: Physiome-based assessment of cardiac toxicity of new drug (13:00-15:00)	**Organizer / Session chair: *임채헌 (울산의대)***
1. Improving the assessment of heart toxicity for all new drugs through translational regulatory science	*Seiryo Sugiura (Univ. Tokyo)*
2. Assessment of cardiac safety with hiPSC derived cardiomyocytes	*조건식 (넥셀)*
3. Three-Dimensional Heart Model-Based Screening of Proarrhythmic Potential by In Silico Simulation of Action Potential and Electrocardiograms	*심은보 (강원대)*
4. Optimum pulse protocol to identify ion channel kinetics	*임채헌 (울산의대)*
5. Cell model application to assess cardiac toxicity	*염재범 (인제의대)*

Contents	
Symposium 6: Stem cell physiology (13:00-15:00)	**Organizer / Session chair: *권상모 (부산의대)***
1. MSC-derived, exsosome-mimetic extracellular nanovesicle-based therapeutics	*김병수 (서울대)*
2. Energy metabolism in stem cells	*도정태 (건국대)*
3. Engineered stem cell therapy for cardiac repair	*박훈준 (가톨릭의대)*
4. Pluripotent stem cell based disease modeling	*차혁진 (서울약대)*

Contents	
Symposium 7: The Physiology of Ageing (16:00-18:00)	**Organizer / Session chair: *차승규 (원주의대)***
1. Nuclear Barrier as the Principle of Biological Aging	*박상철 (전남대)*
2. Molecular genetic dissection of longevity in C. elegans	*이승재 (KAIST)*
3. CD9, cellular senescence, and atherosclerosis	*김재룡 (영남의대)*
4. Lipid signature drives skeletal muscle aging by modulating membrane fluidity	*권기선 (KRIBB)*

Contents	
Symposium 8: Biophysics and Physiology of the Membrane Proteins (16:00-18:00)	**Organizer / Session chair: *임현호 (KBRI)***
1. Watching single alpha-helical membrane proteins fold	*윤태영 (서울대)*
2. Time-resolved Conformational Analysis during GPCR-Gs Coupling	*정가영 (성균관약대)*
3. Structure-function relationship of a CLC-type Cl^-/H^+ antiporter	*임현호 (KBRI)*
4. Analysis of Phototoxin Taste Closely Correlates Nucleophilicity to Type-I Phototoxicity	*강경진 (KBRI)*
5. The role of STIM1 in the innate immune system	*우진석 (UCLA/가톨릭의대)*

Contents	
Symposium 9: Workshop-Simulation-based physiologic education (16:00-18:00)	**Organizer / Session chair: *임채헌 (울산의대), 염재범(인제의대)***
1. Simulation-based electrophysiology education using HHmodel 2.1	*임채헌 (울산의대)*
2. Using an open-source human physiology model (Quantitative Circulatory Physiology, QCP) in the physiology practicum of medical students	*김성준 (서울의대)*
3. Simulation-based lecture of cardiac, skeletal, and smooth muscle contraction	*염재범 (인제의대)*
4. Experiences of simulation-based physiologic education	*연세대학교 원주의대 생리학교실*

▶ 2020 Yudang Academic Award

Contents	
Alzheimer's – a disease beyond the brain: Insights from sphingolipid metabolism	*배재성 (경북대)*

Special Session

Environmental Physiology

S 20 SS-1

Necessity of environmental physiology research according to environmental change
Jeong-Beom Lee, Hye-Jin Lee, Tae-Hwan Park, Eon-Ah Choo
Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

S 20 SS-2

Effect of thermotherapy on biological profile - focusing on circulating irisin and lipid profile
Tae-Hwan Park, Jeong-Beom Lee
[1]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

S 20 SS-3

Korean women divers, 'Haenyeo': aging, thermoregulatory responses and cold adaptation
Joo Young Lee
Department of Textiles, Merchandising and Fashion Design, College of Human Ecology, Seoul National University, Korea

S 20 SS-4

A social history of environmental physiology: The sea women and physiological adaptation research during the Cold War
Jaehwan Hyun
Institute of General Education, Pusan National University, Korea

Symposium

Symposium 1: TRP Channels

S 21 S-1-1

The structure-function relationship of TRPC4/5 channels
Jinsung Kim, Juyeon Ko, Hana Kang, Insuk So
Department of Physiology, College of Medicine, Seoul National University, Seoul, Korea

S 21 S-1-2

TRPC6 channel in fibrosis
Kyu-Hee Hwang[1,2,3], Ji-Hee Kim[1,2,3], Kyu-Sang Park[1,2,3], Seung-Kuy Cha[1,2,3]
[1]Department of Physiology, [2]Department of Global Medical Science, [3]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea

S 22 S-1-3

Lysosome rupture by obinutuzumab binding is mediated by TRPML2 inhibition
Narae Jin, JeongRyeol Kim, Sun Hyung Kwon, Donghyuk Lee, Joo Young Kim
Department of Pharmacology and BK21 Plus, Yonsei University College of Medicine, Seoul, Korea

S 22 S-1-4

Autophagy regulation by the intracellular Ca^{2+} channel TRPML3
So Woon Kim, Mi Kyung Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

Symposium 2: Neuron-glia interaction

S 22 S-2-1

Noradrenergic modulation of cerebellar glial activity during nociception
Seung Ha Kim[1,2], Jaegon Lee[1,2], Jae Yoon Hwang[1,2], Young Gi Min[1,3], Chang-up Kim[5], GeeHoon Chung[4], Sun Kwang Kim[4], Sang Jeong Kim[1,2]
Departments of [1]Physiology, [2]Biomedical Science, [3]Neurology, Seoul National University College of Medicine, [4]Department of Physiology, Kyung Hee University College of Korean Medicine, [5]Department of Physiology, Gachon University College of Korean Medicine, Seoul, Korea

S 22 S-2-2

Role of astrocytic ion channels in cognitive functions
C. Justin Lee
Center for Cognition and Sociality, Institute for Basic Science, Daejeon, Korea

S 23 S-2-3

Astrocyte-neuron interaction promotes synaptic reorganization after brain injury
Jong-Heon Kim[1], Hyun-Gug Jung[2,3], Hyun Soo Shim[4], Seung Jae Hyeon[4], Young-Sun Lee[3], Jin Han[5], Jong Hoon Jung[6], Jaekwang Lee[6], Hoon Ryu[4,7,8], Jae-Yong Park[3], Eun Mi Hwang[2], Kyoungho Suk[1,5]
[1]Brain Science & Engineering Institute, Kyungpook National University, Daegu, [2]Center for Functional Connectomics, Brain Science Institute, Korea Institute of Science and Technology, Seoul, [3]School of Biosystems and Biomedical Sciences, College of Health Science, Korea University, Seoul, [4]Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology, Seoul, [5]Department of Pharmacology and Department of Biomedical Science, School of Medicine, Kyungpook National University, Daegu, [6]Research Group of Functional Food Materials, Korea Food Research Institute, Wanju, Korea, [7]VA Boston Healthcare System, [8]Boston University Alzheimer's Disease Center and Department of Neurology, Boston University School of Medicine, Boston, MA, USA

S 23 S-2-4

Regulation of learning and memory by astrocyte-mediated constant reorganization of synaptic structures
Hyungju Park
Research group for Neurovascular Unit, Korea Brain Research Institute, Korea

Symposium 3: Metabolism

S 23 S-3-1 Lysosomal response to metabolic or cellular stress
Myung-Shik Lee
Severance Biomedical Science Institute & Department of Internal Medicine, Yonsei University College of Medicine, Korea

S 23 S-3-2 Role of CRTC2 in lipid homeostasis
Hye-Sook Han[1], Yong-Min Kwon[1], Kae Won Cho[2], Seung-Hoi Koo[1]
[1]Division of Life Sciences, Korea University, Seoul, [2]Soonchunhyang Institute of Medi-Bioscience (SIMS), Soonchunhyang University, Cheonan, Korea

S 24 S-3-3 The connection of SREBP-1c and H_2S signaling in lipid metabolism
Thuy T.P. Nguyen[1], Seung-Soon Im[2], Tae-Il Jeon[1]
[1]Department of Animal Science, Chonnam National University, Gwangju, [2]Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

S 24 S-3-4 Microbiome plays a pivotal role in the gut-adipose tissue signaling axis
Sungsoon Fang
Severance Biomedical Science Institute, Yonsei University College of Medicine, Korea

Symposium 4: Pathophysiology of vascular contractility and microcirculation

S 24 S-4-1 Differential NO/sGC pathways between pulmonary and systemic arteries
Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Hypoxic/Ischemic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 24 S-4-2 Involvement of autophagy in vascular dysfunction of angII-induced hypertensive mice
Soo-Kyoung Choi
Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

S 25 S-4-3 Altered Na^+ permeability and reactivity of hypertensive arterial smooth muscle
성동준[1], 박상웅[2], 노현주[2], 전민아[3], 강현수[4], 조하나[4], 배영민[3]
[1]Department of Sport and Health Science, Konkuk University, [2]Department of Emergency Medical Services, Eulji University, [3]Department of Physiology, Konkuk University School of Medicine, [4]Department of Physiology, Sungkyunkwan University College of Medicine, Korea

S 25 S-4-4 Oxidative stress and nonselective cation channels in arteries and their contractile regulation
전민아[1], 강현수[2], 조하나[2], 배영민[1], 박상웅[3]
[1]Department of Physiology, Sungkyunkwan University College of Medicine, [2]Department of Physiology, Konkuk University School of Medicine, [3]Department of Emergency Medical Services, Eulji University, Korea

S 25 S-4-5 Mechanisms of connexin-related lymphedema
Michael J. Davis[1], Jorge A. Castorena-Gonzalez[2]
[1]Department of Pharmacology & Physiology, University of Missouri, Columbia, Missouri, [2]Department of Pharmacology, Tulane University, New Orleans, Louisiana, USA

Symposium 5: Physiome based assessment of cardiac toxicity of new drug

S 26 S-5-1 Improving the assessment of heart toxicity for all new drugs through translational regulatory science
Seiryo Sugiura[1], Jun-ichi Okada[1], Takashi Yoshinaga[2], Junko Kurokawa[3], Takumi Washio[1], Tetushi Furukawa[4], Kohei Sawada[5], Toshiaki Hisada[1]
[1]UT-Heart Inc., [2]Eisai Co. ltd., [3]University of Shizuoka, [4]Tokyo Medical and Dental University, [5]University of Tokyo, Japan

S 26 S-5-2 Assessment of cardiac safety with hiPSC derived cardiomyocytes
Gun Sik Cho
넥셀

S 26 S-5-3 Three-dimensional heart model-based screening of proarrhythmic potential by in silico simulation of action potential and electrocardiograms
Eun Bo Shim[1,3], Minki Hwang[3], Chul-hyun Lim[1], Chae Hun Leem[2]
[1]Department of Mechanical and Biomedical Engineering, Kangwon National University, [2]Department of Physiology, University of Ulsan, [3]AI Medic Inc. R&D Department, Korea

S 26 S-5-4 Optimum pulse protocol to identify ion channel kinetics
Young-Seon Lee, Duong Duc Pham, Yunwan Jeon, Kyuri Kim, Chae-Hun Leem
Department of Physiology, University of Ulsan College of Medicine/Asan Medical Center, Seoul, Korea

S 27 S-5-5 Cell model application to assess cardiac toxicity
Jae Boum Youm
Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

Symposium 6: Stem cell physiology

S 27 S-6-1

MSC-derived, exosome-mimetic extracellular nanovesicle-based therapeutics
Ju-Ro Lee[1], Han Young Kim[1], Bong-Woo Park[2], Hun-Jun Park[3], Byung-Soo Kim[1]
[1]School of Chemical and Biological Engineering, Seoul National University, [2]Department of Medical Life Science, College of Medicine, The Catholic University of Korea, [3]Division of Cardiology, Department of Internal Medicine, Seoul St. Mary's Hospital, Korea

S 27 S-6-2

Mitochondrial dynamics and energy metabolism in stem cells
Bong Jong Seo[1], Joonhyuk Choi[1], Jeong Eon Lee[1], Hyeonwoo La[1], Omer Habib[2], Kwonho Hong[1], Jeong Tae Do[1]
[1]Department of Stem Cell and Regenerative Biotechnology, Konkuk Institute of Technology, Konkuk University, Seoul, [2]Department of Chemistry, Hanyang University, Seoul, Korea

S 27 S-6-3

Engineered stem cell therapy for cardiac repair
Hun-Jun Park
Division of Cardiology, Department of Internal medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

S 28 S-6-4

Safe scarless cassette-free selection of genome-edited human pluripotent stem cells using temporary drug resistance
Keun-Tae Kim[1], Ju-Chan Park[2], Hyeon-Ki Jang[3,7], Haeseung Lee[4], Seokwoo Park[5], Ok-Seon Kwon[2], Young-Hyun Go[2], Yan Jin[6], Wankyu Kim[4], Jeongmi Lee[6], Sangsu Bae[3,7], Hyuk-Jin Cha[2]
[1]Department of Life Sciences, Sogang University, Seoul, [2]College of Pharmacy, Seoul National University, Seoul, [3]Research Institute for Convergence of Basic Sciences, Hanyang University, Seoul, [4]Ewha Research Center for Systems Biology, Division of Molecular & Life Sciences, Ewha Womans University, Seoul, [5]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [6]School of Pharmacy, Sungkyunkwan University, Suwon, [7]Department of Chemistry, Hanyang University, Seoul, Korea

Symposium 7: The Physiology of Ageing

S 28 S-7-1

Metazoan principle of aging, based on nuclear barrier hypothesis
Sang Chul Park
Endowed Professor, Chonnam National University, Korea

S 28 S-7-2

Molecular genetic dissection of longevity in *C. elegans*
Seung-Jae V. Lee
Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Korea

S 28 S-7-3

CD9, cellular senescence, and atherosclerosis
Jae-Ryong Kim
Department of Biochemistry and Molecular Biology, Smart-aging Convergence Research Center, College of Medicine, Yeungnam University, Korea

S 29 S-7-4

Lipid signature drives skeletal muscle aging by modulating membrane fluidity
Ki-Sun Kwon[1,2,3]
[1]Aging Research Center, Korea Research Institute of Bioscience and Biotechnology, [2]Department of Functional Genomics, KRIBB School, Korea University of Science and Technology, [3]Aventi Inc., Korea

Symposium 8: Biophysics and Physiology of the Membrane Proteins

S 29 S-8-1

Watching single helical membrane proteins fold
Tae-Young Yoon
School of Biological Sciences, Seoul National University, Seoul, Korea

S 29 S-8-2

Time-resolved conformational analysis during GPCR-G protein coupling
Ka Young Chung
School of Pharmacy, Sungkyunkwan University, Korea

S 29 S-8-3

Structure-function relationship of a CLC-type Cl^-/H^+ antiporter
Hyun-Ho Lim
Neurovascular Research Group, Korea Brain Research Institute (KBRI), Korea

S 30 S-8-4

Analysis of phototoxin taste closely correlates nucleophilicity to type 1 phototoxicity
Eun Jo Du[1,2], KyeongJin Kang[2]
[1]Department of Biological Sciences, Sungkyunkwan University, [2]Neurovascular Unit Research Group, Korea Brain Research Institute, Korea

S 30 S-8-5

The role of STIM1 in the innate immune system

Jin Seok Woo[1], Sonal Srikanth[1], Beibei Wu[1], Yasser M. El-Sherbiny[2,3], Jennifer Leung[1], Koollawat Chupradit[4,5,6], Laura Rice[7], Gil Ju Seo[8], Guillaume Calmettes[9], Chandran Ramakrishna[10], Edouard Cantin[10], Dong Sung An[4,5,6], Ren Sun[11], Ting-Ting Wu[11], Jae U. Jung[8], Sinisa Savic[2,12], Yousang Gwack[1]

[1]Department of Physiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA, [2]National Institute for Health Research—Leeds Biomedical Research Centre and Leeds Institute of Rheumatic and Musculoskeletal Medicine (LIRMM), Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds, UK, [3]Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt, [4]Division of Hematology-Oncology, David Geffen School of Medicine at UCLA, Los Angeles, [5]School of Nursing, University of California at Los Angeles, Los Angeles, [6]UCLA AIDS Institute, Los Angeles, CA, USA, [7]Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds, UK, [8]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, [9]Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA, Los Angeles, [10]Department of Molecular Immunology, City of Hope Beckman Research Institute, Duarte, [11]Department of Molecular and Medical Pharmacology, UCLA, Los Angeles, CA, USA, [12]Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK

Symposium 9: Workshop-Simulation-based physiologic education

S 30 S-9-1

Simulation-based electrophysiology education using HHmodel 2.1

Chae-Hun Leem

Department of Physiology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea

S 31 S-9-2

Using an open-source human physiology model (Quantitative Circulatory Physiology, QCP) in the physiology practicum of medical students

Young Keul Jeon, Sung Joon Kim

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

S 31 S-9-3

Simulation-based lecture of cardiac, skeletal, and smooth muscle contraction

Jae Boum Youm

Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

S-9-4

Experiences of simulation-based physiologic education

Department of Physiology, Yonsei University Wonju College of Medicine

2020 Yudang Academic Award

S 31

Alzheimer's - a disease beyond the brain: Insights from sphingolipid metabolism

Jae-sung Bae, KARI Members

KNU Alzheimer's disease Research Institute, Kyungpook National University, Daegu, Korea; Department of Physiology, School of Medicine, Kyungpook National University, Daegu, Korea

Poster Presentation

P01: Basic Neurophysiology and Pain

S 32 P20-01-01

Layer-specific serotonergic and cholinergic induction of long-term depression in the prefrontal cortex of rats

Dongchul Shin[1], Kwang-Hyun Cho[1], Kayoung Joo[1], Duck-Joo Rhie[1,2]

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Catholic Neuroscience Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea

S 32 P20-01-02

Peripheral pain modulation of Chrysaora pacifica jellyfish venom requires both Ca^{2+} influx and TRPA1 channel activation in rats

Hye-Ji Kim[1], Khulan Amarsanaa[1], Eun-A Ko[1], Young-Joon Kang[2], Sung-Cherl Jung[1]

[1]Department of Physiology, School of Medicine, Jeju National University, Jeju, [2]Department of Emergency Medicine, School of Medicine, Jeju National University, Jeju, Korea

S 32 P20-01-03

The antinociceptive efficacy of low frequency stimulation in chemotherapy-induced peripheral neuropathy mice model

Dong-Wook Kang, Jae-Gyun Choi, Jaehyuk Kim, Cuk-Seong Kim, Sang Do Lee, Jin Bong Park, Byeong Hwa Jeon, Hyun-Woo Kim

Department of Physiology and Medical Science, School of Medicine, Chungnam National University, Daejeon, Korea

S 32 P20-01-04

Chronic angiotensin converting enzyme (ACE) inhibition causes mechanical allodynia in mice that is mediated by substance P

Jae-Gyun Choi, Dong-Wook Kang, Jaehyuk Kim, Jin Bong Park, Hyun-Woo Kim

Department of Physiology and Medical Science, College of Medicine and Brain Research Institute Chungnam National University, Korea

S 33 P20-01-05

Nav1.7 in the trigeminal ganglion plays an important role in the induction of pulpitis pain

Myeounghoon Cha[1], Minjee Kwon[2], Il Young Jung[3], Bae Hwan Lee[1]

[1]Department of Physiology, Yonsei University College of Medicine, [2]Department of Nursing, Kyungil University, [3]Department of Conservative Dentistry and Oral Science Research Center, Yonsei University College of Dentistry, Korea

S 33 P20-01-06
Altered behavioral coping strategy in rats with chronic neuropathic pain
Geehoon Chung[1], Daxian Li[2], Seunghui Woo[2], Nari Kim[2], Sun Kwang Kim[1,2]
[1]Department of Physiology, Kyung Hee University, College of Korean Medicine, [2]Department of Science in Korean Medicine, Kyung Hee University, College of Korean Medicine, Korea

S 33 P20-01-07
Activity-dependent regulation of global Ca^{2+} level and tonic firing rate by TRPC3 channels in SNc dopamine neurons
Ki Bum Um, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, Korea

S 34 P20-01-08
Upregulation of TRESK channel contributes to motor and sensory recovery after spinal cord injury
Gyu-Tae Kim[1], Eun-Jin Kim[1], Eun-Shin Lee[2], Marie Nyiramana[1,3], Adrian Siregar[1,3], Young-Sool Hah[4], Jaehee Han[1], Dawon Kang[1,3]
[1]Department of Physiology, College of Medicine, Gyeongsang National University, [2]Department of Rehabilitation Medicine, College of Medicine, Gyeongsang National University, [3]Convergence Medical Science, Gyeongsang National University, [4]Biomedical Research Institute, Gyeongsang National University Hospital, Korea

S 34 P20-01-09
Alleviation of neuropathic pain via glial regulation in the insular cortex of rats
Kyeongmin Kim, Songyeon Choi, Myeounghoon Cha, Bae Hwan Lee
Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

S 34 P20-01-10
Voltage-gated calcium channels trigger spontaneous glutamate release via nanodomain coupling
Byoung Ju Lee[1,2], Che Ho Yang[1,2], Seung Yeon Lee[1,2], Suk-Ho Lee[1,2], Yujin Kim[2], Won-Kyung Ho[1,2]
[1]Department of Biomedical Sciences, Seoul National University College of Medicine, [2]Department of Physiology, Seoul National University College of Medicine, Korea

S 35 P20-01-11
Antiallodynic effects of KDS2010 on chemotherapy-induced peripheral neuropathy (CIPN) model mice
Su Eun Park[1,2,3], Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Hyun Jin Shin[1,2,3], Ki Duk Park[4], Jin Bong Park[1,2,3]
[1]Department of Medical Sciences, Chungnam National University, Daejeon, [2]Department of BK21Plus, CNU Integrative Biomedical Education Initiative, Chungnam National University, Daejeon, [3]Department of Physiology, School of Medicine and Brain Research Institute, Korea Institute of Science and Technology (KIST), [4]Convergence Research Center for Diagnosis, Treatment and Care System of Dementia, Korea

S 35 P20-01-12
Decoding of spontaneous ongoing pain from primary somatosensory neuronal calcium actiivities using two-photon microscopy
Heera Yoon[1,2], Myeong Seong Bak[2], Seung Ha Kim[3], Ji Hwan Lee[2], Geehoon Chung[1,2], Haney Park[2], Sang Jeong Kim[3], Sun Kwang Kim[1,2]
[1]Department of Physiology, College of Korean Medicine, Kyung Hee University, [2]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, [3]Department of Physiology, Seoul National University College of Medicine, Korea

S 35 P20-01-13
Effects of laser and electro acupuncture treatment with GB30 · GB34 on change in arthritis rat
Yu-Mi Lee, Changsu Na, Mirae Kim, Donghee Choi, Daehwan Youn
Department of Korean Medicine, Dongshin University, Korea

S 35 P20-01-14
JAK3-mediated lamellipodia formation promotes the tangential migration of interneurons in embryonic brain development
A Young Kim, Eun Joo Baik
Department of Physiology, Ajou University School of Medicine, Suwon, Korea

S 36 P20-01-15
Analgesic effects of C. Cortex and its phytochemical against oxaliplatin induced neuropathic pain: suppressing activated glia and released pro-inflammatory cytokines in spinal cord
Ji Hwan Lee[1], Haney Park[1], Nari Kim[1], Sun Kwang Kim[1,2]
[1]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul, [2]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, Korea

S 36 P20-01-16
GluN2B antagonist attenuates mechanical hypersensitivity in the development phase of neuropathic pain after peripheral nerve injury
Youngkyung Kim, Young Ju Ahn, Young Wook Yoon
Department of Physiology, Korea University College of Medicine, Korea

S 36 P20-01-17
Age-dependent expression of satellite glial cell-specific markers in rat sympathetic ganglia
Huu Son Nguyen[1], Seong Jun Kang[1], So Hyun Kim[2], Seong-Woo Jeong[1]
[1]Department of Physiology, Yonsei University Wonju College of Medicine, [2]Department of Physiology, Yonsei University College of Medicine, Korea

S 37 P20-01-18
Characterizing neural selectivity in multidimensional sensory feature space
Sa-Yoon Park[1], Yoo Rim Kim[2], Sang Jeong Kim[2], Chang-Eop Kim[1]
[1]Department of Physiology, College of Korean Medicine, Gachon University, [2]Department of Physiology, College of Medicine, Seoul National University, Korea

S 37 P20-01-19
The role of mitochondria calcium uniporter in C. elegans odor learning
Hee Kyung Lee, Saebom Kwon, Jessica Antonio, Kyoung-hye Yoon
Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

P02: Neuronal Pathophysiology

S 37 P20-02-01

TNF-α mediated progressive neuroinflammation associated with UPRmt in kaolin induced hydrocephalus mouse model

Jiebo Zhu[1,2,3], Min Joung Lee[1,2,3], Hee Jin Chang[1,4], Yunseon Jang[1,2,3], Xianshu Ju[1,3], Jianchen Cui[1,3], Yu Lim Lee[1,3], Eunji Namgung[1,2,3], Dahyun Go[1,2,3], Changjun Seo[1,2,3], Hyoeun Kang[1,2,3], Hyeongseok Kim[1,2,3], Woosuk Chung[1,5,6], Eungseok Oh[1,4], Jun Young Heo[1,2,3]

[1]Department of Medical Science, [2]Department of Biochemistry, [3]Infection Control Convergence Research Center, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University School of Medicine, [5]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, [6]Department of Neurology, Chungnam National University Hospital, Korea

S 37 P20-02-02

Peptide hormone A is a mediator of leptin signaling in hypothalamus to increase POMC expression and α-MSH content

Yunseon Jang[1,2], Xianshu Ju[1,3,4], Min Joung Lee[1,2], Jianchen Cui[1,3,4], Jiebo Zhu[1,2], Eunji Namgung[1,2], Changjun Seo[1,2], Hyoeun Kang[1,2], Da Hyun Go[1,2], Yu Lim Lee[1,3,4], Min Jeong Ryu[1,5], Woosuk Chung[1,3,4], Hyeongseok Kim[1,2], Gi Ryang Kweon[1,2,5], Jun Young Heo[1,2,3]

[1]Department of Biochemistry, Chungnam National University School of Medicine, Daejeon, [2]Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon, [3]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, [5]Research Institute for Medical Science, Chungnam National University School of Medicine, Daejeon, Korea

S 38 P20-02-03

Aggravation of levodopa-induced dyskinesia by injection of mutant α-synuclein in MPTP induced mice

Eunji Namgung[1,2,3], Yunseon Jang[1,2,3], Min Joung Lee[1,2,3], Xianshu Ju[1,2,3], Jianchen Cui[1,2,3], Yu Lim Lee[1,2,3], Jeibo Zhu[1,2,3], Dahyun Go[1,2,3], Chang Jun Seo[1,2,3], Woosuk Chung[4], Hyung seock Kim[1,2,3], Jun Young Heo[1,2,3]

[1]Department of Biochemistry, Chungnam National University School of Medicine, Daejeon, [2]Department of Medical Science, Chungnam National University School of Medicine, Daejeon, [3]Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, Korea

S 38 P20-02-04

Repetitive injection of corticosterone to mother rats during pregnancy induces memory impairment via dysregulating BDNF signaling and NMDA-mediated responses in postnatal rats

Hye-Ji Kim, Sang-Chan Jeon, Eun-A Ko, Sung-Cherl Jung

Department of Physiology, School of Medicine, Jeju Natl'. University, Jeju, Korea

S 38 P20-02-05

Different action of antioxidant between old and young hippocampus following oxidative injury

KyungHee Lee[1], UnJeng Kim[2], Bae Hwan Lee[2,3]

[1]Division of Health Science, Department of Dental Hygiene, Dongseo University, [2]Department of Physiology, College of Medicine, Yonsei University, [3]Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, Korea

S 39 P20-02-06

Long-Lasting and additive analgesic effects of combined treatment of bee venom acupuncture and venlafaxine on paclitaxel-induced allodynia in mice

Daxian Li[1], Geehoon Chung[2], Seunghui Woo[1], Nari Kim[1], Sun Kwang Kim[1,2]

[1]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul, [2]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, Korea

S 39 P20-02-07

The impacts of early social experience on social recognition and its related neural circuits

Gaeun Park[1,2], Changhyeon Ryu[1,2], Soobin Kim[1,2], Yong-Seok Lee[1,2,3], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [3]Neuroscience Research Institute, Seoul National University College of Medicine, Seoul, Korea

P03: Electrophysiology and Ca^{2+} Signaling

S 39 P20-03-01

Higher expression of KCNK10 (TREK-2) K^+ channels and their functional upregulation by lipopolysaccharide treatment in mouse peritoneal B1a cells

Si Won Choi[1], Joohan Woo[2], Kyung Sun Park[3], Juyeon Ko[1], Young Keul Jeon[1], Seong Woo Choi[1,4], Hae Young Yoo[5], Inseong Kho[6], Tae Jin Kim[6], Sung Joon Kim[1,4]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology and Ion Channel Disease Research Center, Dongguk University College of Medicine, Seoul, [3]Wide River Institute of Immunology, Seoul National University College of Medicine, Hongcheon, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, [5]Department of Nursing, Chung-Ang University, Seoul, [6]Department of Immunology, Sungkyunkwan University School of Medicine, Suwon, Korea

S 40 P20-03-02

Inter-spike mitochondrial Ca^{2+} release enhances high frequency synaptic transmission

Che Ho Yang[1], Kyu-Hee Lee[1,3], Won-Kyung Ho[1,2], Suk-Ho Lee[1,2]

[1]Department of Physiology, Seoul National University Medical Research Center, Cell Physiology Lab., Seoul National University College of Medicine and Neuroscience Research Institute, Seoul, [2]Department of Brain and Cognitive Science, Seoul National University, [3]Center for Cognition and Sociality, Institute for Basic Science, Daejeon, Korea

S 40 P20-03-03

Effects of PIP2 and KCNQ activators on the activity of novel KCNQ4 variant channels

Il Soon Choi[1], Hyun Been Choi[1], Heon Yung Gee[2], Sang-Yeon Lee[3], Byung Yoon Choi[3], Tong Mook Kang[1]

[1]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, [2]Department of Pharmacology, Brain Korea 21 PLUS Project for Medical Sciences, Yonsei University College of Medicine, Seoul, [3]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

S 40 P20-03-04

Non-selective cation currents mediated by Cx43 hemichannel-P2X4 receptor signaling pathway in rat atrial myocytes under shear stress

Sun-Hee Woo, Min-Jeong Son, Joon-Chul Kim, Anh TV Vu
Pathophysiology Lab., Chugnam National University, College of Pharmacy, Daejeon, Korea

S 41 P20-03-05

Temperature-dependent facilitation of the voltage-dependent activation of calcium homeostasis modulator 1 ion channel

Young Keul Jeon[1], Si Won Choi[1], Jae Won Kwon[1], Seong Woo Choi[1], Sang Jeong Kim[1,3,4], Sung Joon Kim[1,3,4]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology, Dongguk University College of Medicine, Gyeongju, [3]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 41 P20-03-06

Identification of extracellular disulfide bonds essential for proper folding and function of calcium homemostasis modulator channel

Young Keul Jeon[1,2], Jae Won Kwon[1,2], Sung Joon Kim[1,2,3]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [3]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 41 P20-03-07

The inhibitory effect of the tricyclic antidepressant imipramine on voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells

Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 41 P20-03-08

Dual regulatory effects of $PI(4,5)P_2$ on TREK-2 K^+ channel through antagonizing interaction between the alkaline residues (K^{330} and $R^{355-357}$) in the cytosolic C-terminal helix

Sung Eun Kim[1], Myoung-Hwan Kim[2], Joohan Woo[3], Sung Joon Kim[4]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Physiology, Seoul National University College of Medicine, [3]Department of Physiology, Dongguk University College of Medicine, [4]Department of Physiology, Seoul National University College of Medicine, Korea

S 42 P20-03-09

Electrophysiological assessment of molecular interaction between TRPC4 channel and Exo70

Christine Haewon Park, Insuk So
Department of Physiology, Seoul National University College of Medicine, Korea

S 42 P20-03-10

Cell cycle-associated SK4 activity in head and neck squamous cell carcinoma cells: Role in cell proliferation and its potential clinical applicability

Ji Young Kim[1], Young Keul Jeon[1], Joo Hyun Nam[2,3], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Physiology, Dongguk University College of Medicine, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Korea

S 42 P20-03-11

NALCN channel regulates burst intensities of dopaminergic neurons in the substantia nigra pars compacta

Suyun Hahn, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, Korea

S 43 P20-03-12

The role of lysosomal Ca^{2+} channels in direct cell death ***via*** obinutuzumab binding

Jeong Ryeol Kim, Narae Jin, Sun Hyung Kwon, Donghyuk Lee, Joo Young Kim
Department of Pharmacology and BK21 Plus, Yonsei University College of Medicine, Korea

S 43 P20-03-13

Regulation of transient receptor potential canonical 4 activity by phospholipase C-δ1

Juyeon Ko, Jongyun Myeong, Misun Kwak, Insuk So
Department of Physiology, College of Medicine, Seoul National University, Korea

S 43 P20-03-14

The agonistic action of URO-K10 on $K_v7.4$ and 7.5 channels is attenuated by co-expression of KCNE4 ancillary subunit

Jung Lee[1], Christine Haewon Park[1], Insuk So[1], Jinsung Kim[1], Hana Kang[1], Juyeon Ko[1], Suhan Cho[1], JooHan Woo[3], Meree Chae[2], Sungwon Lee[2], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University of College of Medicine, [2]Department of Urology, Samsung Medical Center, [3]Department of Physiology, Dongguk University College of Medicine, Korea

S 44 P20-03-15

Properties of the chimeric TRPM4 channel as its trafficking marker

Eun Mi Hwang[2], Eun-Hye Byeon[1], Seung-Chan Kim[2], Dawon Kang[1], Dong Kun Lee[1], Jaehee Han[1], Seong-Geun Hong[1]
[1]Department of Physiology, Gyeongsang National University College of Medicine, Jinju, [2]Brain Science Institute, KIST, Seoul, Korea

S 44 P20-03-16

Soluble αKlotho downregulates Orai1-mediated store-operated Ca^{2+} entry and tumor cell migration ***via*** PI3K-dependent signaling

Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Bao TN Dang[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2]
Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

S 44 P20-03-17

Roles of metabotropic glutamate receptors in synaptically-induced Ca^{2+}-spikes in cultured rat hippocampal neurons

Ji Seon Yang[1,2], Sujeong Jeon[1,2], Hyun-Jong Jang[1,2], Shin Hee Yoon[1,2]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, [2]Catholic Neuroscience Institute, The Catholic University of Korea, Korea

S 44 P20-03-18
Cerebellar output networks for fear learning and memory
Kyoung-Doo Hwang[1,2], Hyun-Hee Ryu[1], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2], Yong-Seok Lee[1,2]
[1]Department of Physiology, [2]Department of Biomedical Science, Seoul National University College of Medicine, Seoul, Korea

S 45 P20-03-19
Tricyclic antidepressants regulate abnormal colonic motility like diarrhea and constipation through TRPC4/C5 channel depending on the opioid receptor
Byeongseok Jeong[1], Insuk So[2], Chansik Hong[1]
[1]Department of Physiology, Chosun University School of Medicine, Gwangju, [2]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 45 P20-03-20
Distinct shear-induced Ca^{2+} signaling in the left and right atrial myocytes: role of P2 receptor context
Joon-Chul Kim[1,2], Qui Anh Le[1], Kyeong-Hee Kim[1], Anh Thi Van Vu[1], Sun-Hee Woo[1]
[1]Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, [2]Department of New Drug Development, NEXEL Co. Ltd., Seoul, Korea

S 45 P20-03-21
Inhibition of voltage-dependent K^+ channels by tricyclic antidepressant protriptyline in coronary arterial smooth muscle cells
Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 46 P20-03-22
The direct effect of oxybutynin on voltage-gated K^+ channels in coronary arterial smooth muscle cells
Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 46 P20-03-23
The inhibitory effect of iloperidone on voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells
Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 46 P20-03-24
Inhibition of voltage-dependent K^+ channels by ziprasidone in rabbit coronary arterial smooth muscle cells
Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 47 P20-03-25
The anti-cholinergic drug tolterodine blocks voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells
Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 47 P20-03-26
Hydrogen peroxide (H_2O_2) suppresses the gonadotropin-releasing hormone neurons excitability ***via*** ATP-sensitive potassium channels
Santosh Rijal, Soo Joung Park, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Korea

S 47 P20-03-27
Thermal stimulation triggers TRPV3-mediated Ca^{2+} influx in keratinocytes from patients with atopic dermatitis
Sohyun Kim[1], Seong Hoon Seo[2], Sang Eun Lee[2], Seungsoo Chung[1]
[1]Department of Physiology, Brain Korea 21 Plus Project for Medical Science, Yonsei University College of Medicine, Seoul, [2]Department of Dermatology, Gangnam Severance Hospital, Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea

S 48 P20-03-28
Oxidative stress by Ca^{2+} overload is critical for phosphate-induced vascular calcification
Nhung Thi Nguyen[1,2], Tuyet Thi Nguyen[3], Dat Da Ly[1,2], In-Kyu Lee[4], Seung-Kuy Cha[1,2], Kyu-Sang Park[1,2]
[1]Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea, [3]Internal Medicine Residency Program, College of Health Sciences, VinUniversity, Hanoi, Vietnam, [4]Department of Internal Medicine, School of Medicine, Kyungpook National University, Daegu, Korea

S 48 P20-03-29
Effects on tonic GABAA inhibition and memory by pharmacological modulation of STING
Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Hyun Jin Shin[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]
[1]Department of Medical Sciences, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, Chungnam National University, School of Medicine, Korea

S 48 P20-03-30
GluN2D containing NMDA receptors activity in hippocampal GABAergic interneurons modulate development of Status Epilepticus
Ramesh Sharma[1,2,3], Chiranjivi Neupane[1,2,3], Hyun Jin Shin[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]
[1]Department of Medical Sciences, [2]Department of Physiology, [3]Department of BK21Plus CNU Integrative Biomedical Education Initiative, Chungnam National University, School of Medicine, Korea

S 49 P20-03-31
GABA- and glycine-mimetic responses of linalool on the substantia gelatinosa of the trigeminal subnucleus caudalis in mice
Seon Hui Jang, Soo Joung Park, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Korea

S 49 P20-03-32
Pathway-specific cholinergic modulation of synaptic plasticity in rat primary visual cortex ***in vivo***
Kayoung Joo[1], Kwang-Hyun Cho[1], Duck-Joo Rhie[2]
[1]Department of Physiology, College of Medicine, The Catholic University, [2]Department of Physiology, Catholic Neuroscience Institute, College of Medicine, The Catholic University, Korea

S 49 P20-03-33 Enhanced tonic NMDA current in supraoptic neucleus neurons of DOCA-salt hypertension model
Hyun Jin Shin[1,2,3], Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]
[1]Department of Medical Sciences, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, School of Medicine and Brain Research Institute, Chungnam National University, School of Medicine, Korea

S 50 P20-03-34 Direct calcium binding at S2-S3 linker modifies gating of TRPC4, 5 channels
Jin Hyeong Kim, Jinsung Kim, Juyeon Ko, Insuk So
Department of Physiology, Seoul National University College of Medicine, Korea

S 50 P20-03-35 TRPML3-GATE16 interaction regulates both early and late autophagy by the multimerization of TRPML channels
Areum Choi, Suzi Choi, So Woon Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

S 50 P20-03-36 SYT5 is the Ca^{2+} sensor of autophagosome-lysosome fusion
Seokwoo Hong, Suzi Choi, So Woon Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

P04: Muscle Physiology

S 51 P20-04-01 Inhibition of excessive autophagy ameliorates mesenteric artery dysfunction of angiotensin II- induced hypertensive mice
Youngin Kwon, Soo-Kyoung Choi, Chae Eun Haam, Seonhee Byeon, Young-Ho Lee
Department of Physiology, Yonsei University, College of Medicine, Korea

S 51 P20-04-02 DPP-4 class anti-diabetic drug gemigliptin induces vasodilation via the activation of voltage-dependent K^+ channels and SERCA pumps
Hee Seok Jung, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 51 P20-04-03 SGLT2 inhibitor empagliflozin induces vasodilation via activation of PKG and voltage-gated K^+ channels
Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 52 P20-04-04 Physiological function and molecular composition of ATP-sensitive K^+ channel in human gastric smooth muscle
Hyo-Yung Yun[3], Sang Eok Lee[1], Young Chul Kim[2], Dae Hoon Kim[3], Seung Myeung Son[4], Song-Yi Choi[5], Ra Young You[2], Chan Hyung Kim[6], Woong Choi[6], Hun Sik Kim[6], Sang Jin Lee[2]
[1]Department of Surgery, College of Medicine, Konyang University, Daejeon, [2]Department of Physiology, College of Medicine, CBNU, Cheongju, [3]Department of Surgery, College of Medicine, CBNU, Cheongju, [4]Department of Pathology, CBNU, Cheongju, [5]Department of Pathology, School of Medicine, Chungnam National University, Daejeon, [6]Department of Pharmacology, College of Medicine, CBNU, Cheongju, Korea

S 52 P20-04-05 A muscular hypotonia-associated STIM1 mutant at R429 induces abnormalities in intracellular Ca^{2+} movement and extracellular Ca^{2+} entry in skeletal muscle
Jun Hee Choi[1,2], Mei Huang[1,2], Changdo Hyun[1,2], Mi Ri Oh[1,2], Keon Jin Lee[1,2], Chung-Hyun Cho[3], Eun Hui Lee[1,2]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Department of Biomedicine & Health Sciences, Graduate School, The Catholic University of Korea, Seoul, [3]Department of Biomedical Sciences and Pharmacology, College of Medicine, Seoul National University, Seoul, Korea

S 52 P20-04-06 DPP-4 inhibitor sitagliptin induces vasorelaxation via the activation of PKA and voltage-gated K^+ channels in aortic smooth muscle
Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

S 53 P20-04-07 Identification of a potential exercise-induced myokine in C. elegans
Jessica Antonio, Jae Seung Chang, Kyoung-hye Yoon
Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

P05: Organ Physiology

S 53 P20-05-01 The role of smooth muscle cell mineralocorticoid receptor in heart failure
Seung Kyum Kim[1,2], Iris Jaffe[2]
[1]Seoul National University of Science and Technology Sports Science, [2]Tufts Medical Center Molecular Cardiology Research Institute

S 53 P20-05-02 Discordant interventricular differences in the action potentials, Ca^{2+} transients, and myocyte contractions explained by the lower levels of troponin expression and Ca^{2+} buffering capacity in the right ventricle of rats
Young Keul Jeon[1], Jae Won Kwon[1], Ji Hyun Jang[1], Jae Boum Youm[2], Yin Hua Zhang[1,3,4], Sung Joon Kim[1,3,4]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, [3]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 53 P20-05-03 How to say NO to the excitation-contraction coupling; differential expression of nNOS in the right ventricular myocytes of rats
Jae Won Kwon[1,2], Young Keul Jeon[1,2], Sung Joon Kim[1,2,3]
[1]Department of Physiology, [2]Department of Biomedical Sciences, [3]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 54 P20-05-04
Downregulation of soluble guanylate cyclase and protein kinase G in the pulmonary artery leads to the sensitization to thromboxane A2 in the monocrotaline-induced pulmonary hypertensive rats
Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 54 P20-05-05
Diphosphorylation of myosin regulatory light chain delays relaxation in the pulmonary arteries from monocrotaline-induced pulmonary hypertensive rats
Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 54 P20-05-06
Endogenous catalase prevents obesity by attenuating hypertrophy of white adipocytes
Su-Kyung Shin, Hyun-Woo Cho, Seung-Eun Song, Seung-Soon Im, Jae-Hoon Bae, Dae-Kyu Song
Department of Physiology & Obesity-mediated Disease Research Center, Keimyung University School of Medicine, Daegu, Korea

S 55 P20-05-07
Deletion of TRPC6 aggravates lipid accumulation and insulin resistance in mice
Phan Anh Nguyen[1,2], Kyu-Hee Hwang[1,2], Ji-Hee Kim[1,2], Bao TN Dang[1,2], Kwon Jin[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2]
[1]Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea

S 55 P20-05-08
Role of CXCR4 in differentiation of embryonic submandibular gland
Junchul Kim, Sang Woo Lee, Kyungpyo Park
Department of Physiology, School of Dentistry, Seoul National University and Dental Research Institute, Seoul, Korea

S 55 P20-05-09
Atrial dilation and dysfunction are events accompanied with ventricular hypertrophy at early stage of aortic constriction
Nipa Eslenur, Qui Le, Sun-Hee Woo
Chungnam National University College of Pharmacy, Korea

P06: Endocrine and Energy Metabolism

S 56 P20-06-01
Impaired fatty acid-dependent mitochondrial oxygen consumption was modulated by reduced nNOS activity in atrial myocardium from hypertensive rat
Yu Na Wu, Yin Hua Zhang
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 56 P20-06-02
Lactate accelerates fatty acid oxidation as an anti-obesity metabolite
Jin-Ho Koh[1], Sol-Yi Park[1], Soo-Ryun Jung[1], Jong-Yeon Kim[1], Yong-Woon Kim[1], Hoon-Ki Sung[2], So-Young Park[1], Kyung-Oh Doh[1]
[1]Department of Physiology, Yeungnam University College of Medicine, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto

S 56 P20-06-03
Estrogen-regulated miR-10a/b as gender- and diabetes-associated biomarkers in Korean diabetes mellitus patients
Min Seob Kim[1], Hyun Seok Choi[1], Moxin Wu[1], JiYeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[2], Han-Seung Ryu[2], Suck Chei Choi[2], Tae Yang Yu[3], Se Eun Ha[4], Seungil Ro[4], Moon Young Lee[1]
[1]Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Department of Gastroenterology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, [3]Department of Endocrinology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, Korea, [4]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, USA

S 57 P20-06-04
The suppressive effect of carbon monoxide on ANP secretion via Akt pathway
Weijian Li, Suhn Hee Kim
Department of Physiology, Research Institute for Endocrine Sciences Jeonbuk National University Medical School, Jeonju, Korea

S 57 P20-06-05
Ca^{2+} inhibition on proteasomal degradation of mitochondrial proteins in mouse brown adipocytes
Dat Da Ly[1,2], Nuoc Non Tran[3], Hanh Minh Thi Nguyen[1,2], Nhung Thi Nguyen[1,2], Minho Shong[4], Byung-Hoon Lee[3], Kyu-Sang Park[1,2]
[1]Mitohormesis Research Center, [1]Department of Physiology, Yonsei University Wonju College of Medicine, [3]Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, [4]Research Center for Endocrine and Metabolic Diseases, Chungnam National University School of Medicine, Korea

S 58 P20-06-06
Ca^{2+}-activated mitochondrial biogenesis and functions improve stem cell fate in Rg3-treated human mesenchymal stem cells
Taeui Hong[1,2], Dat Da Ly[1,2], Su Jung Park[1,3], Young Woo Eom[4], Moon Young Kim[1,3,4], Soon Koo Baik[1,3,4], Kyu-Sang Park[1,2]
[1]Mitohormesis Research Center, [2]Department of Physiology, [3]Department of Internal Medicine, [4]Regeneration Medicine Research Center, Yonsei University Wonju College of Medicine, Korea

S 58 P20-06-07
The effects of neurophysiological social engagement system DMT on psychological and physical·physiological function of the juvenile delinquent
Eon-Ah Choo[1], Hyun-Woo Nam[2], Jeong-Beom Lee[3]
[1]Department of Physiology, College of Medicine, Soonchunhyang University, [2]Department of Youth Education and Counseling, Soonchunhyang University, [3]Department of Physiology, College of Medicine, Soonchunhyang University, Korea

S 58 P20-06-08
Senotherapeutic agent attenuates obesity and insulin resistance in high-fat diet-fed mice
Han-Byul Jung, Hye-Na Cha, Min-Ah Choi, Soyoung Park, Eok-Cheon Kim, Jae-Ryong Kim, So-Young Park
Department of Physiology, Yeungnam University of Medicine, Korea

S 59 P20-06-09 Effect of melatonin on Dapagliflozin-induced diabetic ketoacidosis in type 2 diabetic mice
Jae-Hyung Park[1], Hae-Min Shim[1], Hochan Cho[2]
[1]Department of Physiology, [2]Division of Endocrinology, Department of Internal Medicine, Keimyung University School of Medicine, Korea

S 59 P20-06-10 Melatonin prevents transforming growth factor-β1-stimulated transdifferentiation of renal interstitial fibroblasts to myofibroblasts by suppressing reactive oxygen species-dependent mechanisms
Jae-Hyung Park[1], Jung-Yeon Kim[2], Jaechan Leem[2]
[1]Department of Physiology, Keimyung University School of Medicine, [2]Department of Immunology, Catholic University of Daegu School of Medicine, Korea

S 59 P20-06-11 Melatonin inhibits transforming growth factor-β1-induced epithelial-mesenchymal transition in AML12 hepatocytes
Jae-Hyung Park[1], Jung-Yeon Kim[2], Jaechan Leem[2]
[1]Department of Physiology, Keimyung University School of Medicine, [2]Department of Immunology, Catholic University of Daegu School of Medicine, Korea

S 60 P20-06-12 The effects of thermotherapy on the C-reactive protein, FGF-21, adiponectin, irisin and orexin in obese subjects
Hye-Jin Lee[1], Mi-Young Lee[2], Jeong-Beom Lee[3]
[1]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, [2]Global Graduate School of Healthcare, Soonchunhyang University, Asan, [3]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

S 60 P20-06-13 Mitochondrial activation by acute exposure of thyroid hormone in brown adipocytes
Minh-Hanh Thi Nguyen[1,2], Dat Da Ly[1,2], Nhung Thi Nguyen[1,2], Ha Thu Nguyen[1,2], Soo-Jin Kim[1,2], Seung-Kuy Cha[1,2], Kyu-Sang Park[1,2]
[1]Mitohormesis Research Center, [2]Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 60 P20-06-14 The transcriptome analysis of the isolated brain microvessles in endothelial-specific mitochondrial OXPHOS defect mouse
Min Joung Lee[1,2,4], Yunseon Jang[1,2,4], Jiebo Zhu[1,2,4], Da Hyun Go[1,2,4], Changjun Seo[1,2,4], Eunji Namgung[1,2,4], Xianshu Ju[1,4], Yu Lim Lee[1,4], Jianchen Cui[1,4], Hyoeun Kang[1,2], Woosuk Chung[1,4,5], Hyeongseok Kim[1,2], Gi Ryang Kweon[1,2], Jun Young Heo[1,2,4]
[1]Department of Medical Science, [2]Department of Biochemistry, [3]Department of Anesthesiology and Pain Medicine, Chungnam National University School of Medicine, [4]Infection Control Convergence Research Center, College of Medicine, Chungnam National University, [5]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Korea

S 60 P20-06-15 Effects of thermotherapy on plasma irisin levels and FGF21 and metabolic of glucose regulating factors in overweight and obesity
RyeoWon Kwon[1], HyunWoo Nam[2], JinSun Park[1], EonAh Choo[1], HyeJin Lee[1], Jeong-Beom Lee[1]
[1]Department of Physiology, College of Medicine, Soonchunhyang University, [2]Department of Youth Education and Counseling, Soonchunhyang University, Korea

P07: Epithelium and Exocrine Physiology

S 61 P20-07-01 Blood pressure and feeding behavior change in tas2r108 knock-out mouse
Ji-Young Heo[1], Ki-Myung Chung[1,2], Young-Kyung Cho[1,2], Kyung-Nyun Kim[1,2]
[1]Department of Physiology & Neuroscience, [2]Research Institute for Oral Science, Gangneung-Wonju National University, Korea

S 61 P20-07-02 Study the relationship of isocytrate dehydrogenase 2 and mitophagy in human umbilical vein endothelial cells
Su-Jeong Choi[1,2], Harsha Nagar[1], Shuyu Piao[1], Seonhee Kim[1,2], Ikjun Lee[1,2], Jeen-Woo Park[3], Byeong Hwa Jeon[1,2], Hee-Jung Song[4], Cuk-Seong Kim[1,2]
[1]Department of Physiology, College of Medicine, Chungnam National University, [2]BK21Plus CNU Integrative Biomedical Education Initiative, College of Medicine, Chungnam National University, [3]Department of Thoracic and Cardiovascular Surgery, School of Life Sciences, College of Natural Science, Kyungbook National University, [4]Department of Neurology, School of Medicine, Chungnam National University Hospital, Korea

S 61 P20-07-03 The establishment of an in vitro three-dimensional human conjunctival tissue model
Ji Woo Im, Chae Young Lee, Hae-Rahn Bae
Department of Physiology, College of Medicine, Dong-A University, Korea

S 61 P20-07-04 Differential expressions of aquaporin subtypes in female reproductive tract of mice
Ji Woo Im, Hae-Rahn Bae
Department of Physiology, College of Medicine, Dong-A University, Korea

S 62 P20-07-05 Significance of hyaluronate during organogenesis of salivary glands and its application in tissue engineering
Sang-woo Lee, Kyungpyo Park
Department of Oral Physiology, School of Dentistry, Seoul National University, Korea

P08: Inflammation and Immune Physiology

S 62 P20-08-01 Apoptosis inhibitor of macrophage (AIM) contributes to IL-10-induced anti-inflammatory response in LPS-induced acute peritonitis through inhibition of inflammasome activation
Kyungwon Yang[1,2], Jihee Lee Kang[1,2]
[1]Department of Physiology, [2]Inflammation-Cancer Microenvironment Research Center, Ewha Womans University, College of Medicine, Korea

S 62 P20-08-02 Reducing effects of anthocyanin-rich red Chinese cabbage extract on vascular inflammation in atherosclerosis-induced mouse model
Eun Ok Lee, Hee Kyoung Joo, Yu Ran Lee, Sung Min Kim, Hao Jin, Yeon Hee Choi, Byeong Hwa Jeon
Department of Physiology, Chungnam National University School of Medicine, Korea

S 63 P20-08-03 SCAP deficiency in macrophage promotes M1 polarization and obesity in adipose tissue
Jae-Ho Lee, Dae-Kyu Song, Jae-Hoon Bae, Seung-Soon Im
Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

S 63 P20-08-04 Protective effect of kaempferol in RINm5F β-cells under exposure to inflammatory cytokines
Seo-Yoon Chang[1], Yong-Jun Ko[1], Dong-Bin Kim[2], Myung-Jun Kim[1]
[1]Departments of Physiology, [2]Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Korea

S 63 P20-08-05 STAT6 induces PPARγ expression and activation to resolve acute sterile inflammation
Ye-Ji Lee, Bo-Min Kim, Jihee Lee
Department of Physiology, Inflammation-Cancer Microenvironment Research Center, College of Medicine, Ewha Womans University, Seoul, Korea

S 64 P20-08-06 The THIK-1 C-terminus is responsible for LPS-induced reduction of THIK-1 expression levels in macrophages and sensory neurons
Marie Merci Nyiramana[1,2], Eun Jin Kim[1], Adrian S. Siregar[1,2], Dong Kun Lee[1,2], Seong-Geun Hong[1,2], Jaehee Han[1], Dawon Kang[1,2]
[1]Department of Physiology, College of Medicine and Institute of Health Sciences, Gyeongsang National University, Jinju, [2]Department of Convergence Medical Science, Gyeongsang National University, Jinju, Korea

S 64 P20-08-07 Secretory Ref-1 inhibits the inflammatory responses in lipopolysaccharide-induced septic mice
Hee Kyoung Joo, Yu Ran Lee, Eun-Ok Lee, Sungmin Kim, Hao Jin, Yeon Hee Choi, Byeong Hwa Jeon
Department of Physiology, School of Medicine, Chungnam National University, Korea

S 64 P20-08-08 Chios Mastic Gum suppresses the LPS-induced upregulation of inflammatory cytokine via regulating MAPK pathway in the HDPCs
Ye-Won Seol, Sam Young Park, Kyung Joo Seong, Sun-Woong Bae, Song-Yeon Park, So-Ra Kim, Won-Jae Kim, Ji-Yeon Jung
Department of Physiology, School of Dentistry, Dental Science Research Institute, Hard-tissue Biointerface Research Center, Chonnam National University, Korea

S 64 P20-08-09 The role of APE1/Ref-1 in atherosclerotic mice model
Sungmin Kim[1,2,3], Hao Jin[1,2,3], Hee Kyoung Joo[2], Yu Ran Lee[2], Eun Ok Lee[2], Yeon Hee Choi[1,2,3], Byeong Hwa Jeon[1,2,3]
[1]Department of Physiology, [2]Research Institute for Medical Sciences, [3]Department of BK21Plus CNU Integrative Biomedical Education Initiative, College of Medicine, Chungnam National University, Daejeon, Korea

S 65 P20-08-10 APE1/Ref-1 associated with chronic inflammation and fibrosis in the renal tubular and interstitial tissues in mouse CKD model
Soo Yeon An[1,2], Sungmin Kim[1,3], Hee Kyong Joo[1,3], Hao Jin[1,3], Yu Ran Lee[1,3], Eon Ok Lee[1,3], Yeon Hee Choi[1,3], Byeong Hwa Jeon[1,3]
[1]Department of Physiology, College of Medicine, Chungnam National University, [2]Department of Internal Medicine, Chungnam National University Hospital, [3]Research Institute for Medical Sciences, College of Medicine, Chungnam National University, Korea

P09: Cellular Physiology and Cancer

S 65 P20-09-01 CR6-interacting factor 1 Deficiency Promotes Premature Senescence via SIRT3 Inhibition in endothelial cells
Seonhee Kim, Shuyu Piao, Ikjun Lee, Harsha Nagar, Su-Jeong Choi, Cuk-Seong Kim
Department of Physiology, Chungnam National University, Korea

S 66 P20-09-02 Enhanced expression of GABRD predicts poor prognosis in patients with colon adenocarcinoma
Moxin Wu[1,4], Keun Young Kim[2], Won Cheol Park[2], Han-Seung Ryu[3], Suck Chei Choi[3], Min Seob Kim[4], Ji Yeon Myung[4], Hyun Seok Choi[4], Eui Joong Kim[4], Moon Young Lee[4]
[1]Department of Medical Laboratory, Affiliated Hospital of Jiujiang University, Jiujiang, China, [2]Department of General Surgery, Wonkwang University, School of Medicine, Wonkwang Digestive Disease Research Institute, Iksan, [3]Department of Gastroenterology, Wonkwang University, School of Medicine, Wonkwang Digestive Disease Research Institute, Iksan, [4]Department of Physiology, School of Medicine, Wonkwang University, Wonkwang Digestive Disease Research Institute & Institute of Wonkwang Medical Science, Iksan, Korea

S 66 P20-09-03 Propyl gallate induces human pulmonary fibroblast cell death through the regulation of Bax and caspase-3
Mina Ryu, Woo Hyun Park
[1]Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Korea

S 66 P20-09-04 Propyl gallate induces cell death in human pulmonary fibroblast through increasing reactive oxygen species levels and depleting glutathione
Mina Ryu, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Korea

S 67 P20-09-05 Auranofin induces cell death via increasing intracellular oxidative stress and GSH depletion in human lung cancer cells
Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

S 67 P20-09-06
Enhanced cell death effects of MAP Kinase inhibitors in propyl gallate-treated lung cancer cells are related to increased ROS levels and GSH depletion
Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

S 67 P20-09-07
Effects of N-acetyl cysteine and buthionine sulfoximine in propyl gallate-treated lung cancer cells: cell death, reactive oxygen species, and glutathione
Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

S 67 P20-09-08
Tetrahydrobiopterin regulated cardiac energy metabolism in diabetic hearts
Hyoung Kyu Kim, Nam Mi Park, Dae Yun Seo, Pham Trong Kha, Sun-Woo Kim, Jae Boum Youm, Nari Kim, Jong Chul Won, Jin Han
Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Korea

S 68 P20-09-09
Macrophages induce migration in A549 cells
Hee Ju Song, Taehee Kim, Sang Do Lee
Department of Physiolosy, Medical Science, Chungnam National University, Korea

S 68 P20-09-10
The effects of autophagy regulators on Auranofin-treated lung cancer cells in relation to cell growth and reactive oxygen species
XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

S 68 P20-09-11
Auranofin inhibits the proliferation of lung cancer cells via necrosis and caspase-dependent apoptosis
XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

S 68 P20-09-12
Propyl gallate reduces the growth of lung cancer cells through caspase-dependent apoptosis and G1 phase arrest of the cell cycle
XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

S 69 P20-09-13
The anti-apoptotic effects of caspase inhibitors in propyl gallate-treated lung cancer cells are somewhat related to changes in reactive oxygen species and glutathione levels
XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

S 69 P20-09-14
Propyl gallate inhibits the proliferation of Calu-6 and A549 lung cancer cells via affecting ROS and GSH levels
XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

S 69 P20-09-15
CRBN overexpression reverses drug resistance of multiple myeloma cells by regulating mitochondrial function
Jubert Marquez, Nam-mi Park, Hyoung Kyu Kim, Jin Han
Department of Physiology, National Research Laboratory for Mitochondrial Signaling, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

S 70 P20-09-16
NecroX-5 mitigates hypoxia/reoxygenation injury by preserving PGC1α expression levels and protecting phosphorylation capacity during mitochondrial oxidation
Vu Thi Thu[1,2], Hyoung Kyu Kim[1], Maria Victoria Faith Garcia[1], Le Thanh Long[1], Bayalagmaa Nyamaa[1], In-Sung Song[1], To Thanh Thuy[2], Nguyen Quang Huy[2], Jubert Marquez[1], Soon Ha Kim[3], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], Jin Han[1]
[1]Department of Physiology, National Research Laboratory for Mitochondrial Signaling, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea, [2]Faculty of Biology, VNU University of Science, Hanoi, Vietnam, [3]Product Strategy and Development, LG Life Sciences Ltd, Seoul, Korea

S 70 P20-09-17
CR6-interacting factor 1 deficiency inhibits activity of endothelial nitric oxide synthase activity by impeding biosynthesis of tetrahydrobiopterin
Ikjun Lee, Seonhee Kim, Harsha Nagar, Su-jeong Choi, Byeonghwa Jeon, Shuyu Piao, Cuk-Seong Kim
Department of Phyisology & Medical Science, Chungnam National University, Korea

S 70 P20-09-18
Study on the regulation of mitochondrial dynamics and vascular regeneration in BM-derived mesenchymal stem cells for the treatment of ischemic disease
Jin su Kim, Seung Taek Ji, Jin Sup Jung, Sang-Mo Kwon
Pusan National University School of Medicine, Korea

S 70 P20-09-19
Inhibitory effect of sirtuin 6 on cancer proliferation and metastasis in hepatocellular carcinoma
Congshan Li, Soomi Kim
Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

S 71 P20-09-20
3,3'-diindolylmethane induces synergistic anticancer effect with 5-fluorouracil in gastric carcinoma cancer
Congshan Li, Soomi Kim
Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

S 71 P20-09-21 Ursolic acid-enhanced antitumor effect of DIM via activation of Hippo signaling pathway in esophageal cancer cells
Ruoyu Meng, Soomi Kim
Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

S 71 P20-09-22 Chemosensitizing and cytotoxic effects of ursolic acid and doxorubicin on colorectal cancer cells
Dan Hu, Soomi Kim
Department of Physiology, Institute for Medical Sciences, Jeonbuk National University Medical School, Jeonju, Korea

S 71 P20-09-23 Knockdown of catalase promotes adipocyte differentiation and expression of the NADPH oxidase NOX4
Hyun-Woo Cho[1], Su-Kyung Shin[1], Seung-En Song[1], Sang Pyo Kim[2], Seung-Soon Im[1], Jae-Hoon Bae[1], Dae-Kyu Song[1]
[1]Department of Physiology & Obesity-mediated Disease Research Center, [2]Department of Pathology, Keimyung University School of Medicine, Daegu, Korea

S 72 P20-09-24 Activation of ERK1/2-mTORC1-NOX4 mediates TGF-β1-induced epithelial-mesenchymal transition and fibrosis in retinal pigment epithelial cells
Soo-Jin Kim, Dat Da Ly, Ha Thu Nguyen, Hanh Minh T. Nguyen, Seung-Kuy Cha, Kyu-Sang Park
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 72 P20-09-25 Acetylated APE1/Ref-1 is secreted through ATP-binding cassette transporter A1
Yu Ran Lee[1,2], Hee Kyoung Joo[1,2], Eun-Ok Lee[1,2], Sungmin Kim[1,2], Hao Jin[1,2], Byeong Hwa Jeon[1,2]
[1]Department of Physiology, [2]Research Institute for Medical Sciences, College of Medicine, Chungnam National University, Korea

S 72 P20-09-26 Hematopoietic- and neurologic-expressed 1 inhibited autophagy in colorectal cancer
Ruoyu Meng, Soomi Kim
Department of Physiology, Institute for Medical Sciences, Jeonbuk National University Medical School, Korea

S 73 P20-09-27 Recombinant human BMP-2 induced cell apoptosis in Human colorectal cancer cells
Ruoyu Meng, Soomi Kim
Department of Physiology, Jeonbuk National University Medical School, Korea

S 73 P20-09-28 Hematopoietic- and eurologic-expressed sequence 1 reduced Autophagy via Akt/mTOR sinaling pathway in Hepatocellular Carcinoma cell
Ruoyu Meng, Soomi Kim
Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

S 73 P20-09-29 Ursolic acid plus paclitaxel inhibited cell proliferation via Akt signaling pathway in esophageal cancer cells
Ruoyu Meng, Soomi Kim
Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

S 73 P20-09-30 Two-pore domain K^+ channels involve autophagy in bladder cancer cell lines
Yangmi Kim
Department of Physiology, College of Medicine, Chungbuk National University, Cheongju, Korea

P10: Exercise and Integrative Physiology

S 74 P20-10-01 Association between serum irisin concentration and bone stiffness in Korean adults
Jae Seung Chang[1], Jong-Whan Choi[2], In Deok Kong[1]
[1]Department of Physiology, [2]Department of Biochemistry, Yonsei University Wonju College of Medicine, Korea

S 74 P20-10-02 Resistance exercise improves cardiac contractility by preserving mitochondrial function, which ameliorates diabetic cardiomyopathy
Tae Hee Ko, Seung Hun Jeong, Hyoung Kyu Kim, Min Sung Kim, Jubert C. Marquez, SungRyul Lee, Jae Boum Youm, Dae Yun Seo, Byoung Doo Rhee, Kyung Soo Ko, Nari Kim, Jin Han
Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

P11: Physiomes and Systems Biology

S 74 P20-11-01 Co-occurrence of acute food restriction and microinjection of D1 dopamine receptor agonist in the nucleus accumbens core produces sensitized-locomotor activity in amphetamine pre-exposed rat
Seohyun Lee, Hyung Shin Yoon, Jeong-Hoon Kim
Department of Physiology, Yonsei University College of Medicine, Korea

S 75 P20-11-02 Deep learning based parameter estimation for ion channel kinetics
Yun Wan Jeon[1], Young Seon Lee[1], Kyu Ri Kim[1], Jae Boum Youm[2], Chae Hun Leem[1]
[1]Department of Physiology, University of Ulsan College of Medicine, Seoul, [2]Department of Physiology, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, Korea

P12: Others: Drugs, Phytochemicals, Miscellaneous

S 75 P20-12-01

Hidden translational initiation of 453delC-KCNH2 mutant in LQT2 patient generates hERG K^+ channels with reduced activity

Na kyeong Park, Seong Woo Choi, Sung Joon Kim
Department of Physiology, Department of Biomedical Sciences, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Korea

S 75 P20-12-02

The changes of colonic contractility in chronic sertraline treated mice

Min Seob Kim[1], Hyun Seok Choi[1], Moxin Wu[1], JiYeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[2], Seung Ho Jang[3], Moon Young Lee[1]
[1]Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Department of Gastroenterology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, [3]Department of Psychiatry, Wonkwang University School of Medicine, Iksan, Korea

S 76 P20-12-03

Effect of oral famotidine with mosapride compared with famotidine alone on the intragastric pH in the rat stress model

Hyeon Seok Choi[1], Min Seob Kim[1], Jiyeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[1,2], Moon Young Lee[1]
Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Good Breath Clinic, Gunpo, Korea

S 76 P20-12-04

Human physio-types: from body shape to inner functions

Duong Duc Pham, Chae Hun Leem
Department of Physiology, University of Ulsan College of Medicine, Korea

S 76 P20-12-05

Lipid emulsion provides neuroprotection in an animal model of stroke.

Motomasa Tanioka[1,2], Wyun Kon Park[3], Joohyun Park[2,4], Jong Eun Lee[2,4], Bae Hwan Lee[1,2]
[1]Department of Physiology, [2]Brain Korea 21 PLUS Project for Medical Science, [3]Department of Anesthesiology and Pain Medicine, Anesthesia and Pain Research Institute, [4]Department of Anatomy, Yonsei University College of Medicine, Seoul, Korea

S 77 P20-12-06

Dipeptide YA is responsible for the positive effect of oyster hydrolysates on alcohol metabolism in single ethanol binge rodent models

Adrian S. Siregar[1,2], Marie Merci Nyiramana[1,2], Eun-Jin Kim[1], Eui-Jung Shin[1,2], Min Seok Woo[1,2], Jin-Mok Jin-Mok[3], Jung Hwan Kim[4], Dong Kun Lee[1,2], Jong Ryeal Hahm[5], Hyun Joon Kim[2,6], Chang-Woon Kim[7], Nam-Gil Kim[8], Si-Hyang Park[9], Yeung Joon Choi[10], Sang Soo Kang[2,6], Seung-Geun Hong[1], Jaehee Han[1], Dawon Kang[1,2]
[1]Department of Physiology and Institute of Health Sciences, College of Medicine, Gyeongsang National University, [2]Department of Convergence Medical Science, Gyeongsang National University, Jinju, [3]Department of Clinical Laboratory Science, Masan University, Changwon, [4]Department of Premedicine, [5]Department of Internal Medicine, Hospital and Institute of Health Sciences, [6]Department of Anatomy and Institute of Health Sciences, College of Medicine, Gyeongsang National University, Jinju, [7]Department of Obstetrics and Gynecology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, [8]Department of Marine Biology and Aquaculture and Institute of Marine Industry, Gyeongsang National University, Tongyeong, [9]Sunmarin Biotech, [10]Ocean-Pep, Jinju Bioindustry Foundation, Jinju, Korea

S 77 P20-12-07

Effects of Cheonwangbosimdan (CBD) of cognitive function induced by Scopolamine

Sungyoung Cho, Changsu Na
Department of Korea Medicine, Dongshin University, Korea

S 77 P20-12-08

Generation of a gene edited Hemophilia A patient-derived iPSC line, YCMi001-B-1, by targeted insertion of coagulation factor FVIII using CRISPR/Cas9

Sanghyun Park[1,2], Jin Jea Sung[1,2,3], Sang-Hwi Choi[1,2], Jongwan Kim[4], Myung Soo Cho[4], Dong-Wook Kim[1,2,3]
[1]Department of Physiology, [2]Brain Korea 21 PLUS Program for Medical Science, [3]Severance Biomedical Research Institute, Yonsei University College of Medicine, [4]ES Team, S. Biomedics Co., Korea

S 78 P20-12-09

Echinochrome A increases mitochondrial mass and function by modulating mitochondrial biogenesis regulatory genes

Seung Hun Jeong[1,2,3], Hyoung Kyu Kim[1,2,3], In-Sung Song[1,2,3], Jubert Marquez[3], Amy H. Kim[3], Kyung Soo Ko[1,2,3], Byoung Doo Rhee[1,2,3], Natalia P. Mishchenko[4], Sergey A. Fedoreyev[4], Valentin A. Stonik[4], Jin Han[1,2,3]
[1]Cardiovascular and Metabolic Disease Center (CMDC), National Research Laboratory for Mitochondrial Signaling, Inje University, Busan, [2]Department of Physiology, College of Medicine, Inje University, Busan, [3]Department of Health Sciences and Technology, Graduate School of Inje University, Busan, Korea, [4]George B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Science, Vladivostok, Russia

S 78 P20-12-10

Generation of a human induced pluripotent stem cell line, YCMi002-A, from a Factor VII deficiency patient carrying F7 mutations and an isogenic control line

Do-Hun Kim[1,2], Chul-Yong Park[3], Sung-Rae Cho[4], Dong-Wook Kim[1,2,5]
[1]Department of Physiology, [2]Brain Korea 21 PLUS Program for Medical Science, Yonsei University College of Medicine, Seoul, [3]Center for Genome Engineering, Institute for Basic Science, Daejeon, [4]Department and Research Institute of Rehabilitation Medicine, [5]Severance Biomedical Research Institute, Yonsei University College of Medicine, Seoul, Korea

S 78 P20-12-11

Early cardiomyocyte differentiation from mouse embryonic stem cell is regulated by mitochondrial pyruvate dehydrogenase phosphatase 1

Sun Woo Kim[1], Hyoung Kyu Kim[1], Hye Jin Heo[1], Jae Boum Youm[1], Sung Woo Cho[2], In-Sung Song[1], Sun Young Lee[1], Tae Hee Ko[1], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], Jin Han[1]
[1]Department of Physiology, National Research Laboratory for Mitochondrial Signaling, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, [2]Laboratory of Vascular Biology and Stem Cell, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea

SS-1

Necessity of environmental physiology research according to environmental change

Jeong-Beom Lee, Hye-Jin Lee, Tae-Hwan Park, Eon-Ah Choo
Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

Purpose: Sudomotor activity is modified by repetitive physical and/or thermal training. Physical activities induce internal thermal loads and then raise the overall body temperature. Environmental heat or passive heating, such as hot water immersion, also increases body temperature and results in sweating. Repetitive physical activity or thermal load induces physiological heat adaptation in parallel with modified sudomotor activity. Sweating sensitivity depends on the extension of the period of environmental acclimation. Tropical natives sweat less and preserve more body fluid than temperate natives, tolerating heat stress. However, the mechanisms involved in such sweating reduction has not been fully elucidated.
Methods: We examined sudomotor responses of tropical natives (Africans, males) and temperate natives (Koreans, males) subjects during hot water (43℃) leg and half bath, immersion (central sudomotor response). Correlations between mean body temperature, basal metabolic rate (BMR) activated sweat gland density (ASGD), activated sweat gland output (ASGO) sweat onset time, local sweat volume and sweat rate (SR) were also examined. All procedures were done in an automated climate chamber. Local skin temperatures and BMR were measured and mean body temperature was calculated. Sweating activities which include evaporative loss rate, sweat onset time, sweat rate, sweat volume and whole body sweat loss volume were examined.
Results: In the heat load test, Africans showed lower mean body and local skin temperatures than Koreans before and after heating. Before and after heating, BMR declined significantly in Africans, while that of Koreans declined less. Local sweat onset time increased more in Africans than in Koreans. Local evaporative loss rate, local sweat volume, local sweat rate, ASGD, ADGO and whole body sweat loss volume reduced in Africans than in Koreans. There were positive associations of mean body temperature and resting BMR with mean sweat rate.
Conclusions: In conclusion, we observed the larger reduction of sudomotor activity in tropical Africans than in temperate Koreans, which was associated with their lower mean body temperature and lower BMR.

Keywords: Thermal sweating, Heat acclimatization, Mean body temperature, BMR, Environmental acclimation

SS-2

Effect of thermotherapy on biological profile - focusing on circulating irisin and lipid profile

Tae-Hwan Park, Jeong-Beom Lee
[1]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

Purpose: The aim of this study is to identify the effect of repeated thermotherapy on the circulating irisin, free fatty acid and adiponectin level as a preventive and alternative treatment to replace exercise in unfavorable conditions, such as air pollution and pandemic.
Methods: Total 15 times of thermotherapies were performed on female subjects. The heat load was applied via half-body immersion into a hot water bath. After 5 minutes of process, 5 minutes break was taken, resulting in a passive heat load of 30 minutes out of a total of 60 minutes of thermotherapy. Lipid profile and blood collection were performed only before and after the first and last therapy.
Results: Circulating irisin and free fatty acid levels showed a significant increase after thermotherapy. The level of adiponectin, which decreases fat deposition by increasing fat oxidation, was also increased.
Conclusions: Heat loading may activate the sympathetic nervous system and alter body temperature, causing changes in circulating irisin and lipid profile.

Keywords: Thermotherapy, Irisin, Adiponectin, Free fatty acid, Body mass index

SS-3

Korean women divers, 'Haenyeo': aging, thermoregulatory responses and cold adaptation

Joo Young Lee
Department of Textiles, Merchandising and Fashion Design, College of Human Ecology, Seoul National University, Korea

Purpose: We have been studying the thermoregulatory responses of Korean breath-hold women divers, called haenyeo, in terms of aging, thermoregulatory responses, and cold adaptation. During the 1960s to the 1980s, haenyeos received attention from environmental physiologists due to their unique ability to endure cold water while wearing only a thin cotton bathing suit. However, their overall cold-adaptive traits have disappeared since they began to wear wetsuits and research has waned since the 1980s. For social and economic reasons, the number of haenyeos rapidly decreased and the average age of haenyeos is about 75 years old at present. The purpose of the present study was to introduce our publications on haenyeos.
Methods: For the past several years, we revisited and explored older haenyeos in terms of environmental physiology, beginning with questionnaire and field studies and later advancing to thermal tolerance tests in conjunction with cutaneous thermal threshold tests in a climate chamber. Older non-diving females, older-retired haeneyo females, and young non-diving females were compared with older haenyeos in the controlled experiments. We reviewed our eight publications.
Results: Our findings were that older haenyeos and retired-older haenyeos still retain local cold tolerance on the extremities despite their aging. Finger cold tests supported more superior local cold tolerance for older haenyeos than for older non-diving females. However, thermal perception under cold stress reflected aging effects rather than local cold acclimatization. An interesting finding was the possibility of positive cross-adaptation which might be supported by greater heat tolerance and cutaneous warm perception thresholds of older haenyeos who adapted to cold water.
Conclusions: It was known that cold-adaptive traits of haenyeos disappeared, but we have confirmed that cold-adaptive traits are still retained on the face and hands which could be interpreted by a mode switch to local adaptation from the overall adaptation to cold. Further studies on cross-adaptation between chronic cold stress and heat tolerance are needed.

Keywords: Haenyeo, Aging, Cold adaptation, Cotton bathing suits, Cold tolerance, Cross-adaptation

SS-4

A social history of environmental physiology: The sea women and physiological adaptation research during the Cold War

Jaehwan Hyun
Institute of General Education, Pusan National University, Korea

Purpose: Focusing on the history of physiological research on sea women (*Haenyeo* in Korean), this paper examines the emergence of environmental physiology during the Cold War.
Methods: It positions this scientific discipline within the social context in which indigenous peoples became the central focus of human physiologists.
Results: The human condition was drastically changed after World War II; decolonization and rapid modernization in Africa and Asia transformed the material landscape in those regions. Space and marine technologies opened outer space and deep-sea for human exploration. Nuclear weapons made new environments with radioactive contamination. The rise of

such posthuman conditions pushed contemporary human biologists to investigate indigenous people as a "living fossil" of human adaptability to extreme environments. While studying the sea women's adaptability with his Korean colleague Suk Ki Hong (1928-1999), Hermann Rahn (1912-1990), who had switched his career from experimental zoology to respiratory physiology, turned again into the new field of environmental physiology.
Conclusions: This paper will present how their interest in the diving women was shaped along the line of indigenous people research on the one hand, and their research served to construct their research subjects as the "primitive" population, on the other.

Keywords: Environmental physiology, Physiological adaptation, Haenyeo, Cold War

S-1-1

The structure-function relationship of TRPC4/5 channels

Jinsung Kim, Juyeon Ko, Hana Kang, Insuk So
Department of Physiology, College of Medicine, Seoul National University, Seoul, Korea

The study of the structure-function relationship of ion channels has been one of the most challenging goals in contemporary physiology. Revelation of the three-dimensional (3D) structure of ion channels has facilitated our understanding of many of the submolecular mechanisms inside ion channels, such as selective permeability, voltage dependency, agonist binding, and inter-subunit multimerization. Identifying the structure-function relationship of the ion channels is clinically important as well since only such knowledge can imbue potential therapeutics with practical possibilities. In a sense, recent advances in the understanding of the structure-relationship of transient receptor potential canonical (TRPC) channels look promising since human TRPC channels are calcium-permeable, non-selective cation channels expressed in many tissues such as the gastrointestinal (GI) tract, kidney, heart, vasculature, and brain. TRPC channels are known to regulate GI contractility and motility, pulmonary hypertension, right ventricular hypertrophy, podocyte injury, seizure, fear, anxiety-like behavior, and many others. In this presentation, we tried to elaborate recent findings of Cryo-EM (cryogenic-electron microscopy) based structural information of TRPC 4 and 5 channels and domain-specific functions of the channel, such as G-protein mediated activation mechanism, extracellular modification of the channel, homo/hetero-tetramerization, and pharmacological gating mechanisms.

Keywords: TRPC, Structure-function relationship, Transient receptor potential canonical

Acknowledgement: This work was supported by by the National Research Foundation of Korea, which is funded by the Ministry of Science, ICT (Information & Communication Technology), and Future Planning (MSIP) of the Korean government (2018R1A4A1023822).

S-1-2

TRPC6 channel in fibrosis

Kyu-Hee Hwang[1,2,3], Ji-Hee Kim[1,2,3], Kyu-Sang Park[1,2,3], Seung-Kuy Cha[1,2,3]
[1]Department of Physiology, [2]Department of Global Medical Science, [3]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea

Fibrosis is an accelerated tissue repair process that causes serious injury and damage leading to organ failure. Deregulation of Ca^{2+} signaling has been postulated as pathological event in fibrosis. TRPC6 channel has been implicated in pathogenesis of cardiac and renal fibrosis, but its pathological role in hepatic fibrosis is unknown. A primary culprit for hepatic fibrosis is hepatic stellate cell (HSC) activation. Aberrant Ca^{2+} influx mediates either directly or indirectly HSC activation and leads to hepatic fibrosis. Here, we show that TRPC6 is overexpressed in cirrhotic models by bile duct ligation (BDL) and thioacetamide (TAA) administration and in *in vitro* HSC activation model. Of note, TRPC6 expression is strongly correlated with Laennec scoring system for stating fibrosis in human liver biopsy specimens. Mechanistically, injury stress triggers mitochondrial oxidative stress aggravating TRPC6 upregulation leading to activation of HSC. TRPC6 activates MRTF-A/SRF pathway to initiate fibrosis. Deletion of *Trpc6* prevents stress-induced fibrotic changes, oxidative stress, and activation of MRTF-A/SRF pathway. Together, these data demonstrate that TRPC6-mediated Ca^{2+} influx causes hepatic fibrosis via HSC activation. These provide a new perspective on the pathogenesis of fibrosis and offers clues for therapeutic strategies for fibrotic diseases.

Keywords: Calcium signaling, Hepatic fibrosis, MRTF-A, Oxidative stress

Acknowledgement: This study was supported by the Basic Science Research Program through the National Research Foundation of Korea, funded by the Ministry of Education (NRF-2017R1A5A2015369 & 2019R1A2C1084880).

S-1-3

Lysosome rupture by obinutuzumab binding is mediated by TRPML2 inhibition

Narae Jin, JeongRyeol Kim, Sun Hyung Kwon, Donghyuk Lee, Joo Young Kim

Department of Pharmacology and BK21 Plus, Yonsei University College of Medicine, Seoul, Korea

Obinutuzumab is a type II, humanized, anti-CD20 antibody used as a first-line treatment for chronic lymphocytic leukemia and follicular lymphoma. The major cell death mechanism of obinutuzumab is known as ADCC (antibody-dependent cell mediated cytotoxicity) driven by glyco-engineering in its Fc region. Obinutuzumab also cause the cell death through the direct binding to its target, CD20 in surface of B cells, this characteristic also contributes the high ADCC efficacy. Since CD20 is a Ca^{2+} channel, the Ca^{2+} influx through CD20 after obinutuzumab binding might be easily considered the cell death mechanism, but Ca^{2+} influx through CD20 is not involved in the cell death because obinutuzumab causes more cell death in extracellular Ca^{2+} free condition. In this direct cell death process, lysosomal rupture and released cathepsin have been known to be essential, but the mechanism of lysosomal rupture is unknown. TRPML protein family is an endo-lysosomal Ca^{2+} permeable cation channel consisting of TRPML1, TRPML2, and TRPML3, the similarity between them is 75%. TRPML1 is causative gene of lysosomal storage disorder disease, Mucolipidosis IV, and it maintains the endo-lysosome homeostasis. TRPML1 is known to play an important role in endo-lysosomal fusion and fission. Trafficking of endocytic vesicles and movement of lysosome also are controlled by TRPML1 protein. Based on the high similarity with TRPML1 proteins, TRPML2 is expected to performed the similar function in endo-lysosomal trafficking of immune cells, the TRPML2 highly expressed cells. Since B cells mainly have TRPML2 as a Ca^{2+} channel in their endo-lysosome, analysis of changes in TRPML2 function by obinutuzumab binding can provides clues to the molecular mechanism of lysosome rupture process. In this talk, I will present our effort to elucidate the molecular mechanism of the lysosome rupture in B cell death by obinutuzumab binding. In particular, TRPML2 functions in the initial change of lysosomes by obinutuzumab binding will be discussed.

Keywords: TRPML2, Obinutuzumab, B cell, Lysosome rupture, Cell death

Acknowledgement: This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (No. NRF-2019R1A2C4002468).

S-1-4

Autophagy regulation by the intracellular Ca^{2+} channel TRPML3

So Woon Kim, Mi Kyung Kim, Hyun Jin Kim

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

Autophagy involves multiple fusion events, initiating with autophagosome formation and culminating with fusion with endo-lysosomes in a Ca^{2+}-dependent manner. However, the source of Ca^{2+} and the molecular mechanism by which Ca^{2+} is provided for the process is still not clear. The intracellular Ca^{2+} permeable channel TRPML3 localizes in autophagosome membranes and interacts with the mammalian ATG8 homologue GATE16 to regulate autophagy. Therefore, we hypothesized that TRPML3 may be the channel providing the Ca^{2+} necessary for autophagy. To test this hypothesis, we generated a TRPML3-GCaMP6 fusion protein as a specific reporter of the TRPML3 intracellular Ca^{2+} compartment and the compartment identity. Notably, TRPML3-GCaMP6 showed minimal overlap with LC3, but mainly localized in the phagophores, suggesting that TRPML3 supplies Ca^{2+} in the early stage of autophagy. Importantly, TRPML3-GCaMP6 localization in a vesicular compartment increased in response to nutrient starvation, suggesting that TRPML3 activity is regulated by autophagy signaling. Indeed, lipid binding assay revealed that TRPML3 interacts with phosphatidylinositol-3-phosphate (PI3P), an essential signal for autophagosome formation. Confocal imaging and current measurement showed that TRPML3 is directly activated by PI3P, which leads to increased autophagy. Inhibition of TRPML3 suppressed autophagy even in the presence of excess PI3P upon induction of autophagy, while activation of TRPML3 reversed inhibition of autophagy caused by blocking PI3P. Moreover, disruption of the TRPML3-PI3P interaction abolished both TRPML3 activation by PI3P and the increase of autophagy. Taken together, these results suggest that TRPML3 is a key regulator of autophagy as a downstream effector of PI3P. Activation of TRPML3 by PI3P is the critical step providing Ca^{2+} for the fusion process essential for autophagosome biogenesis.

Keywords: Autophagy, Ca^{2+} channel, GCaMP6, PI3P, TRPML3

Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean Government (NRF-2017R1D1A1B03032877 and NRF-2018R1D1A1A02048295).

S-2-1

Noradrenergic modulation of cerebellar glial activity during nociception

Seung Ha Kim[1,2], Jaegon Lee[1,2], Jae Yoon Hwang[1,2], Young Gi Min[1,3], Chang-up Kim[5], GeeHoon Chung[4], Sun Kwang Kim[4], Sang Jeong Kim[1,2]

Departments of [1]Physiology, [2]Biomedical Science, [3]Neurology, Seoul National University College of Medicine, [4]Department of Physiology, Kyung Hee University College of Korean Medicine, [5]Department of Physiology, Gachon University College of Korean Medicine, Seoul, Korea

The cerebellar activation during noxious stimulations and removal of the cerebellum resulting in somatosensory dysfunction have been revealed in clinical studies. The cerebellum is a part of the pain matrix. However, it is still difficult to define the connection between the cerebellum and pain pathways as the exact role of the cerebellum in the process of pain perception is yet to be fully understood. Here, by utilizing two-photon calcium imaging, we showed the global calcium activation of Bergmann glia (BG) through electrical noxious stimuli and c-fiber-mediated capsaicin injection. This calcium response was induced by adrenergic input via locus coeruleus and was blocked by the BG-specific chemogenetic suppression. Moreover, capsaicin-induced paw licking behavior attenuated over time, indicating the relationship between BG calcium activity and pain behavior. The blocker test has confirmed that the alpha 1 adrenergic receptor (a1-AR) mediates the BG calcium response during pain states. Interestingly, the a1-AR was genetically suppressed in a BG-specific manner, blocking its calcium response. Similarly, capsaicin-induced pain behavior was attenuated in the BG-specific a1-AR knockdown mice. Taken together, we suggest that the pain processing in the cerebellum may be mediated by BG activity through glial adrenergic signaling, leading to the conclusion that the cerebellum is actively involved in pain processing.

Keywords: Cerebellum, Bergmann Glia, Adrenergic, Nociception, Locus coeruleus

S-2-2

Role of astrocytic ion channels in cognitive functions

C. Justin Lee

Center for Cognition and Sociality, Institute for Basic Science, Daejeon, Korea

In the brain ion channels are critical for membrane excitability, cell signaling, information processing and perception and cognition. Although many of the ion channels that contribute neuronal functions are studied in detail, most of the ion channels that are expressed in astrocytes are not well studied. In the past 15 years, I have focused on various ion channels that are specifically expressed in astrocytes. So far, we have identified several key ion channels that are expressed in astrocytes. These include the Ca^{2+}-activated anion channel, Best1, stretch-activated Ca^{2+} channel, TRPA1, volume-regu-

lated anion channel, TTYH1, 2, 3, the passive conductance channel, a heterodimer of TREK-1/TWIK-1 and the astrocyte-specific water channel, Aqp-4. I will present the overview of these channels in terms of identification, characterization, and function. Finally, I will highlight their role in cognition.

Keywords: Astrocyte, Best1, TRPA1, VRAC, TTYH, TREK1, TWIK1, Aqp-4

S-2-3

Astrocyte-neuron interaction promotes synaptic reorganization after brain injury

Jong-Heon Kim[1], Hyun-Gug Jung[2,3], Hyun Soo Shim[4], Seung Jae Hyeon[4], Young-Sun Lee[3], Jin Han[5], Jong Hoon Jung[6], Jaekwang Lee[6], Hoon Ryu[4,7,8], Jae-Yong Park[3], Eun Mi Hwang[2], Kyoungho Suk[1,5]

[1]Brain Science & Engineering Institute, Kyungpook National University, Daegu, [2]Center for Functional Connectomics, Brain Science Institute, Korea Institute of Science and Technology, Seoul, [3]School of Biosystems and Biomedical Sciences, College of Health Science, Korea University, Seoul, [4]Center for Neuroscience, Brain Science Institute, Korea Institute of Science and Technology, Seoul, [5]Department of Pharmacology and Department of Biomedical Science, School of Medicine, Kyungpook National University, Daegu, [6]Research Group of Functional Food Materials, Korea Food Research Institute, Wanju, Korea, [7]VA Boston Healthcare System, [8]Boston University Alzheimer's Disease Center and Department of Neurology, Boston University School of Medicine, Boston, MA, USA

Hevin, also known as SPARC-like protein 1 (SPARCL1 or SC1), is a synaptogenic protein secreted by astrocytes and modulates the formation of glutamatergic synapses in the developing brain by interacting with synaptic adhesion proteins, such as neurexin and neuroligin. Here, we identified the neuron-specific vesicular protein calcyon as a novel interaction partner of hevin and demonstrated that this interaction played a pivotal role in synaptic reorganization after an injury in the mature brain. Astrocytic hevin was upregulated post-injury in a photothrombotic stroke model. Hevin was fragmented by MMP3 induced during the acute stage of brain injury, and this process was associated with severe gliosis. At the late stage, the functional hevin level was restored as MMP3 expression decreased. The C-terminus of hevin interacted with the N-terminus of calcyon. By using RNAi and binding competitor peptides in an ischemic brain injury model, we showed that this interaction was crucial in synaptic and functional recoveries in the sensory-motor cortex, based on histological and electrophysiological analyses. Regulated expression of hevin and calcyon and interaction between them were confirmed in a mouse model of traumatic brain injury and patients with chronic traumatic encephalopathy. Our study provides direct evidence for the causal relationship between the hevin-calcyon interaction and synaptic reorganization after brain injury. This neuron-glia interaction can be exploited to modulate synaptic reorganization under various neurological conditions.

Keywords: Calcyon, Hevin, Stroke, Synaptic recovery, Protease

S-2-4

Regulation of learning and memory by astrocyte-mediated constant reorganization of synaptic structures

Hyungju Park

Research group for Neurovascular Unit, Korea Brain Research Institute, Korea

In the adult brain, synapses undergo constant formation and elimination processes, but the exact function of this constant synapse reorganization and how synapse elimination is regulated in the adult brain are largely unknown. Here, we reveal a significant function of astrocytic phagocytosis in maintaining proper hippocampal synaptic connectivity. By utilizing mCherry-eGFP phagocytosis reporters, we found a dominant role of astrocytes in neuronal activity-dependent elimination of excitatory synapses. Knocking-out *Megf10* in the adult astrocytes reduced their ability to eliminate excitatory synapses and induces the accumulation of excessive synapses with abnormal multiple connections between pre- and post-synapses, indicating that astrocytic phagocytosis mechanism is required for synapse reorganization. Finally, we found that *Megf10* knock-out mice exhibit enhanced excitatory synaptic transmission but defective long-term synaptic plasticity with impaired hippocampal memory acquisition. Taken together, our data provide evidence that astrocytes constantly eliminate unnecessary excitatory pre- and post-synaptic connections in the adult hippocampus, and that this astrocytic function is critical for homeostasis of the circuit connectivity and cognitive functions.

S-3-1

Lysosomal response to metabolic or cellular stress

Myung-Shik Lee

Severance Biomedical Science Institute & Department of Internal Medicine, Yonsei University College of Medicine, Korea

The physiological roles of ER and mitochondria in cellular and systemic metabolism are well characterized. Contribution of the dysfunction or stress of ER and mitochondria in the development of metabolic diseases and diabetes is also well established. However, the role of lysosome in the physiology of cellular or systemic metabolism is less well known. Contribution of lysosomal dysfunction or stress in the development of common metabolic diseases or diabetes has also not been studied well except genetic disorders such as lysosomal storage diseases. Now it is clear that lysosome functions as a hub for metabolic signal transduction such as mTOR or AMPK signaling and also as an effector organelle for execution of autophagy. On the functional aspect, autophagy rejuvenates organelles such as mitochondria or ER by degrading dysfunctional or senescent organelles, and replenishes nutrients in nutrient deficiency. Dysregulated autophagy is associated with various diseases including neurodegenerative diseases, cancer and metabolic disorders. Autophagy is tightly controlled by coordinated activity of diverse regulatory circuits including transcription factor EB (TFEB), a master regulator of autophagy- and lysosomal biogenesis-related gene expression. In nutrient deficiency, inhibition of mTORC1 and activation of calcineurin by lysosomal Ca^{2+} release lead to TFEB dephosphorylation and nuclear translocation. Besides nutrient deficiency, organelle stress such as mitochondrial stress may also induce TFEB activation through lysosomal Ca^{2+} release, leading to activation of autophagy. TFEB increases expression of autophagy- and lysosomal biogenesis-related genes by binding to coordinated lysosomal expression and regulation (CLEAR) element of target gene promoters. Here, the role of lysosomal response to metabolic or cellular stress in the adaptation to those stresses will be discussed together with the implication of such response to the development of the diseases.

Keywords: Lysosome, TFEB, Autophagy, Calcium, Calcineurin

S-3-2

Role of CRTC2 in lipid homeostasis

Hye-Sook Han[1], Yong-Min Kwon[1], Kae Won Cho[2], Seung-Hoi Koo[1]

[1]Division of Life Sciences, Korea University, Seoul, [2]Soonchunhyang Institute of Medi-Bioscience (SIMS), Soonchunhyang University, Cheonan, Korea

CREB regulated transcription coactivator 2 (CRTC2) is a master transcriptional regulator of hepatic gluconeogenesis. In conjunction with CREB or its related bZIP factors, CRTC2 promotes increased expression of gluconeogenic genes under fasting conditions as well as diet-induced obesity and insulin resistant state. Surprisingly, recent study also revealed a potential role of this factor in the regulation of lipid metabolism, prompting us to address this issue by utilizing liver-specific CRTC2 knockout mice.
We found that hepatic CRTC2 expression is elevated in mice under high fat diet (HFD)-feeding, with concomitant activation of mammalian target of rapamycin complex (mTORC) 1 pathway, suggesting a potential correlation between the two pathways. Indeed, depletion of CRTC2 in the liver reduced

fatty liver phenotype in diet-induced obesity (DIO) setting, in part via the reduction in mTORC1 pathway. Mechanistically, we found that reduced miR-34a expression in CRTC2-depleted mice activates TSC2, leading to the inactivation of mTORC1 in the liver. Finally, we demonstrated that CRTC2-miR-34a-mTOR axis is also stimulated in nonalcoholic fatty liver disease (NAFLD) patients. These data collectively suggest that activation of CRTC2 in the liver could promote the incidence of NAFLD by activating miR-34a-mTORC1 pathway in mammals..

Keywords: CRTC2, Liver, Lipid metabolism, mTOR, NAFLD

S-3-3

The connection of SREBP-1c and H_2S signaling in lipid metabolism

Thuy T.P. Nguyen[1], Seung-Soon Im[2], Tae-Il Jeon[1]

[1]Department of Animal Science, Chonnam National University, Gwangju, [2]Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

A metabolic imbalance between lipid synthesis and degradation can lead to hepatic lipid accumulation, a characteristic of patients with nonalcoholic fatty liver disease (NAFLD). Sterol regulatory element-binding proteins (SREBPs) are well characterized as key transcription factors in regulation of lipid biosynthesis. Here, we report that SREBP-1c impairs autophagic lipid catabolism by H_2S signaling in hepatic steatosis. SREBP-1c directly upregulates miR-216a, which targets cystathionine gamma-lyase (CSE), a key enzyme responsible for H_2S production, in diet-induced murine NAFLD models. Decreased hepatic and circulating H_2S levels attenuate sulfhydration of Unc-51 like autophagy activating kinase 1 (ULK1), disrupting autophagy activity. By contrast, SREBP-1c deficiency maintains CSE/H_2S-mediated autophagy activity to protect mice from liver steatosis. We further establish that sulfhydration of ULK1 Cys951 stabilizes its binding to Atg13, allowing the full kinase activation of it. Remarkably, mutation of Cys951 suppresses autolysosome formation, thereby inducing hepatic lipid accumulation in mice, suggesting that the loss of ULK1 sulfhydration relates to the pathology of NAFLD. Moreover, the inhibition of CSE attenuates the protective effects of SREBP-1c deficiency in NAFLD mouse model. These results imply that SREBP-1c reciprocally regulates hepatic lipid biosynthesis and catabolic lipid degradation in NAFLD, and ULK1 sulfhydration-mediated autophagy is identified as a novel mechanism of SREBP-1c-regulated lipid catabolism.

Keywords: SREBP-1c, Autophagy, ULK1, Protein sulfhydration, NAFLD

S-3-4

Microbiome plays a pivotal role in the gut-adipose tissue signaling axis

Sungsoon Fang

Severance Biomedical Science Institute, Yonsei University College of Medicine, Korea

Here we report the human gut microbial composition in a population sample of 49 non-obese and 50 obese Korean individuals. We find that only a few bacterial species are sufficient to distinguish between individuals with various metabolic markers. The abundance of these bacteria is markedly reduced with increase of adiposity, BMI, WC, blood TG, and fatty liver while microbial gene richness has not been markedly changed between individuals. Animal studies reveal that oral treatment of those species enhances bile acid metabolism and increases OXPHOS in adipose tissues to protect against diet-induced obesity. Using comprehensive genomic analysis, we have noticed that gene expression profile of effector bacteria is strain-specific that contributes to differential metabolic responses in animal models. Furthermore, we have revealed that carbohydrate metabolic process is effector strain-specific to prevent against diet-induced obesity. Our findings clearly support that strain-specific metabolic processes of microbiota are responsible for host metabolic homeostasis to prevent against diet-induced obesity.

Keywords: Gut microbiome, Obesity, Bile acid signaling

S-4-1

Differential NO/sGC pathways between pulmonary and systemic arteries

Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]

[1]Department of Physiology, [2]Hypoxic/Ischemic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Compared to systemic arteries (e.g. mesenteric arteries (MAs)), pulmonary arteries (PAs) are exposed to lower pressure with the same flow rate, which implies low peripheral resistance. Regarding with the contractile agonists, TXA2 and angiotensin II (Ang II) induce potent contraction in PAs while not in MAs. Previously, we found that the unconventional expression of eNOS in the muscular layer of PAs have physiological roles; treatment with L-NAME (NOS inhibitor) enhanced and prolonged the transient contraction by Ang II in PAs. In our recent studies, the critical role of signaling pathways distal to NO, i.e. soluble guanylate cyclase (sGC) and cGMP-dependent kinase (PKG) could be demonstrated from the dramatic prolongation of the recovery from the high-K+ (80 mM KCl)-induced contraction by sGC inhibitor in PAs while not in MAs. Interestingly, the contractile responses of PAs from monocrotalin-induced pulmonary hypertensive (MCT-PAH) rats showed similar pattern to the normal PAs treated with the sGC inhibitor, which was reversed by the application of membrale permeable 8-Br-cGMP. Using immunoblot assays, we confirmed the translational changes of sGG, PKG and ROCK in the MCT-PAH PAs. Finally, we found that the diphosphorylated state of the regulatory myosin light chain was increased in the PAs of MCT-PAH, which might explain the markedly prolonged relxation by washout of contractile conditions (e.g. high-K^+). The intrinsic roles of NO/sGC pathways attenuating the excessive contractile responses are more crucial in PAs than systemic arteries, and the downregulation of associated molecules could underlie the pathophysiology of pulmonary hypertension.

Keywords: Pulmonary artery, Smooth muscle, NO, Soluble guanylate cyclase, PKG

S-4-2

Involvement of autophagy in vascular dysfunction of angII-induced hypertensive mice

Soo-Kyoung Choi

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

Aims: Autophagy is an intracellular degradation system that disassembles cytoplasmic components through autophagosomes fused with lysosomes. Recently, the involvement of autophagy in cardiovascular diseases, including pulmonary hypertension, atherosclerosis, and myocardial ischemia, has attracted considerable research attention. However, the role of autophagy in hypertension is not well understood. In the present study, we hypothesized that autophagy contributes to the dysfunction of mesenteric arteries in angiotensin II (Ang II)-induced hypertensive mice.
Methods and Results: Ang II induced an increase in beclin1 and LC3 II expression and a decrease in p62 expression in vascular smooth muscle cells. The expression of these proteins was increased by using an autophagy inhibitor chloroquine. Ang II induced an increase in beclin1 and LC3 II expression and a decrease in p62 expression, and these expressions were reversed using an autophagy inhibitor 3-methyladenine. Chloroquine and 3-methyladenine dose-dependently dilated arteries pre-contracted by U46619. To determine the in vivo role of autophagy in hypertension, we treated Ang II-induced hypertensive mice with 3-methyladenine (30 $mg.kg^{-1}day^{-1}$). The blood pressure of Ang II-treated mice was significantly higher than that of vehicle-treated mice. Interestingly, 3-methyladenine reduced the blood pressure of Ang II-treated mice. Endothelium-dependent relaxation was significantly impaired in Ang II-treated mice, which was recovered by treat-

ment with 3-methyladenine. We measured the fluorescence intensity of DAF-FM diacetate, an indicator of nitric oxide, to detect the production of nitric oxide. The fluorescence intensity of DAF-FM diacetate was reduced in the mesenteric arteries of Ang II-induced hypertensive mice, which was restored by treatment with 3-methyladenine. p-eNOS (S1177) expression decreased in the mesenteric arteries of Ang II-treated mice, which was reversed by treatment with 3-methyladenine.
Conclusions: Therefore, autophagy inhibition ameliorates elevated blood pressure in Ang II-induced hypertensive mice, which is associated with improvement in endothelium-dependent relaxation. These results suggest that autophagy inhibition exerts beneficial effects on the dysfunction of mesenteric arteries in hypertension.

S-4-3

Altered Na^+ permeability and reactivity of hypertensive arterial smooth muscle

성동준[1], 박상웅[2], 노현주[2], 전민아[3], 강현수[4], 조하나[4], 배영민[3]

[1]Department of Sport and Health Science, Konkuk University, [2]Department of Emergency Medical Services, Eulji University, [3]Department of Physiology, Konkuk University School of Medicine, [4]Department of Physiology, Sungkyunkwan University College of Medicine, Korea

Altered salt homeostasis is one of major risk factors of hypertension. Although the increased renal Na^+ retention and intravascular volume overload by altered activity of mineralocorticoid (MC) hormone in kidneys contribute to hypertension, a non-renal mechanism, likely related to the vasculature, is also involved. In this study, we examined the non-renal hypothesis that increased functions of Na^+-permeable, non-selective cation channels (NSCCs) in arterial smooth muscle contribute to the increased vasoconstriction of MC (deoxycorticosterone acetate, DOCA)-salt hypertensive rat.
Membrane currents and membrane potential (*Em*) were measured by nystatin-perforated patch-clamp technique. Isometric vasoconstriction was measured from mesenteric arterial rings. Effects of extracellular Na^+ reduction and pharmacological blockers of Na^+-permeable channels such as flufenamic acid (FFA) and amiloride/benzamil were examined.
The basal tone and serotonin (5-HT)-induced vasoconstrictions were substantially increased in DOCA-salt hypertensive mesenteric arteries. Comparison of membrane conductance (*Gm*) of sham and DOCA-salt hypertensive arteries indicated that resting *Gm* was lager and that *Gm* increased even more in response to 5-HT in DOCA-salt hypertensive arterial smooth muscle than in sham arterial smooth muscle. Reduction in $[Na^+]_{out}$ normalized the increased vasoconstriction of the DOCA-salt hypertensive arteries as well as normalizing both the depolarized resting Em and the exaggerated 5-HT-induced *Em* depolarization. Effects of FFA were similar to those of low $[Na^+]_{out}$. However, ENaC blockers amiloride and benzamile were without effect.
These results indicate that the enhanced activity of the Na^+-permeable NSCCs other than ENaC in DOCA-salt hypertensive rat arterial smooth muscle critically contribute to the hyper-reactivity of DOCA-salt hypertensive rat. The molecular candidates for the Na^+-permeable NSCCs will be discussed.

Keywords: Hypertension, Mineralocorticoid, Epithelial Na^+ channel (ENaC), Non-selective cation channel, Vascular smooth muscle

S-4-4

Oxidative stress and nonselective cation channels in arteries and their contractile regulation

전민아[1], 강현수[2], 조하나[2], 배영민[1], 박상웅[3]

[1]Department of Physiology, Sungkyunkwan University College of Medicine, [2]Department of Physiology, Konkuk University School of Medicine, [3]Department of Emergency Medical Services, Eulji University, Korea

Oxidative stress is associated with many diseases, such as hypertension and diabetes mellitus. Oxidative stress reportedly activates the L-type voltage-gated calcium channel (VDCCL) and elevates $[Ca^{2+}]i$ in various kinds of cells. However, how oxidative stress activates VDCCL under clinical setting and the consequence for arterial contractions are unclear. Here, we examined the hypothesis that hydrogen peroxide (H_2O_2) alters membrane potential (E_m) by altering Na^+ influx through cation channels, which consequently activates VDCCL to induce vasoconstriction in rat mesenteric arteries. To measure the tone of the endothelium-denuded arteries, a conventional isometric organ chamber was used. Membrane currents and Em were recorded by the patch clamp technique. $[Ca^{2+}]_i$ and $[Na^+]_i$ were measured with microfluorometry using Fura2-AM and SBFI-AM, respectively. We found that H_2O_2 (10 and 100 μM) increased arterial contraction, and nifedipine blocked the effects of H_2O_2 on isometric contraction. H_2O_2 increased $[Ca^{2+}]_i$ as well as $[Na^+]_i$, and depolarised Em. Gd^{3+} (1 μM) blocked all these H_2O_2-induced effects including Em depolarisation and increases in $[Ca^{2+}]_i$ and $[Na^+]_i$. Although both nifedipine (30 nM) and low Na+ bath solution completely prevented the H_2O_2-induced increase in $[Na^+]_i$, they only partly inhibited the H_2O_2-induced effects on $[Ca^{2+}]_i$ and Em. Taken together, the results suggested that H_2O_2 constricts rat arteries by causing Em depolarisation and VDCCL activation through activating Gd^{3+}-and nifedipine-sensitive, Na^+-permeable channels as well as Gd^{3+}-sensitive Ca^{2+} permeable cation channels. We suggest that unidentified Na^+-permeable cation channels as well as Ca^{2+}-permeable cation channels may function as important mediators for oxidative stress-induced vascular dysfunction. We are also going to suggest some molecular candidates for these H_{22}-activated cation channels.

S-4-5

Mechanisms of connexin-related lymphedema

Michael J. Davis[1], Jorge A. Castorena-Gonzalez[2]

[1]Department of Pharmacology & Physiology, University of Missouri, Columbia, Missouri, [2]Department of Pharmacology, Tulane University, New Orleans, Louisiana, USA

Mutations in *GJC2* and *GJA1*, encoding connexins Cx47 and Cx43 respectively, are linked to human lymphedema, but the underlying mechanisms are unknown. We tested two possible mechanisms whereby Cx deficiency might lead to: 1) electrical uncoupling of the lymphatic muscle cell layer and interfering with entrainment of the contraction waves normally necessary for efficient lymph transport; and/or 2) defective lymphatic valves normally required for prevention of lymph backflow. We generated mouse models with global or tissue-specific deletion of the four major lymphatic connexin isoforms, Cx47, Cx43, Cx37 (*GJA4*) or Cx45 (*GJC1*) and developed new, ex vivo methods to quantify contraction wave entrainment and valve function in mouse popliteal lymphatic vessels. Deletion of Cx45, but none of the other Cx isoforms, from lymphatic smooth muscle (but not from endothelium) led to ~15-fold reduction in conduction speed of spontaneous contraction waves, an increase in the number of pacemaking initiation sites and a reduction in the percentage of waves conducting over the entire vessel length (i.e. entrainment). Deletion of Cx43, Cx37 or Cx47 from the endothelium led to reductions in lymphatic valve density, and deletion of Cx43, Cx37 or Cx45 (but not Cx47) resulted in some degree of valve dysfunction, as manifested by increased back leak and/or an abnormally high value of adverse pressure required for valve closure. The hierarchy of importance in terms of maintaining normal valve function was Cx43 > Cx37 > Cx45 > Cx47. Our results suggest that *GJC2* and *GJA1* mutations cause human lymphedema not by lymphatic contractile dysfunction or loss of contraction wave entrainment but rather by reducing valve density (Cx43, Cx47) and promoting valve incompetence (Cx43).

S-5-1

Improving the assessment of heart toxicity for all new drugs through translational regulatory science

Seiryo Sugiura[1], Jun-ichi Okada[1], Takashi Yoshinaga[2], Junko Kurokawa[3], Takumi Washio[1], Tetushi Furukawa[4], Kohei Sawada[5], Toshiaki Hisada[1]
[1]UT-Heart Inc., [2]Eisai Co. ltd., [3]University of Shizuoka, [4]Tokyo Medical and Dental University, [5]University of Tokyo, Japan

Safety issue is a challenging part of the drug discovery. In particular, cardiovascular events are one of the leading causes of premature termination of the development, thus a significant amount of time and money are spent for the *in vivo* screening of the arrhythmogenicity of drug candidates. We have developed an in silico screening system for the cardiotoxicity, which potentially replaces the expensive and time-consuming *in vivo* testing. The inhibitory effects of compounds on major ion currents were evaluated over a wide range of concentrations using an automated patch clamp system. Based on the concentration-inhibition relations thus obtained, we simulated the human cardiac electrophysiology while increasing the plasma concentration of compounds using the multi-scale heart simulator "UT-Heart". This hybrid-system coupling the *in vitro* experiments and the *in silico* heart model could accurately predict the arrhythmogenicity of compounds. Extending this approach, we also developed an arrhythmic hazard map under multiple ion channel block, which can be available on line. We will present the details of these approaches at the symposium.

S-5-2

Assessment of cardiac safety with hiPSC derived cardiomyocytes

Gun Sik Cho
넥셀

Failure to correctly predict adverse cardiotoxic effects of new pharmaceuticals is the major cause of compound attrition during drug development as well as for withdrawal of drugs already on the market. With recent advances in the stem cell field it is now possible to generate human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) that recapitulate the native behavior and accurately assess the pro-arrhythmic potentials of candidate drugs. At present, hiPSC-CMs are being actively investigated with high-throughput technology, especially through the CiPA initiative, for their potential use as a model system for complete cardiac safety screening. hiPSC-CMs displayed spontaneous beating and expressed the major cardiac markers and ion channels such as Potassium, Sodium and Calcium channel. Upon exposure to CiPA Phase II Validation Study Compounds identified as High, Medium or Low risk for manifesting human TdP, hiPSC-CMs showed an accurate ability to predict cardiotoxic effects and we could find consistency with clinical study results. In this presentation, I will show how can we use hiPSC-derived cardiomyocytes for predicting cardiotoxicity of drug candidates and the benefits of hiPSC-CMs based cardiotoxicity testing assay compare with current hERG safety assay.

S-5-3

Three-dimensional heart model-based screening of proarrhythmic potential by in silico simulation of action potential and electrocardiograms

Eun Bo Shim[1,3], Minki Hwang[3], Chul-hyun Lim[1], Chae Hun Leem[2]
[1]Department of Mechanical and Biomedical Engineering, Kangwon National University, [2]Department of Physiology, University of Ulsan, [3]AI Medic Inc. R&D Department, Korea

Purpose: The proarrhythmic risk is a major concern in drug development. The Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative has proposed the JTpeak interval on electrocardiograms (ECGs) and qNet, an in silico metric, as new biomarkers that may overcome the limitations of the hERG assay and QT interval. In this study, we simulated body-surface ECGs from patch-clamp data using realistic models of the ventricles and torso to explore their suitability as new in silico biomarkers for cardiac safety.
Methods: Human ventricular geometry was reconstructed from computed tomography (CT) images, and a Purkinje fiber network was mapped onto the endocardial surface. The electrical wave propagation in the ventricles was obtained by solving a reaction-diffusion equation using finite-element methods.
Results: We tested seven drugs in this study: dofetilide (high proarrhythmic risk), ranolazine, verapamil (QT increasing, but safe), bepridil, cisapride, mexiletine, and diltiazem. The body-surface ECG data were calculated using a torso model that included the ventricles. The effects of the drugs were incorporated in the model by partly blocking the appropriate ion channels. The effects of the drugs on single-cell action potential were examined first, and three-dimensional (3D) body-surface ECG simulations were performed at free Cmax values of 1x, 5x, and 10x. In the single-cell and ECG simulations at 5x Cmax, dofetilide, but not verapamil or ranolazine, caused arrhythmia. However, the non-increasing JTpeak caused by verapamil and ranolazine that has been observed in humans was not reproduced in our simulation.
Conclusions: Although validation is needed to improve the predictive capability of the model, this study provided an effective biomarker to examine the effects of drugs on body-surface ECG parameters using realistic 3D models of the ventricles and torso. This step could lead to our ultimate goal of creating a comprehensive in silico drug-safety testing system.

Keywords: 3D heart model, ECG simulation, Computational simulation, Toxicity analysis

S-5-4

Optimum pulse protocol to identify ion channel kinetics

Young-Seon Lee, Duong Duc Pham, Yunwan Jeon, Kyuri Kim, Chae-Hun Leem
Department of Physiology, University of Ulsan College of Medicine/Asan Medical Center, Seoul, Korea

Purpose: We aim to develop a method to optimize the time duration of voltage-clamp protocol by applying parameter sensitivity analysis of model parameters.
Methods: A mathematical model of cardiac I_{Kr} kinetics and sinusoidal voltage-clamp protocol of 8 second duration developed by Mirams group have been used in this study. This model has 8 kinetic parameters and one conductance parameter. We performed parameter sensitivity analysis by randomly varying 9 parameters at evenly spaced time points of voltage-clamp protocol. Cumulative parameter sensitivities with the absolute values of sensitivity at each time point were calculated. We have also used an experimental data of I_{Kr} with the sinusoidal voltage clamp by Mirams group.
Results: The cumulative parameter sensitives showed that sensitivities do not increase in the region between 2 and 3 seconds and between 7 and 8 seconds of the entire protocol. This result suggests that all of 9 parameters have little contribution to the change in the I_{Kr} current during the two-time ranges. Thus, this result prompted us to test if those two-time ranges may be unnecessary for parameter estimation with the sinusoidal voltage clamp protocol. Then we have eliminated the portions of the original sinusoidal voltage clamp protocol of 8 seconds to a reduced sinusoidal voltage protocol of 6 seconds. The experimental data of Mirams group, was also reduced manually from 8 seconds to 6 seconds. We ran an optimization procedure using CME-S to fit 9 model parameters. We found that there was little difference between the model parameters fitted with the experimental data with 8 second and 6 second duration.
Conclusions: In this paper we suggest that parameter sensitivity analysis of ionic current model can be applied for the optimization of voltage-clamp protocol.

Keywords: I_{Kr} current model, Mathematical model, Voltage-clamp protocol,

Parameter sensitivity analysis, Ion channel, Ion current

Acknowledgement: This work was partially supported by a grant (No. 19172MFDS168) from the Ministry of Food and Drug Safety of Korea in 2019 and the EDISON Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016-936606).

S-5-5

Cell model application to assess cardiac toxicity

Jae Boum Youm

Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

Patch-clamp studies on cardiac ion channels have been used to screen the arrhythmogenic risk of drugs developed by pharmaceutical companies. Several drugs have been withdrawn from the market as they were found to affect main ion channels responsible for cardiac arrhythmias. The cardiac arrhythmogenicity, however, could not be attributed to a single change in ion channel. The harmony of multiple ion channels and intracellular events is the key which maintains a normal sinus cardiac rhythm. Therefore, computational cell models have been developed and proposed to be used to predict cardiac toxicity of drugs. Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative emerged to quantify the arrhythmogenic risk of drugs by using the qNet score which is the amount of electronic charge carried by net current. The CiPA initiative has also the ability of developing the statistical framework to translate experimental variability into prediction uncertainty. Although the CiPA initiative is very convincing, it remains to be further considered. For example, the proarrhythmic risk of drugs could be higher under the condition such as beta-adrenergic stimulation and channel mutations. In this study, the beta-adrenergic stimulation and mutation in SCN5A were integrated into the CiPA initiative to quantify the proarrhythmic risk of drugs under the arrhythmia-prone condition. This approach is expected to provide a quantitative arrhythmogenic score of drugs under various risk conditions.

Keywords: Comprehensive in vitro Proarrhythmia Assay (CiPA), Cardiac toxicity, Cell model, Beta-adrenergic stimulation, Channel mutation

S-6-1

MSC-derived, exosome-mimetic extracellular nanovesicle-based therapeutics

Ju-Ro Lee[1], Han Young Kim[1], Bong-Woo Park[2], Hun-Jun Park[3], Byung-Soo Kim[1]

[1]School of Chemical and Biological Engineering, Seoul National University, [2]Department of Medical Life Science, College of Medicine, The Catholic University of Korea, [3]Division of Cardiology, Department of Internal Medicine, Seoul St. Mary's Hospital, Korea

Owing to poor engraftment and safety concerns regarding mesenchymal stem cell (MSC) therapy, MSC-derived exosomes have emerged as an alternative cell-free therapy for myocardial infarction (MI). However, the diffusion of exosomes out of the infarcted heart following injection and the low productivity limit the potential of clinical applications. Here, we developed exosome-mimetic extracellular nanovesicles (NVs) derived from iron oxide nanoparticles (IONPs)-incorporated MSCs (IONP-MSCs). The retention of injected IONP-MSC-derived NVs (IONP-NVs) within the infarcted heart was dramatically augmented by magnetic guidance. Furthermore, IONPs significantly increased the levels of therapeutic RNAs and proteins in IONP-MSCs as well as IONP-NVs, which can reduce the concern of low exosome-productivity. The injection of IONP-NVs into the infarcted heart and magnetic guidance induced an early shift from the inflammation phase to the reparative phase, reduced apoptosis and fibrosis, and enhanced angiogenesis and cardiac function recovery. This approach can enhance the therapeutic potency of an MSC-derived NV therapy and may pave the way for the clinical application of MI.

Keywords: Cardiac repair, Iron oxide nanoparticle, Mesenchymal stem cell, Myocardial infarction, Nanovesicle

S-6-2

Mitochondrial dynamics and energy metabolism in stem cells

Bong Jong Seo[1], Joonhyuk Choi[1], Jeong Eon Lee[1], Hyeonwoo La[1], Omer Habib[2], Kwonho Hong[1], Jeong Tae Do[1]

[1]Department of Stem Cell and Regenerative Biotechnology, Konkuk Institute of Technology, Konkuk University, Seoul, [2]Department of Chemistry, Hanyang University, Seoul, Korea

Mitochondria are important organelles that regulate many cellular events, including energy metabolism, cell cycle, differentiation, and cell survival. Mitochondria can proliferate and dynamically change their morphology through fusion and fission. Mitochondrial fusion is mediated by proteins encoded by *Mfn1*, *Mfn2*, and *Opa1*, while mitochondrial fission is mediated by proteins encoded by *Fis1* and *Dmn1L*. During the differentiation of pluripotent embryonic stem cells (ESCs), the globular shape of mitochondria progressively changed to elongated shape. We found that *Mfn2/Dnm1L* ratio was correlated with mitochondrial elongation during the ESC differentiation. Next, we established three different stem cells from blastocyst, such as ESCs, extraembryonic endoderm (XEN) cells, and trophoblast stem cells (TSCs), and compared mitochondrial morphology and energy metabolism. Our results revealed that ESCs and TSCs share mitochondrial characteristics, such as the mitochondrial morphology, energy metabolism, and the expression profiles of mitochondria-related gene set, but differ from XEN cells. We further investigated the function of mitochondria-fission related genes, such as *Fis1*, *Mff*, and *Dnm1l*. Using the homozygous knockout ESC lines, namely, *Fis1*$^{-/-}$, *Mff*$^{-/-}$, and *Dnm1l*$^{-/-}$ ESCs, we found that knockout of *Dnml1* showed more significant change in the mitochondrial elongation, energy metabolism, and ATP production compared with *Fis1* and *Mff*.

Keywords: Embryonic stem cells, Extraembryonic stem cells, Trophoblast stem cells, Mitochondria, Energy metabolism, *Dnm1l*

S-6-3

Engineered stem cell therapy for cardiac repair

Hun-Jun Park

Division of Cardiology, Department of Internal medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

The clinical use of human mesenchymal stem cells (hMSCs) has been hampered by their poor performance after transplantation into failing hearts. Here, to improve the therapeutic potential of hMSCs, we manufactured genetically engineered hMSCs expressing hepatocyte growth factor (HGF-eMSCs) and stromal derived factor-1α (SDF1α-eMSCs), respectively. At first, we worked out a strategy termed **"1) In-vivo priming"** in which hMSCs are primed in-vivo in myocardial infarction (MI)-induced hearts through HGF-eMSCs that was encapsulated within an epicardially implanted 3D cardiac patch. Primed hMSCs through HGF-eMSCs exhibited improved vasculogenic potential and cell viability, which ultimately enhanced vascular regeneration and restored cardiac function to the MI hearts. Histological analyses further demonstrated that the primed hMSCs survived longer within a cardiac patch and conferred cardioprotection evidenced by substantially higher numbers of viable cardiomyocytes in the MI hearts. Next, we developed **"2) Dual approach"** platform to regenerate vasculatures by simultaneously promoting postnatal vasculogenesis and angiogenesis, which are two core mechanisms of neovascularization. We introduced intramyocardial injection of human induced pluripotent stem cell-derived endothelial cells (hiPSC-EC-IN) for de-novo vasculogenesis, whereas epi-

cardially implantation of SDFα-eMSC patch (SDF1α-eMSC-PA) for the angiogenesis of pre-existing vessels. As expected, SDF1α-eMSC-PA improved capillary density through the prolonged secretion of paracrine factors including SDF1α as well as the improved vasculogenic potential of hiPSC-ECs, ultimately achieving comprehensive neovascularization and restoring cardiac function to the MI hearts. These results provide compelling evidence that high performance combinational cell therapy with engineered stem cells will ensure the achievement of a proper instructive cellular niche and facilitate cell retention, survival, and integration into the host heart tissue. We convince that this strategy may constitute a new avenue and hope for the treatment of MI.

Keywords: Myocardial infarction, Mesenchymal stem cell, Cell engineering, Cardiac regeneration

S-6-4

Safe scarless cassette-free selection of genome-edited human pluripotent stem cells using temporary drug resistance

Keun-Tae Kim[1], Ju-Chan Park[2], Hyeon-Ki Jang[3,7], Haeseung Lee[4], Seokwoo Park[5], Ok-Seon Kwon[2], Young-Hyun Go[2], Yan Jin[6], Wankyu Kim[4], Jeongmi Lee[6], Sangsu Bae[3,7], Hyuk-Jin Cha[2]

[1]Department of Life Sciences, Sogang University, Seoul, [2]College of Pharmacy, Seoul National University, Seoul, [3]Research Institute for Convergence of Basic Sciences, Hanyang University, Seoul, [4]Ewha Research Center for Systems Biology, Division of Molecular & Life Sciences, Ewha Womans University, Seoul, [5]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [6]School of Pharmacy, Sungkyunkwan University, Suwon, [7]Department of Chemistry, Hanyang University, Seoul, Korea

An efficient gene editing technique for use in human pluripotent stem cells (hPSCs) would have great potential value in regenerative medicine, as well as in drug discovery based on isogenic human disease models. However, the extremely low efficiency of gene editing in hPSCs is a major technical hurdle that remains to be resolved. Previously, we demonstrated that YM155, a survivin inhibitor developed as an anti-cancer drug, induces highly selective cell death in undifferentiated hPSCs. In this study, we demonstrated that the high cytotoxicity of YM155 in hPSCs, which is mediated by selective cellular uptake of the drug, is due to high expression of *SLC35F2* in these cells. Consistent with this, knockout of SLC35F2 with CRISPR-Cas9 or depletion with siRNAs made hPSCs highly resistant to YM155. Simultaneous gene editing of a gene of interest and transient knockdown of SLC35F2 following YM155 treatment enabled genome-edited hPSCs to survive because YM155 resistance was temporarily induced, thereby achieving enriched selection of clonal populations with gene knockout or knock-in. This precise and efficient genome editing approach took as little as 3 weeks without cell sorting or introduction of additional genes.

Keywords: Gene editing, CRISPR-Cas9, SLC35F2, YM155, Human pluripotent stem cells, Disease modeling

S-7-1

Metazoan principle of aging, based on nuclear barrier hypothesis

Sang Chul Park

Endowed Professor, Chonnam National University, Korea

When the aging-dependent cellular behaviors toward growth factors and toxic stress have been analyzed, the perinuclear accumulation of the activated signals, either mitogenic or apoptotic, has been observed, suggesting the aging-dependent inefficiency of the nucleocytoplasmic trafficking of the signals. Thereby, it would be natural to assume the operation of the functional nuclear barrier in aging-dependent manner, which would be designated as "Park and Lim's Barrier." This novel mechanism of aging-dependent operation of the functional nuclear barrier is proposed as the ultimate checking mechanism for cellular protection against toxic environment and the general mechanism for the trade-off growth to survival in aging. Comparative transcriptome analysis revealed that nuclear barrier-induced senescence (NBIS) was most similar in gene expression changes to RS, compared to senescence induced by other stresses tested (oxidative stress, DNA damage and oncogene), implying that the nuclear barrier would be a critical driver of RS-like physiological senescence-associated changes. Shared senescence-related processes between NBIS and RS included lysosomal degradation, nuclear transport, and translation, resulting in coordinated reduction in transmission of extrinsic signals to the nucleus and intracellular protein and mRNA supply from the nucleus. This nature of aging mechanism was observed to be operative in yeast, the primitive eukaryotic organism, as well. Therefore, we propose that nuclear barrier hypothesis of aging as the fundamental principle of physiological aging in metazoan eukaryotes.

S-7-2

Molecular genetic dissection of longevity in *C. elegans*

Seung-Jae V. Lee

Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Korea

Many environmental and genetic factors that influence lifespan have been identified for the last three decades. My laboratory aims to elucidate molecular mechanisms by which intrinsic and extrinsic factors regulate lifespan by using molecular genetics of the roundworm *Caenorhabditis elegans*. In this talk, I will present our latest work on signaling pathways that mediate the effects of internal and external factors on lifespan and aging. In particular, I will talk about our findings regarding a novel longevity-promoting protein kinase VRK-1, which activates the master cellular energy sensor AMPK via phosphorylation. In addition, I will also present our work on crucial functions of lipid homeostasis, which appears as important as DNA and protein homeostasis, in organismal aging. Because many findings on aging regulation in *C. elegans* have been shown to be evolutionarily conserved, homologous mechanisms may exist in mammals including humans.

Keywords: Aging, *C. elegans*, VRK-1, AMPK, Lipidostasis

References

1. Park, S.#, Artan, A.#, Han, S.H.#, Park, H.E., Kwon, S., Hwang, A.B., Shin, W.S., Kim, K.T.*, and Lee, S.J.V.* (2020) VRK-1 Extends Lifespan by Activation of AMPK via Phosphorylation. Sci. Adv. doi: 10.1126/sciadv.aaw7824.
2. Jung, Y.#, Kwon, S.#, Ham S.#, Lee, D., Park, H.E.H., Yamaoka, Y., Jeong, D.E., Artan, M., Altintas, O., Park, S., Hwang, W., Lee, Y., Son, H.G., An, S.W.A., Kim, E.J.E., Seo, M., and Lee, S.J.V.* (2020) C. elegans Lipin 1 moderates the lifespan-shortening effects of dietary glucose by maintaining ω-6 polyunsaturated fatty acids. Aging Cell 19(6):e13150.
3. Lee, D. #, An, S.W.A.#, Jung Y.#, Yamaoka, Y., Ryu, Y., Goh, G.Y.S., Beigi, A., Ma, D., Ha, C.M., Taubert, S., Lee, Y., and Lee, S.J.V.* (2019) MDT-15/MED15 permits longevity at low temperature via enhancing lipidostasis and proteostasis. PLoS Biol. 17(8): e3000415.

S-7-3

CD9, cellular senescence, and atherosclerosis

Jae-Ryong Kim

Department of Biochemistry and Molecular Biology, Smart-aging Convergence Research Center, College of Medicine, Yeungnam University, Korea

CD9, a 24 kDa tetraspanin membrane protein, is known to regulate cell adhesion and migration, cancer progression and metastasis, immune and allergic responses, and viral infection. CD9 is upregulated in senescent endothelial cells, neointima hyperplasia, and atherosclerotic plaques. How-

ever, its role in cellular senescence and atherosclerosis remains undefined. We investigated the potential mechanism for CD9-mediated cellular senescence and its role in atherosclerotic plaque formation. CD9 knockdown in senescent human umbilical vascular endothelial cells significantly rescued senescence phenotypes, while CD9 upregulation in young cells accelerated senescence. CD9 regulated cellular senescence through a phosphatidylinositide 3 kinase-AKT-mTOR-p53 signal pathway. CD9 expression increased in arterial tissues from humans and rats with age, and in atherosclerotic plaques in humans and mice. Anti-mouse CD9 antibody noticeably prevented the formation of atherosclerotic lesions in *ApoE*$^{-/-}$ mice and *Ldlr*$^{-/-}$ mice. Furthermore, CD9 ablation in *ApoE*$^{-/-}$ mice decreased atherosclerotic lesions in aorta and aortic sinus. These results suggest that CD9 plays critical roles in endothelial cell senescence and consequently the pathogenesis of atherosclerosis, implying that CD9 is a novel target for prevention and treatment of vascular aging and atherosclerosis.

Keywords: CD9, Cellular senescence, Atherosclerosis

Acknowledgement: This work was supported by the Basic Science Research Program (2014R1A2A2A01004559), Medical Research Center Program (2015R1A5A2009124), and Bio and Medical Technology Development Program (2013M3A9B6076413) through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT.

S-7-4

Lipid signature drives skeletal muscle aging by modulating membrane fluidity

Ki-Sun Kwon[1,2,3]

[1]Aging Research Center, Korea Research Institute of Bioscience and Biotechnology, [2]Department of Functional Genomics, KRIBB School, Korea University of Science and Technology, [3]Aventi Inc., Korea

Aging gives rise to the loss of muscle mass and strength, called sarcopenia. Chronic inflammation, mitochondrial defect, hormonal deregulation, and malnutrition are all causes of sarcopenia. Abnormal lipid accumulation may also contribute to sarcopenia, mechanisms in which are totally unknown. We found membrane lipid saturation in aged muscle by comparative lipidome profiling and discovered a lipid chaperone FABP3 upregulated in aged muscles by proteome profiling. FABP3 overexpression induced membrane lipid saturation in young mice, while FABP3 knockdown reversed the lipid composition in aged mice. Lipid saturation mediated by FABP3 triggered an unfolded protein response (UPR) via PERK activation, eIF2α phosphorylation, and reduced protein translation, resulting in the deterioration of muscle mass and contractility, which was ameliorated via FABP3 knockdown. Further, FABP3 overexpression reduced membrane fluidity and knockdown increased membrane fluidity in myotubes, potentially causing ER stress. Taken together, we establish a mechanical aging model in which FABP3 drives age-dependent lipidome remodeling in skeletal muscle and deteriorates muscle mass and contractility by modulating membrane fluidity and ER stress signaling.

Keywords: Sarcopenia, Lipidomics, Phospholipids, Fluidity, Aging

S-8-1

Watching single helical membrane proteins fold

Tae-Young Yoon

School of Biological Sciences, Seoul National University, Seoul, Korea

Tracking the entire folding pathways of integral membrane proteins is essential for understanding their biogenesis, which yet remains to be demonstrated. Here we describe the development of an experimental method that allows us to observe *in situ* folding of integral membrane proteins in a bilayer-like environment. We first completely unfold the protein at high force and then lower the force to initiate folding from a loosely stretched state where the transmembrane helices are aligned in a zigzag manner within a bicelle, thereby imposing minimal constraints on the reconstituted folding process. The new approach allowed us to characterize the folding pathway of two integral membrane proteins: the *Escherichia coli* rhomboid protease GlpG and the human β_2-adrenergic receptor. Despite their great evolutionary distance, the revealed folding pathways share striking similarities, including usage of helical hairpins as the basic folding unit and a highly ordered accrual of structure in an N- to C-terminal direction. These common features suggest that the folding pathways of integral membrane proteins have evolved to maximize their fitness with co-translational folding.

S-8-2

Time-resolved conformational analysis during GPCR-G protein coupling

Ka Young Chung

School of Pharmacy, Sungkyunkwan University, Korea

Protein-protein interactions and conformational changes of a signaling protein are major mechanisms of cellular signal transduction. To understand the precise signaling mechanism, studies have investigated the structural mechanism of signaling proteins using various biochemical and/or biophysical techniques such as X-ray crystallography, nuclear magnetic resonance (NMR), electron microscopy, and electron paramagnetic resonance. In addition to these techniques, surface labeling mass spectrometry has been successfully used for conformational analysis of signaling proteins. Exposed or flexible regions have higher labeling rates and buried or ordered regions have lower labeling rates. Therefore, surface labeling mass spectrometry is a useful tool for studying protein-protein interaction interfaces and conformational changes during a signaling protein activation. Although surface labeling mass spectrometry does not provide 3-D structural information, it analyzes dynamic protein conformations that are difficult to be analyzed with other techniques. GPCR signal transduction involves extensive protein-protein interactions and conformational changes of related signaling proteins. In this seminar, I will discuss the conformational mechanisms of GPCR signaling analyzed by surface labeling mass spectrometry.

Keywords: GPCR, G protein, Structure, Signal transduction

References

1. Kim HR, Xu J, Maeda S, Duc NM, Ahn D, Du Y*, and Chung KY*. Structural mechanism underlying primary and secondary coupling between GPCRs and the Gi/o family. Nature Communications. 2020:11:3160. (*Corresponding authors)
2. Du Y, Duc NM, Rasmussen SGF, Hilger D, Kubiak X, Wang L, Bohon J, Kim HR, Wegrecki M, Asuru A, Jeong KM, Lee JM, Chance MR, Lodowski DT*, Kobilka BK*, Chung KY*. Assembly of a GPCR-G protein complex. Cell. 2019:177(5):1232-1242.e11. (*Corresponding authors)

S-8-3

Structure-function relationship of a CLC-type Cl^-/H^+ antiporter

Hyun-Ho Lim

Neurovascular Research Group, Korea Brain Research Institute (KBRI), Korea

The CLC family proteins are involved in a variety of cellular processes, where chloride homeostasis needs to be controlled. Two distinct classes of CLC proteins, Cl^- channels and Cl^-/H^+ antiporters, have been functionally and structurally investigated over the last several decades. By subjecting purified CLC proteins to Cl^- and H^+ transport measurements, electrophysiological recording, equilibrium ligand-binding studies and X-ray crystallography, we have revealed the molecular mechanism of strange anion selectivity, an anion binding site and conformational intermediate of a CLC antiporter hitherto unknown. In this symposium, I will discuss about the functional heterogeneities and structural uniformity of CLC family proteins and share recent preliminary data on the uncharacterized bacterial CLC protein.

Keywords: CLC Cl^-/H^+ antiporter, X-ray crystallography, Electrophysiology

S-8-4

Analysis of phototoxin taste closely correlates nucleophilicity to type 1 phototoxicity

Eun Jo Du[1,2], KyeongJin Kang[2]

[1]Department of Biological Sciences, Sungkyunkwan University, [2]Neurovascular Unit Research Group, Korea Brain Research Institute, Korea

Although pigment molecules, which exhibit colors by photo-absorbing specific wavelengths via pi-bond electron conjugation, often show toxicity due to generation of free radicals and reactive oxygen species (ROS) upon photo-illumination, the molecular mechanism by which organisms perceive phototoxins for avoidance is unknown. Here, we discover that nucleophile-sensing Transient Receptor Potential Ankyrin 1-A isoform (TRPA1(A)), previously shown to serve as a receptor for free radicals and ROS induced by photochemical reactions, enables Drosophila melanogaster to aphotically taste potentially phototoxic pigments for feeding deterrence. Thus, TRPA1(A) works for both cause (phototoxins) and effect (free radicals and ROS) of photochemical reactions. The pigment molecules, riboflavin, methylene blue, rose bengal and porphines, activate TRPA1(A) in darkness, and, upon light illumination, are able to initiate acrylamide polymerization, a consequence of free radical generation as known with type 1 phototoxins. On the other hand, other tested pigments are little capable of either task. Such pre-photochemical detection of phototoxins requires the nucleophile-sensing ability of TRPA1, since the phototoxin-induced activities of nucleophile-insensitive TRPA1 variants, such as the TRPA1(B) isoform and site-specific TRPA1(A) mutants, were much attenuated. When heterologously expressed in Xenopus oocytes, TRPA1(A) from the malaria-transmitting mosquito, Anopheles gambiae, shows larger current responses to phototoxins than Drosophila TRPA1(A), consistent with their previously characterized disparate nucleophile responsiveness. Collectively, in addition to detection of light-induced free radicals, nucleophile sensitivity of TRPA1(A) allows insects to minimize photochemical injuries through aphotic detection of phototoxins. Conversely, pigments need to bear high nucleophilicity (electron-donating propensity) to act as type-I phototoxins, which is consistent with the fact that transferring photo-excited electrons from phototoxins to other molecules causes free radicals. Thus, identification of a novel sensory mechanism in Drosophila reveals a property fundamental to type-I phototoxins.

Keywords: Nucleophile, Phototoxins, Pigments, Photochemical reactions, Drosophila

S-8-5

The role of STIM1 in the innate immune system

Jin Seok Woo[1], Sonal Srikanth[1], Beibei Wu[1], Yasser M. El-Sherbiny[2,3], Jennifer Leung[1], Koollawat Chupradit[4,5,6], Laura Rice[7], Gil Ju Seo[8], Guillaume Calmettes[9], Chandran Ramakrishna[10], Edouard Cantin[10], Dong Sung An[4,5,6], Ren Sun[11], Ting-Ting Wu[11], Jae U. Jung[8], Sinisa Savic[2,12], Yousang Gwack[1]

[1]Department of Physiology, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA, [2]National Institute for Health Research—Leeds Biomedical Research Centre and Leeds Institute of Rheumatic and Musculoskeletal Medicine (LIRMM), Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds, UK, [3]Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt, [4]Division of Hematology-Oncology, David Geffen School of Medicine at UCLA, Los Angeles, [5]School of Nursing, University of California at Los Angeles, Los Angeles, [6]UCLA AIDS Institute, Los Angeles, CA, USA, [7]Leeds Institute of Biomedical and Clinical Sciences, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Beckett Street, Leeds, UK, [8]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, [9]Department of Medicine (Cardiology), David Geffen School of Medicine at UCLA, Los Angeles, [10]Department of Molecular Immunology, City of Hope Beckman Research Institute, Duarte, [11]Department of Molecular and Medical Pharmacology, UCLA, Los Angeles, CA, USA, [12]Department of Clinical Immunology and Allergy, St James's University Hospital, Leeds, UK

Stimulator of interferon genes (STING) is an endoplasmic reticulum (ER) signaling adaptor that is essential for the type I interferon response to DNA pathogens. Aberrant activation of STING is linked to the pathology of autoimmune and autoinflammatory diseases. The rate-limiting step for the activation of STING is its translocation from the ER to the ER-Golgi intermediate compartment. Here, we found that deficiency in the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) caused spontaneous activation of STING and enhanced expression of type I interferons under resting conditions in mice and a patient with combined immunodeficiency. Mechanistically, STIM1 associated with STING to retain it in the ER membrane, and coexpression of full-length STIM1 or a STING-interacting fragment of STIM1 suppressed the function of dominant STING mutants that cause autoinflammatory diseases. Furthermore, deficiency in STIM1 strongly enhanced the expression of type I interferons after viral infection and prevented the lethality of infection with a DNA virus in vivo. This work delineates a STIM1-STING circuit that maintains the resting state of the STING pathway.

Keywords: Stromal interaction molecule 1 (STIM1), Stimulator of interferon genes (STING), Type I interferon

S-9-1

Simulation-based electrophysiology education using HHmodel 2.1

Chae-Hun Leem

Department of Physiology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea

인체 내 전기현상은 다양한 세포에서 정보를 처리하고 기능을 조절하는 핵심기전으로 작용하지만 의과대학생을 대상으로 짧은 시간 내에 해당 지식을 전달하기가 쉽지는 않다. 이는 전기생리학이라는 학문이 여러 복합적 학문지식이 요구되고 또한 이온채널 동역학의 비선형적 현상을 연계하여 세포가 보이는 여러 전기적 현상을 이해하여야 하기 때문이다. 비선형현상을 단순한 수식과 도표로 이해를 시키기에는 한계가 있으며 이러한 비선형적 현상을 좀 더 쉽게 이해하고 받아들이기 위해 Hodgkin-Huxley의 신경세포 모델 기반의 시뮬레이션을 활용하여 막전압형성, 이온농도에 따른 막전압의 변화, 막전압고정, 전압변화에 의한 K^+이온통로와 Na^+이온통로의 활성변화와 이에 따른 활동전압의 발생기전 및 특징들에 대한 이해, 활동전압의 역치, 순응 현상, 그리고 활동전압의 전도현상 및 이에 미치는 여러 요소들에 대한 이해를 도모할 수 있다. 이러한 모델은 전기생리학 강의 및 전산실습과정을 통해 해당 지식체계를 좀 더 공고히 쌓을 수 있도록 도와줄 수 있어 전기생리학 교육에 큰 도움이 되고 있다.

Keywords: Hodgkin-Huxley, Nerve action potential, Electrophysiology ed-

ucation, Ion channel

Acknowledgement: This work was supported by the EDISON Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2016-936606).

S-9-2

Using an open-source human physiology model (Quantitative Circulatory Physiology, QCP) in the physiology practicum of medical students

Young Keul Jeon, Sung Joon Kim

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

인체생리학은 생체항상성을 유지하는 기능적 측면을 이해하는 학문이며, 의과대학 교육과정에서 중요한 기초학문이다. 학생들은 장기와 계를 기준으로 나눈 각론을 공부하며, 더 나아가 세포와 분자 수준의 기능에 대해 학습한다. 특히, 인체 기능은 서로 긴밀하게 연결되어 있기에, 장기와 조직의 기능을 유기적으로 이해하는 것이 중요하다. 그러나 인체생리학 강좌를 이수한 학생들이라 하더라도 각 장기와 계들의 유기적 상호작용을 통합적으로 이해하는데 여전히 어려움을 겪는다. 학생들이 자기주도적으로 복습하는 과정에서 생리학적 개념들을 연결하여 이해를 하는데 도움을 주는 학습 도구 개발이 필요하다. 미국 미시시피대학 생리학연구자들(Guyton 그룹)이 개발한 통합 생리학 시뮬레이션(quantitative circulatory physiology, QCP)은 4000개 이상의 생리 변수들과 각 변수들의 상호작용을 정의하여 가상의 '인체'를 대략 구현하였으며, 초기 버전은 공개된 바 있다. 이 프로그램을 활용하여, 서울의대 1학년 학생을 대상으로 4년간(2017–2020) 순환기계를 중심으로 통합적 인체생리변화를 모사하는 실습을 진행하였다. 학생들은 외부 자극을 조절하여 가상의 인체가 어떻게 반응하는지 예측 및 관찰 할 수 있다. 순환기 뿐만 아니라, 호흡기와 신장, 산–염기균형 등의 장단기 변화를 자율신경계–내분비계의 조절양상과 함께 관찰할 수 있었다. 이 과정을 통해 통합적 생리기능 이해에 도움이 되었으며, 소규모 그룹토의에도 유용하였다. 특히, 실제로는 수행하기 어려운 인체생리 실험을 가상적 환경조건들을 변화시키면서 반복할 수 있기 때문에, 극한환경의 인체생리 반응을 공부하는 자극이 되었다. 학생들은 프로그램을 이용한 복습을 통해 지식의 정리와 이해에 큰 도움을 받았으며, 토론과정에서 생리학 과목 자체에 대한 흥미도 얻을 수 있었다. 본 연제에서 이 프로그램에 대한 간략한 설명과 함께, 실제 실습에 사용한 온라인 강의영상 및 리포트 자료의 사례 등을 소개할 것이다.

S-9-3

Simulation-based lecture of cardiac, skeletal, and smooth muscle contraction

Jae Boum Youm

Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

인체의 근육은 기능과 구조에 따라 크게 심근, 골격근, 평활근으로 분류할 수 있으며 수의적인 조절 여부에 따라 수의근, 불수의근으로도 분류할 수 있다. 이전에는 자라, 토끼, 개구리, 쥐 등의 동물을 이용하여 심실근, 비복근, 소장 평활근 등 근육의 수축과 이완을 실습하여 왔으나 인체가 아닌 동물의 생리를 학습한다는 한계와 동물실험윤리와의 충돌을 극복하기 위해 컴퓨터 시뮬레이션을 이용하는 것이 점차 확대되고 있다. 본 연제에서 소개할 심근 시뮬레이션에는 액틴, 미오신 등 수축 단백에 의한 수축역학, 세포내 이온 변화, 미토콘드리아에 의한 ATP 생산, 이온통로에 의한 활동전압 생성 등 흥분-수축 연결에 관련된 모든 요소가 포함되어 있다. 학습내용으로는 세포밖 여러 이온에 의한 활동전압과 수축력의 변화, early after-depolarization과 delayed after depolarization 등 각종 부정맥의 발생원리 등이 다루어 진다. 골격근 시뮬레이션은 가자미근과 손가락폄근을 다루고 있으며 이온통로, 이온수송체, 수축기구 등의 요소를 포함하여 골격근의 흥분-수축 연결을 재현할 수 있다. 학습내용으로는 실무율, 불응기, 가중, 강축현상 및 근피로의 원리 등이 있다. 평활근 시뮬레이션은 혈관 평활근과 자궁 평활근을 다루고 있으며 이온통로, 이온수송체, 수축기구 등의 요소를 가지고 있다. 학습내용으로는 평활근의 수축원리에 해당되는 ca-calmodulin 관계, myosin light chain kinase/phosphatase, latch state 등이 포함된다. 본 연제에서는 각 시뮬레이션 소프트웨어에 대한 간단한 사용 설명과 학습내용을 소개할 것이다.

2020 Yudang Academic Award

Alzheimer's - a disease beyond the brain: Insights from sphingolipid metabolism

Jae-sung Bae, KARI Members

KNU Alzheimer's disease Research Institute, Kyungpook National University, Daegu, Korea; Department of Physiology, School of Medicine, Kyungpook National University, Daegu, Korea

The global burden of Alzheimer's disease (AD), already the most common type of dementia, is expected to increase still further owing to population ageing. Current major challenges in AD include the lack of reliable biomarkers for its early diagnosis, as well as the lack of effective preventive strategies and treatments. Thus, increased understanding of the novel molecular pathogenesis of AD could lead to the development of improved diagnostic and therapeutic strategies. In this talk, we will discuss the development of therapeutics for AD in the context of novel neuropathological mechanisms including inflammation, immune responses, impairment of autophagy, and vascular dysfunction related with sphingolipid metabolism. The novel therapeutic strategies currently in development based on biological principles, especially two kinds of sphingolipid enzymes such as sphingosine kinase1 (SphK1) and acid sphingomyelinase (ASM), will provide promise for the development of a new generation of therapeutics to prevent and treat AD.

Keywords: Alzheimer's disease, Sphingolipid, Microglia, Inflammatory Resolution, Immune Regulation

P20-01-01

Layer-specific serotonergic and cholinergic induction of long-term depression in the prefrontal cortex of rats

Dongchul Shin[1], Kwang-Hyun Cho[1], Kayoung Joo[1], Duck-Joo Rhie[1,2]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Catholic Neuroscience Institute, College of Medicine, The Catholic University of Korea, Seoul, Korea

Purpose: Pyramidal neurons in layers 2/3 (L2/3 PyNs) of the cortex extend their basal dendrites near the soma and as apical dendritic tufts in layer 1, which mainly receive feed-forward and feedback inputs, respectively. Neuromodulators such as acetylcholine and serotonin may regulate the information flow between brain structures depending on the brain state. However, dendritic compartment-specific induction of synaptic transmission in single PyNs is still uncertain.
Methods: Here, we studied layer-specific serotonergic and cholinergic induction of long-term synaptic plasticity in L2/3 PyNs of the agranular insular cortex, a lateral component of the orbitofrontal cortex using whole-cell recordings and FM1-43 dye unloading.
Results: In the experiment of FM1-43 dye unloading, we verified that local electrical stimulation to layers 1 (L1) and 3 (L3) activated axon terminals mostly located in L1 and perisomatic area (L2/3). Independent and AMPA receptor-mediated excitatory postsynaptic potential (EPSP) was evoked by local electrical stimulation of either L1 or L3. Application of serotonin (5-HT, 10 μM) induced activity-dependent long-term depression (LTD) in L2/3 but not in L1 inputs. LTD induced by 5-HT was blocked by the 5-HT2 receptor antagonist ketanserin, an NMDA receptor antagonist and by intracellular Ca^{2+} chelation. The 5-HT2 receptor agonist α-me-5-HT mimicked the LTD induced by 5-HT. However, the application of carbachol induced muscarinic receptor-dependent LTD in both inputs.
Conclusions: The differential layer-specific induction of LTD by neuromodulators might play an important role in information processing mechanism of the prefrontal cortex.

Keywords: Pyramidal cell, Synaptic transmission, Orbitofrontal cortex, Layer 2/3, Cholinergic

P20-01-02

Peripheral pain modulation of Chrysaora pacifica jellyfish venom requires both Ca^{2+} influx and TRPA1 channel activation in rats

Hye-Ji Kim[1], Khulan Amarsanaa[1], Eun-A Ko[1], Young-Joon Kang[2], Sung-Cherl Jung[1]
[1]Department of Physiology, School of Medicine, Jeju National University, Jeju, [2]Department of Emergency Medicine, School of Medicine, Jeju National University, Jeju, Korea

Purpose: The venom of jellyfish triggers severe dermal pain along with inflammation and tissue necrosis, and occasionally, induces internal organ dysfunction. However, the basic mechanisms underlying its cytotoxic effects are still unknown. Here, we report one of the mechanisms involved in peripheral pain modulation associated with inflammatory and neurotoxic oxidative signaling in rats using the venom of jellyfish, Chrysaora pacifica (CpV).
Methods: This jellyfish is identified by brown tentacles carrying nematocysts filled with cytotoxic venom that induces severe pain, pruritus, tentacle marks, and blisters. The subcutaneous injection of CpV into rat forepaws in behavioral tests triggered nociceptive response with a decreased threshold for mechanical pain perception. These responses lasted up to 48 hours and were completely blocked by verapamil and TTA-P2, T-type Ca^{2+} channel blockers, or HC030031, a transient receptor potential cation ankyrin 1 (TRPA1) channel blocker, while another Ca^{2+} channel blocker, nimodipine, was ineffective. Also, treatment with Ca^{2+} chelators (EGTA and BaptaAM) significantly alleviated the CpV-induced pain response.
Results: These results indicate that CpV-induced pain modulation may require both Ca^{2+} influx through the T-type Ca^{2+} channels and activation of TRPA1 channels. Furthermore, CpV induced Ca^{2+}-mediated oxidative neurotoxicity in the dorsal root ganglion (DRG) and cortical neurons dissociated from rats, resulting in decreased neuronal viability and increased intracellular levels of ROS. Taken together, CpV may activate Ca^{2+}-mediated oxidative signaling to produce excessive ROS acting as an endogenous agonist of TRPA1 channels in the peripheral terminals of the primary afferent neurons, resulting in persistent inflammatory pain.
Conclusions: These findings provide strong evidence supporting the therapeutic effectiveness of blocking oxidative signaling against pain and cytotoxicity induced by jellyfish venom.

Keywords: Jellyfish venom, Chrysaora pacifica, Inflammatory pain, ROS, TRPA1, T-type Ca^{2+} channel

P20-01-03

The antinociceptive efficacy of low frequency stimulation in chemotherapy-induced peripheral neuropathy mice model

Dong-Wook Kang, Jae-Gyun Choi, Jaehyuk Kim, Cuk-Seong Kim, Sang Do Lee, Jin Bong Park, Byeong Hwa Jeon, Hyun-Woo Kim
Department of Physiology and Medical Science, School of Medicine, Chungnam National University, Daejeon, Korea

Purpose: Docetaxel (DTX), a chemotherapeutic agent derived from taxol, commonly used to treat various cancer, produces unbearable neuropathic pain. For effective control of chemotherapy-induced pain, various treatment techniques are being studied, and electrical stimulation is considered as one of them. Therefore, this study was designed to investigate the antinociceptive effect and related neuronal mechanism of low frequency stimulation (LFS) on the DTX-induced neuropathic pain in mice.
Methods: In order to produce chemotherapy-induced peripheral neuropathy (CIPN), intraperitoneally administered DTX (2.5 mg/kg) for 4 times once every 2 days on male ICR mice. LFS (16 Hz, 800uA) was performed at the right wrist area and pain behavior response was measured using 2g of von Frey filament on both hind paws. In addition, western blot and immunofluorescence staining were performed on the spinal cord and DRG to measure changes of brain derived neurotrophic factor (BDNF).
Results: Repeated LFS significantly attenuated DTX-induced mechanical allodynia. Furthermore, LFS suppressed the enhanced expression of brain derived neurotrophic factor (BDNF) by DTX in dorsal root ganglion (DRG) and spinal dorsal horn.
Conclusions: In conclusion, these results suggest that LFS may be an effective treatment for patients suffering from CIPN.

Keywords: Chemotherapy, Docetaxel, Low frequency stimulation, Antinociception, BDNF

P20-01-04

Chronic angiotensin converting enzyme (ACE) inhibition causes mechanical allodynia in mice that is mediated by substance P

Jae-Gyun Choi, Dong-Wook Kang, Jaehyuk Kim, Jin Bong Park, Hyun-Woo Kim
Department of Physiology and Medical Science, College of Medicine and Brain Research Institute Chungnam National University, Korea

Purpose: Angiotensin converting enzyme inhibitor (ACEi) inhibits the enzyme dipeptidyl carboxypeptidase, which is involved in the conversion of angiotensin I to II and degradation of kinins like substance P (SP) and bradykinin (BK). However, the role of ACEi (Captopril and Enalapril) has not yet been studied much about the generation and maintenance of pain in mice.
Methods: In light of these observations, we hypothesized that: 1) Involve-

ment of the ACEi (i.t. or i.p. injection, s.i.d.) on mechanical allodynia (MA) in mice. 2) Involvement of the substance P (SP) in ACEi (i.t. or i.p. injection, s.i.d.) treated mice. 3) Effect of intraperitoneal (i.p.) injection of NK1R blocker (L-733,060) on the maintenance of the ACEi (i.t. or i.p. injection, s.i.d.) on mechanical allodynia (MA) in mice. 4) Intraplantar injection of substance P (SP) into the hindpaw of mice induced mechanical allodynia to von Frey testing.
Results: Mice treated with vehicle (control) or the remaining four groups received two doses (i.t. 7, and i.p. 60 mg/kg; s.i.d.) of captopril and enalapril, and mechanical allodynia was evaluated for 10 days. Both drug doses dependently increased the paw withdrawal frequency in von-Frey method. The ACEi-induced increase in the levels of SP in dorsal root ganglion (DRG) and spinal cord dorsal horn on mice. Then, in L-733,060, pretreated animals ACEi just decreased the paw withdrawal frequency, and treated with ACE blocked SP-induced allodynia. The administration of SP (25 μg) resulted in a significant increase in the mechanical paw withdrawal frequency compared to the ACE treated with SP, persisting for 3 days.
Conclusions: Our study suggests that ACEi causes mechanical allodynia in mice that is mediated by SP.

Keywords: Angiotensin converting enzyme, Mechanical allodynia, Neurokinin-1 receptor, Pain, Substance P

P20-01-05

Nav1.7 in the trigeminal ganglion plays an important role in the induction of pulpitis pain

Myeounghoon Cha[1], Minjee Kwon[2], Il Young Jung[3], Bae Hwan Lee[1]

[1]Department of Physiology, Yonsei University College of Medicine, [2]Department of Nursing, Kyungil University, [3]Department of Conservative Dentistry and Oral Science Research Center, Yonsei University College of Dentistry, Korea

Purpose: Pulpitis produces significant changes in the peripheral nervous system, which induce hyperalgesia. However, the neuronal activity and Nav1.7 expression following pulpal noxious pain have not yet been investigated in the trigeminal ganglion (TG). The aim of the present study was to verify whether experimentally induced pulpitis activates the expression of Nav1.7 peripherally and to reveal the pain controlled by Nav1.7 channel inhibition.
Methods: Acute pulpitis was induced through pulp exposure and mustard oil (MO, n=12) application in rat maxillary molar tooth pulp. Sham (n=12) and naïve (n=12) rats did not undergo tooth inflammation. Three days post-MO application, abnormal painful behavioral activity was recorded, and the rats were then euthanized to allow for immunohistochemical and molecular analysis and optical imaging of the Nav1.7 expressions in the TG.
Results: We found significantly increased MO-induced pain-like behavior and histological evidence of severe pulp inflammation-induced pain. At the optical recording, the TG of MO group expressed the higher neuronal activities after electrical stimulation on TG. Also Nav1.7 labelling immunoreactivity in the TG were significantly higher in the MO group than in the sham and naïve group rats. In the TG, the immunoreactivity of Nav1.7 was more pronounced in small-size neurons, and there were differences between groups. The change in the expression level of Nav1.7 through western blot was increased in the MO group. In addition, inhibition of Nav.1.7 by ProTxII in the pulpitis model effectively suppressed hyperpolarized activity in the TG caused by electrical stimulation on TG.
Conclusions: These findings indicated that the Nav1.7 channel was significantly involved in nociceptive signal processing in the peripheral nervous system following acute pulp inflammation.

Keywords: Pulpitis, Acute pain, Nav1.7, Trigeminal ganglion

P20-01-06

Altered behavioral coping strategy in rats with chronic neuropathic pain

Geehoon Chung[1], Daxian Li[2], Seunghui Woo[2], Nari Kim[2], Sun Kwang Kim[1,2]

[1]Department of Physiology, Kyung Hee University, College of Korean Medicine, [2]Department of Science in Korean Medicine, Kyung Hee University, College of Korean Medicine, Korea

Purpose: Persistent pain in chronic pain patients affects mental distress. The prolonged stress due to physical pain alters individuals' sensitivity to the environmental stressors and adjusts the coping strategy related to emotional contagion. This study aims to investigate the alteration of behavioral strategy in rats suffering from chronic neuropathic pain, thereby understand the relationship between chronic pain and empathic distress.
Methods: Spinal nerve ligation surgery was performed on Sprague-Dawley rats to induce neuropathic pain. The physical pain was assessed using the von Frey test. The mental distress was assessed with a forced swimming test. The coping strategy of the animal to the mental distress was measured using a series of helping behavior tests toward a trapped conspecific.
Results: Rats with chronic neuropathic pain showed decreased paw withdrawal threshold in the von Frey test, increased immobility time in the forced swimming test, and enhanced success rate of trap opening in the test of helping behavior toward a trapped conspecific.
Conclusions: The prolonged physical pain affects the brain process related to distress and alters behavioral coping strategy.

Keywords: Neuropathic pain, Chronic pain, Empathy, Emotional contagion

P20-01-07

Activity-dependent regulation of global Ca^{2+} level and tonic firing rate by TRPC3 channels in SNc dopamine neurons

Ki Bum Um, Myoung Kyu Park

Department of Physiology, Sungkyunkwan University School of Medicine, Korea

Purpose: Pacemaker dopamine neurons in the substantia nigra pars compacta (SNc) exhibit low-frequency spontaneous firing without any input stimuli. Since the tonic firing of dopamine neurons determines ambient dopamine levels, regulation of tonic firing rate is very important. Although mGluR1 is reported to activate some type of TRP channels, it is still unclear how mGluR1 regulates firing activities and patterns in SNc dopamine neurons. In this study, we present that mGluR1 increases tonic firing rate and Ca^{2+} levels via TRPC3 channels in SNc dopamine neurons.
Methods: We used whole-cell or attached patch-clamp recordings in single isolated or brain slice dopamine neurons from the SNc region. Simultaneously with the electrophysiological recording, cytosolic Ca^{2+} levels were measured by using Flour-4 or Fura-2 calcium indicators.
Results: In SNc dopamine neurons, we observed that DHPG, a mGluR1 agonist, after transient inhibition of spontaneous firing due to store Ca^{2+} release, caused a slow sustained increase in tonic firing rates with a concurrent Ca^{2+} rise. The DHPG-induced sustained Ca^{2+} increases can be induced by two distinct firing-dependent or firing-independent pathways. L-type Ca^{2+} channel blockers suppressed the sustained DHPG-dependent increase in Ca^{2+} without affecting the increase of tonic firing, suggesting that the DHPG-induced increased spontaneous firing is a causal factor for sustained Ca^{2+} rises. TRPC3 channel blocker pyr10 reduced DHPG-induced Ca^{2+} rises dramatically together with elimination of the spontaneous firing enhancement. In TRPC3 KO mice, tonic firing rate was not increased by the application of DHPG or repetitive mGluR1-dependent synaptic stimulation.
Conclusions: From these results, we conclude that TRPC3 channels determine the basal firing rate in synaptic mGluR1-dependent ways in SNc dopamine neurons.

Keywords: Dopamine neuron, mGluR1, TRPC3

P20-01-08

Upregulation of TRESK channel contributes to motor and sensory recovery after spinal cord injury

Gyu-Tae Kim[1], Eun-Jin Kim[1], Eun-Shin Lee[2], Marie Nyiramana[1,3], Adrian Siregar[1,3], Young-Sool Hah[4], Jaehee Han[1], Dawon Kang[1,3]

[1]Department of Physiology, College of Medicine, Gyeongsang National University, [2]Department of Rehabilitation Medicine, College of Medicine, Gyeongsang National University, [3]Convergence Medical Science, Gyeongsang National University, [4]Biomedical Research Institute, Gyeongsang National University Hospital, Korea

Purpose: TWIK (tandem-pore domain weak inward rectifying K^+)-related spinal cord K+ channel (TRESK), a member of the two-pore domain K+ channel family, is abundantly expressed in dorsal root ganglion (DRG) neurons. However, the function of TRESK in spinal cord injury (SCI) has not yet fully understood. In this study, we investigated the role of TRESK during SCI using transgenic mice that overexpress the TRESK gene (TG_{TRESK}).
Methods: SCI rats were made into a contusion model by dropping a rod 2 mm in diameter from 25 mm in height on the ninth thoracic spinal cord segment (T9). SCI mice were generated by acute compression by a one minute epidural compression at T9 using a vascular clamp. TG_{TRESK} mice also received the same SCI operation. In vitro experiments using TRESK overexpressing HEK293 cells and RAW264.7 macrophage cell were designed to identify the role of TRESK in oxidative and inflammatory stresses, pathological conditions of SCI.
Results: TRESK mRNA expression was upregulated in the cerebral cortex, spinal cord, and DRG isolated from the ninth thoracic (T9) spinal cord contusion rats. The expression was significantly upregulated in the spinal cord below the SCI site at acute time points (6, 24, and 48 h) after SCI. In addition, TRESK expression was highly increased in DRGs below and adjacent to the injury site. In HEK-293 cells and RAW264.7 cells overexpressing TRESK, H2O2-induced cell death and LPS-induced production of pro-inflammatory mediators were significantly decreased compared to vector-transfected cells, respectively ($p < 0.05$). TG_{TRESK} mice showed faster behavioral recovery and higher mechanical threshold from T9 SCI compared to sham-operated mice. In addition, the TGTRESK mice had lower TNF-α concentrations than wild-type mice.
Conclusions: These results indicate that TRESK upregulated following SCI contributes to recovery of behavior and mechanical pain threshold by suppressing excitability of motor and sensory neurons and inflammatory processes.

Keywords: Dorsal root ganglion, Inflammation, Oxidative stress, Spinal cord injuries, Two-pore domain K^+ channel

P20-01-09

Alleviation of neuropathic pain via glial regulation in the insular cortex of rats

Kyeongmin Kim, Songyeon Choi, Myeounghoon Cha, Bae Hwan Lee

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

Purpose: Studies on the role of neuroglia in neuropathic pain have been carried out, but a majority of studies focused on the level of the spinal cord. The insular cortex (IC) is known to process pain information and mainly involved in discriminative sensory and motivative emotion. However, the analgesic effects of inhibition of glia in the IC remain unexplored. The aim of this study was to reveal the pain alleviation effect of inhibition of neuroglia (astrocytes or microglia) in the IC during pain development.
Methods: Specifically, fluorocitrate (FC, astrocyte inhibitor) and minocycline (MC, microglia inhibitor) were used to suppress glial cells during both early and late stages of neuropathic pain development. Therefore, we performed two experiments, administration of fluorocitrate (FC, astrocyte inhibitor, 1 nM) and minocycline (MC, microglia inhibitor, 20 μM) into the IC immediately after nerve injury (early inhibition) and application of each drug after establishment of chronic pain (late inhibition).
Results: Both early and late inhibition of neuroglia was effective in attenuating the pain. Subsequently, the role of astrocytes and microglia was demonstrated by observing the changes in protein expressions and morphological alterations. Interestingly, early inhibition of microglia showed enduring analgesic effects, persisting after drug withdrawal. Both glial fibrillary acidic protein (GFAP) and cluster of differentiation molecule 11b and c (CD11b/c) expressions were decreased and morphological alterations of neuroglia were also observed after injection of FC or MC, respectively. Late inhibition of neuroglia activity by using MC and FC showed similar analgesic effects. However, late inhibition of microglia banished the analgesic effect on a day after MC withdrawal. The significant changes in expression level and morphology GFAP and CD11b/c were observed in late inhibition.
Conclusions: These findings indicate that suppression of neuroglia in the IC alleviates the development of neuropathic pain.

Keywords: Neuropathic pain, Insular cortex, Astrocytes, Microglia

P20-01-10

Voltage-gated calcium channels trigger spontaneous glutamate release via nanodomain coupling

Byoung Ju Lee[1,2], Che Ho Yang[1,2], Seung Yeon Lee[1,2], Suk-Ho Lee[1,2], Yujin Kim[2], Won-Kyung Ho[1,2]

[1]Department of Biomedical Sciences, Seoul National University College of Medicine, [2]Department of Physiology, Seoul National University College of Medicine, Korea

Purpose: Neurotransmitter release occurs either synchronously to action potentials or spontaneously, yet whether molecular machineries underlying evoked and spontaneous release are identical, especially whether voltage-gated Ca^{2+} channels (VGCCs) can trigger spontaneous events has been in debate. To elucidate this issue, we characterized Ca^{2+} dependency of miniature excitatory postsynaptic currents (mEPSCs), in autaptic cultured hippocampal neurons, acute hippocampal slices, and acute brain stem slices.
Methods: To measure spontaneous glutamate release at excitatory synapses, we recorded synaptic activities at resting state (-70 mV) under the whole-cell voltage clamp condition using pipette solutions containing 0.1 mM EGTA from hippocampal neurons grown isolated on astrocyte feeder islands at least for 3 weeks for synaptic maturation. To examine whether vesicular Ca^{2+} sensors that operate for spontaneous and evoked release are identical or distinct, we measured mEPSC frequency and eEPSC amplitude from the same cells with intracellular EGTA or BAPTA.
Results: We found that 58 % mEPSC frequency was dependent on extracellular Ca^{2+} ($[Ca^{2+}]_o$), and Ca^{2+} cooperativity of evoked and spontaneous release was comparable. Moreover, most (> 90%) of $[Ca^{2+}]_o$-dependent mEPSCs was attributable to P/Q-, N-, and R-type VGCCs by activation of single subtype or coactivation of multiple subtypes. $[Ca^{2+}]_o$- and VGCC-dependence on spontaneous release was also observed at different areas in hippocampal slices and at the calyx of Held synapses. Interestingly, the contribution of VGCCs increased with depolarization and ages at certain synapses, suggesting a modulatory mechanism. At the calyx of Held synapses, type 1 synapses with tight coupling between Ca^{2+} sensors and Ca^{2+} sources showed VGCC-dependence. Consistently, our simulation based on experiments with two different calcium chelators showed that cultured hippocampal neurons had tight coupling with coupling distance of 22 nm.
Conclusions: These data suggest that the distance between VGCCs and Ca^{2+} sensors is the key factor to determine VGCC dependence of spontaneous release.

Keywords: VGCC, mEPSC, Autapse, Hippocampus, Calyx of held

P20-01-11

Antiallodynic effects of KDS2010 on chemotherapy-induced peripheral neuropathy (CIPN) model mice

Su Eun Park[1,2,3], Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Hyun Jin Shin[1,2,3], Ki Duk Park[4], Jin Bong Park[1,2,3]

[1]Department of Medical Sciences, Chungnam National University, Daejeon, [2]Department of BK21Plus, CNU Integrative Biomedical Education Initiative, Chungnam National University, Daejeon, [3]Department of Physiology, School of Medicine and Brain Research Institute, Korea Institute of Science and Technology (KIST), [4]Convergence Research Center for Diagnosis, Treatment and Care System of Dementia, Korea

Purpose: To investigate the analgesic effect of a new newly developed, highly selective and reversible MAO-B inhibitor, KDS2010, in Chemotherapy-induced peripheral neuropathy (CIPN).
Methods: We developed a new selective and reversible pharmacological inhibitor of MAO-B, a key enzyme implicated in monoamine metabolism and reactive oxygen species (ROS) as a potential therapeutic target for CIPN. After confirmation of successful establishment of a paclitaxel-induced mechanical allodynia in mice, KDS2010 was introduced to the neuropathic mice via oral route with different doses to test its novel therapeutic effect.
Results: Paclitaxel-induced mechanical allodynia were significantly suppressed by KDS2010 administration in a dose-dependent manner. Furthermore, immunostaining of spinal sections showed KDS2010 significantly reduced paclitaxel-induced astrogliosis. Increased immunodensity of GFAP-ir in neuropathic mice was significantly reversed by KDS2010 treatment. In addition, treatment with KDS2010 cased minimal side effects on the body weight, motor co-ordination, motor activity as well as working memory in both the control and neuropathic mice. Sirt1 the functional outcome is decreased senescence, apoptosis, inflammation, and oxidative stress. Further investigations are needed to clarify the hypothesis that KDS2010 may act via mediating ROS production in relation to SIRT1, a negative regulator of oxidative stress and inflammation.
Conclusions: Our results demonstrate that reversible MAO-B inhibitor, KDS2010 attenuated paclitaxel-induced pain behaviors through supressing spinal astrogliosis while reducing unwanted with minimal side effects on the body weight and essential brain functions. These effects make KDS2010 a promising therapeutic treatment of CIPN.

Keywords: KDS2010, CIPN, Reactive astrocyte, ROS

P20-01-12

Decoding of spontaneous ongoing pain from primary somatosensory neuronal calcium actiivities using two-photon microscopy

Heera Yoon[1,2], Myeong Seong Bak[2], Seung Ha Kim[3], Ji Hwan Lee[2], Geehoon Chung[1,2], Haney Park[2], Sang Jeong Kim[3], Sun Kwang Kim[1,2]

[1]Department of Physiology, College of Korean Medicine, Kyung Hee University, [2]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, [3]Department of Physiology, Seoul National University College of Medicine, Korea

Purpose: The objective measurement of spontaneous pain is a long-standing challenging issue in the fields of pain and neuroscience. The pain processing is involved multiple brain regions, including primary somatosensory cortex (S1) which have been suggested for pain perception. In the present study, we hypothesized that neuronal activity patterns in the mouse S1 cortex are distinct between spontaneous pain and non-pain conditions, and that this discrepancy can be used for quantitatively measuring spontaneous pain and then evaluating the analgesic effects of pain killers.
Methods: To explore this hypothesis, we performed in vivo two-photon calcium imaging in the layer 2/3 S1 neurons of awake, head-fixed mice with or without formalin-induced biphasic spontaneous pain. We then applied a deep learning technic, including bidirectional recurrent neural networks, for decoding spontaneous pain information from the recorded neuronal calcium activity patterns.
Results: The deep learning model successfully predicted the intensity of pain induced by various doses of formalin injection. Furthermore, the model of ours could estimate the efficacies of analgesic drugs, such as ketoprofen and lidocaine, on formalin-induced pain. Interestingly, our machine learning model could also discriminate other types of pain, such as topical capsaicin application-induced pain, CFA inflammatory pain, and nerve injury-induced neuropathic pain, even though the model was trained using formalin-induced pain group data.
Conclusions: We believe that our model which aims to decode sensory information from S1 activity could be applied to distinguish the pain condition in general and this method could be a valuable tool for objective assessment of pain and drug efficacy.

Keywords: Pain, Deep learning, S1 cortex, Spontaneous pain, Two-photon microscopy

P20-01-13

Effects of laser and electro acupuncture treatment with GB30 · GB34 on change in arthritis rat

Yu-Mi Lee, Changsu Na, Mirae Kim, Donghee Choi, Daehwan Youn

Department of Korean Medicine, Dongshin University, Korea

Purpose: This study aimed to investigate the effects of electroacupuncture (EA), the laser acupuncture (LA) and the combination therapy (LA+EA) in a collagenase-induced osteoarthritis rat model.
Methods: Osteoarthritis rat model was induced by injection of collagenase into left lower articular cavity (50 μl to knee and 10 μl to ankle). In order to assess the anti-osteoarthritic effects of EA, the 830 nm LA and 830 nm LA+EA, the histopathological findings and plantar withdrawal responses were analyzed.
Results: All of the treatment methods used in this study were effective in reducing pain. All treatment groups were effective in decreasing inflammatory cytokines of TNF-a and IL-6; the 830 nm LA and 830 nm LA+EA groups significantly reduced IL-1β.
Conclusions: 830 nm LA and EA inhibit the production of collagenase-induced inflammatory mediators of osteoarthritis

Keywords: Arthritis, Laser acupuncture, Electroacupuncture, Collagenase, Combination therapy

P20-01-14

JAK3-mediated lamellipodia formation promotes the tangential migration of interneurons in embryonic brain development

A Young Kim, Eun Joo Baik

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

Purpose: During brain development, different types of neurons are originated from spatially and molecularly segregated progenitors and are moved along the distinct migratory pathways. Among them, inhibitory GABAergic interneurons from the subpallium navigate along multiple tangential migration routes to settle into appropriate developing cortical layers. Disturbed neuronal migration gives rise to neurological or neuropsychiatric disorders, such as congenital epilepsy, autism spectrum disorder, and schizophrenia. Here, we identified JAK3 as a modulator of migration and differentiation of interneurons during developmental stage.
Methods: All experiments were performed with the mouse brain at embryonic day 13.5 (E13.5). In ex vivo slice culture, the brain from FVB-Tg(Gad1GFP)45704Swn/J mouse cut coronally at 300 μm and incubated in Neurobasal A media with 1% fetal bovine serum (FBS) for 24 hours. In in vitro culture, the medial ganglionic eminence (MGE) from wild type CD1 mouse brain were isolated and incubated in DMEM/F12 with 10% FBS for 3days after scratch-wound.
Results: Most interneurons produced in the medial ganglionic eminence (MGE) move to the developing cortex in mouse embryonic day E13.5 to

E15.5. At E13.5, pharmacological deficiency of JAK3 delayed the tangential migration of interneurons from subpallium to developing cortex in ex vivo slice culture. In addition, our in vitro wound healing models showed the migration of MGE-derived interneurons reduced by treatment of JAK3 inhibitor. These results are thought that JAK3 modulates the migration of nestin-positive precursor cell from MGE via formation of actin-mediated lamellipodial protrusion. By JAK3 inhibition, the nestin-positive precursor cells showed filopodia-based migration instead of lamellipodia and reduced alignment of focal adhesion molecule. Reduced migration of the precursor cells affected the positioning, distance, and velocity of MGE-derived GABAergic cell migration.
Conclusions: Taken together, the migration of MGE-derived interneurons relies on the motility of the nestin-positive precursor cells from MGE. Then, JAK3 is a proper modulator of lamellipodia-based migration in nestin-positive presursor cells.

Keywords: Interneuron, Migration, Lamellipodia, JAK3

P20-01-15

Analgesic effects of C. Cortex and its phytochemical against oxaliplatin induced neuropathic pain: suppressing activated glia and released pro-inflammatory cytokines in spinal cord

Ji Hwan Lee[1], Haney Park[1], Nari Kim[1], Sun Kwang Kim[1,2]

[1]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul, [2]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, Korea

Purpose: Cinnamomi Cortex (C. Cortex) has been used in East Asia to treat various pain symptoms. Anti-nociceptive effect of Coumarin, a major phytochemical of C. Cortex, on variety of pain was reported. We investigated whether and how C. Cortex and Coumarin alleviate oxaliplatin-induced cold and mechanical allodynia in Sprague Dawley rats.
Methods: The pain behaviors of cold and mechanical allodynia were quantified by a tail immersion test in cold water (4 ˚C) and a von Frey hair test, respectively.
Results: Significant pain behaviors were observed three days after an oxaliplatin injection. C. Cortex (200 mg/kg) and Coumarin (10 mg/kg) were orally administrated for five consecutive days after an oxaliplatin injection. C. Cortex and Coumarin inhibited the activation of spinal astrocytes and microglia by oxaliplatin treatment through immunohistochemistry. C. Cortex down-regulated up-regulated pro-inflammatory cytokines, IL-1β and TNF-α, in the spinal cord after an oxaliplatin injection. Coumarin decreased increase of TNF-α after an oxaliplatin treatment (0.1 mM) on primary cultured astrocytes.
Conclusions: This study suggests that C. Cortex could be an alternative therapeutic agent on oxaliplatin-induced cold allodynia, and Coumarin may play a role in this efficacy of C. Cortex.

Keywords: Oxaliplatin, Glia, Cinnamomi cortex, CIPN

P20-01-16

GluN2B antagonist attenuates mechanical hypersensitivity in the development phase of neuropathic pain after peripheral nerve injury

Youngkyung Kim, Young Ju Ahn, Young Wook Yoon

Department of Physiology, Korea University College of Medicine, Korea

Purpose: N-Methyl-D-aspartate receptor (NMDAR) GluN2B subtype is gaining attention because blocking of its activity is reported to reduce neuropathic pain with minimal side effects. However, the effectiveness of GluN2B antagonist on neuropathic pain following peripheral nerve injury and the signal transduction pathways associated with GluN2B activation are not known.
Methods: After spinal nerve ligation (SNL), we investigated the temporal changes in GluN2B, its phosphosites at Ser1303 (p-Ser1303), calcium/calmodulin-dependent protein kinase II (CaMKII), and postsynaptic density protein 95 (PSD-95) in the dorsal spinal cord. Co-immunoprecipitation was used to examine the interaction between p-Ser1303 and CaMKII, and PSD-95, respectively. Mechanical and cold paw withdrawal responses were measured before and after intrathecal administration of GluN2B antagonist in the early and late phase after SNL.
Results: GluN2B expression increased from 6 hours to 4 days and p-Ser1303 expression increased up to 2 weeks. The interaction between p-Ser1303 and CaMKII was robustly enhanced from 6 hours to 4 days, and that between p-Ser1303 and PSD-95 was increased from 4 days to 2 weeks after injury. GluN2B antagonist Ro 25-6981 reduced phosphorylation of GluN2B Ser1303 and the interaction between p-Ser1303 and PSD-95 in the early phase. It was more effectively increased mechanical paw hypersensitivity in the early phase than late phase.
Conclusions: These results demonstrate that GluN2B signaling may induce mechanical paw hypersensitivity after peripheral nerve injury.

Keywords: GluN2B, PSD-95, Neuropathic pain, Spinal nerve ligation, Peripheral nerve injury

P20-01-17

Age-dependent expression of satellite glial cell-specific markers in rat sympathetic ganglia

Huu Son Nguyen[1], Seong Jun Kang[1], So Hyun Kim[2], Seong-Woo Jeong[1]

[1]Department of Physiology, Yonsei University Wonju College of Medicine, [2]Department of Physiology, Yonsei University College of Medicine, Korea

Purpose: The glial cells including astrocytes have been established as essential for many functions of the central nervous system. Recently, several lines of evidence have suggested that satellite glial cells (SGCs) form distinct functional units with the primary neurons for bidirectional communication in the peripheral sensory and autonomic ganglia. In the sensory ganglia, the SGCs are found to express the glial cell-specific markers including S100 calcium-binding protein ß (S100ß), glutamine synthetase (GS), and inwardly rectifying potassium 4.1 (Kir 4.1) channel which are defined in the central glial cells. To date, however, it is unclear whether in the SGCs of the autonomic ganglia, the glial-specific markers are expressed and if so age-dependently changed in their expression.
Methods: In this regard, we isolated the sympathetic superior cervical ganglia (SCG) from the rats at P0, P28, and P56, and then performed double immunohistochemistry employing the antibodies against tyrosine hydroxylase (TH), a sympathetic neuronal marker, S100ß, GS, Kir4.1, and glial fibrillary acidic protein (GFAP).
Results: Compared with the P28 and P56 rats, the soma size and the number of the SGC-encircled SCG neuron were much smaller in the neonates. Most neonatal SGCs were Kir4.1-negative and about 50% neonatal SGCs were both S100ß- and GS-positive. However, most SGCs from the adolescent and adult rats expressed the aforementioned glial-specific markers. The GFAP-positive SGC was scarce at all ages. Interestingly, LPS treatment of rats significantly increased the number of the GFAP-positive SGCs.
Conclusions: Taken together, we found that the SGCs in the sympathetic ganglia are proliferating and age-dependently express the S100ß, GS, and Kir4.1. In addition, the GFAP appears to be a potential SGC-specific marker under pathological conditions.

Keywords: Satellite glial cell, Autonomic, Sympathetic ganglia, S100ß, Kir 4.1, Glutamine synthetase, Glial fibrillary acidic protein

P20-01-18

Characterizing neural selectivity in multidimensional sensory feature space

Sa-Yoon Park[1], Yoo Rim Kim[2], Sang Jeong Kim[2], Chang-Eop Kim[1]
[1]Department of Physiology, College of Korean Medicine, Gachon University,
[2]Department of Physiology, College of Medicine, Seoul National University, Korea

Purpose: Investigation on how cortical neurons process complex stimuli that have multi-modality features is a fundamental question in sensory neuroscience field. Increasing number of studies focus on complex natural stimuli, rather than simple artificial stimuli, making it harder to characterize the selectivity of the neural response using conventional "outside-in" approach (i.e. using researcher-defined features). Despite the importance of the question, the problem that causes mischaracterization is not defined well enough and the solution has not been proposed yet.
Methods: We suggested possible scenarios for mischaracterizing selectivity by formalizing the problem of characterizing neural selectivity as classifying problem in multidimensional feature space with stimulus-feature matrix and response vector. The stimulus-feature matrix is a binary design matrix that represents stimuli and corresponding features, and the response vector is results of neural response. To solve the problem, we formulated a theoretical framework for characterizing neural selectivity in multidimensional sensory feature space, given the assumption that all feasible features are known. Further, to find all feasible features given stimuli, we devised inside-out feature finding (IOFF) algorithm that suggests possible feature with a normalized value, based on simple linear regression analysis.
Results: To validate the usefulness of the IOFF algorithm, we applied this algorithm to in vivo two-photon Ca^{2+} imaging data of neurons in primary somatosensory cortex and posterior parietal cortex. We confirmed that IOFF can be used for finding missing features and for verifying whether all possible features are known.
Conclusions: This study proposed a theoretical framework for characterizing neural selectivity in multidimensional feature space. Further, to apply this framework to actual data, this study has invented a method to find all feasible features.

Keywords: Sensory neuroscience, Characterizing neural selectivity, Methodology, Theoretical framework, Feasible feature extraction

P20-01-19

The role of mitochondria calcium uniporter in C. elegans odor learning

Hee Kyung Lee, Saebom Kwon, Jessica Antonio, Kyoung-hye Yoon
Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

Purpose: Calcium enters the mitochondria through the mitochondrial calcium uniporter (MCU), where, in addition to buffering cytosolic calcium, it regulates important cellular processes such as ATP synthesis and apoptosis. In this study, we sought to find the role of MCU and mitochondrial calcium in neurons using the model organism C. elegans. We used a simple learning paradigm, called odor adaptation, to test whether MCU plays a role in learning and memory.
Methods: Mutant strains of mcu-1 were pre-exposed to an attractive odor, then were given a choice between the same odor and solvent control, which were placed on either side of a 9 cm plate. Worms on each side were counted to calculate the chemotaxis index. Transgenic strains that expressed mcu-1 under tissue-specific promoters and inducible promoters were generated, to test when and where MCU is needed for odor learning.
Results: I found that, whereas wild type strain shows significantly decreased chemotaxis index in response to 60 min pre-exposure, mcu-1 mutants show only a modest decrease in chemotaxis index. This was the case only for odors detected by the AWC sensory neuron - mcu-1 showed normal learning behavior for odors sensed by the AWA sensory neuron. Restoring MCU-1 in the neurons resulted in a low chemotaxis index similar to wild type, showing that mcu-1 in the neurons contributes to odor learning. In transgenic strains expressing mcu-1 under an inducible promoter, mcu-1 needed to be induced early during larval development (egg or L1 stage) to restore odor learning, and inducing after L4 or adult stage did not improve odor learning.
Conclusions: MCU is required for learning odors detected by one specific sensory neuron, AWC. MCU is required in neurons for odor learning, and it is required during development, which suggests MCU has a role in proper neural development that supports learning. We are currently trying to determine which neuron MCU is needed, and which developmental process is affected that contributes to this phenotype.

Keywords: MCU, C. elegans, Odor learning, AWC neuron, Chemosensory neuron

P20-02-01

TNF-α mediated progressive neuroinflammation associated with UPRmt in kaolin induced hydrocephalus mouse model

Jiebo Zhu[1,2,3], Min Joung Lee[1,2,3], Hee Jin Chang[1,4], Yunseon Jang[1,2,3], Xianshu Ju[1,3], Jianchen Cui[1,3], Yu Lim Lee[1,3], Eunji Namgung[1,2,3], Dahyun Go[1,2,3], Changjun Seo[1,2,3], Hyoeun Kang[1,2,3], Hyeongseok Kim[1,2,3], Woosuk Chung[1,5,6], Eungseok Oh[1,4], Jun Young Heo[1,2,3]
[1]Department of Medical Science, [2]Department of Biochemistry, [3]Infection Control Convergence Research Center, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University School of Medicine, [5]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, [6]Department of Neurology, Chungnam National University Hospital, Korea

Purpose: To identify the association between tumor necrosis factor (TNF)-α signaling and mitochondrial unfolded protein responses (UPRmt) in kaolin induced hydrocephalic mouse model (KIHMM).
Methods: Generated hydrocephalic model, by injected 25% kaolin into C57BL/6J mice cisterna magna with stereotactic surgery. Western blotting, qRT-PCR, ELISA, and immunofluorescence to assess the expression of TNF-α signaling and UPRmt.
Results: TNF-α in reactive microglia and nuclear factor-κB (NF-κB) signaling activated in the prefrontal cortex above the expanded ventricles. Most interestingly, Hsp60 induction preceded to TNF-α mediated neuroinflammation accompanied by an increase of UPRmt expression.
Conclusions: TNF-α and UPRmt were activated in KIHMM after induction of Hsp60, it provides a new sight for the pathogenic target of hydrocephalus.

Keywords: Hydrocephalus, Neuroinflammation, TNF-α, Unfolded protein responses

P20-02-02

Peptide hormone A is a mediator of leptin signaling in hypothalamus to increase POMC expression and α-MSH content

Yunseon Jang[1,2], Xianshu Ju[1,3,4], Min Joung Lee[1,2], Jianchen Cui[1,3,4], Jiebo Zhu[1,2], Eunji Namgung[1,2], Changjun Seo[1,2], Hyoeun Kang[1,2], Da Hyun Go[1,2], Yu Lim Lee[1,3,4], Min Jeong Ryu[1,5], Woosuk Chung[1,3,4], Hyeongseok Kim[1,2], Gi Ryang Kweon[1,2,5], Jun Young Heo[1,2,3]
[1]Department of Biochemistry, Chungnam National University School of Medicine, Daejeon, [2]Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon, [3]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, [5]Research Institute for Medical Science, Chungnam National University School of Medicine, Daejeon, Korea

Purpose: Hypothalamic regulation of appetite governs whole-body energy balance. Satiety is regulated by endocrine factors, including leptin, and im-

paired leptin induction causes obesity. Peptide hormone A (PHA) promotes energy expenditure in the periphery, and systemic reconstitution of PHA antagonizes obesity. However, whether hypothalamic PHA plays a role in controlling food intake remains unknown. Therefore, we analyzed anorectic effect of PHA via leptin signaling.
Methods: To verify anorectic effect of PHA on obesity, we performed cereboventricular injection of recombinant PHA to normal and obese C57BL/6 mice fed with high-fat diet (HFD). Then, we examined alteration of food intake, body weight, anorectic neuronal expression and α-melanocyte-stimulating hormone (α-MSH) content in hypothalamus.
Results: Here, we demonstrated that PHA is expressed in proopiomelanocortin (POMC)-positive neurons located in the arcuate nucleus (ARC) of the hypothalamus. PHA expression was stimulated by leptin-induced STAT3 phosphorylation. Notably, intracerebroventricular injection of PHA significantly reduced food intake by stimulating phosphorylation of CREB in the POMC and increasing α-MSH content in the hypothalamus. We also found that hypothalamic injection of PHA significantly suppressed food intake and decreased body weight in high-fat-diet-induced obese mice, which exhibit leptin insensitivity.
Conclusions: We announce that hypothalamic PHA provokes the anorectic α-MSH production and mediates leptin signaling to prevent obesity.

Keywords: Hypothalamus, Peptide hormone A, Appetite, Leptin, POMC

P20-02-03

Aggravation of levodopa-induced dyskinesia by injection of mutant α-synuclein in MPTP induced mice

Eunji Namgung[1,2,3], Yunseon Jang[1,2,3], Min Joung Lee[1,2,3], Xianshu Ju[1,2,3], Jianchen Cui[1,2,3], Yu Lim Lee[1,2,3], Jeibo Zhu[1,2,3], Dahyun Go[1,2,3], Chang Jun Seo[1,2,3], Woosuk Chung[4], Hyung seock Kim[1,2,3], Jun Young Heo[1,2,3]

[1]Department of Biochemistry, Chungnam National University School of Medicine, Daejeon, [2]Department of Medical Science, Chungnam National University School of Medicine, Daejeon, [3]Infection Control Convergence Research Center, Chungnam National University School of Medicine, Daejeon, [4]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Daejeon, Korea

Purpose: Levodopa (LD) is the primary therapy for Parkinson's disease (PD) to improve PD behavioral symptoms. However, long-term (more than 5 years) LD treatment can cause dyskinesia in more than 80% of PD patients. Levodopa-induced dyskinesia (LID) can be treated with deep brain stimulation (DBS), but because there is a risk of bleeding and the infection involves surgery, the development of drugs to relieve LID symptoms is essential. For preclinical purposes, the development of an animal model that reproduces the symptoms of LID patients should be preceded, but the LID models currently mainly used are neurotoxin (MPTP and 6-OHDA) induced PD animal models. Therefore, an LID animal model for the genetic causative factor of PD should be developed.
Methods: To develop the LID animal model based on the causative factor of PD, we administrated Levo/carbi dopa until appearance of dyskinetic movement after mutant alpha synuclein viral injection to SN area.
Results: We established a new combined LID animal model by confirming more severe abnormal involuntary movement scale (AIMS) than only α-syn or MPTP treated model.
Conclusions: This result will provide an important experimental platform that serves for clinical trial as animal models that most closely recapitulate patients with LID symptoms.

Keywords: Levodopa-induced dyskinesia, Parkinson's disease, α-synuclein, MPTP

P20-02-04

Repetitive injection of corticosterone to mother rats during pregnancy induces memory impairment via dysregulating BDNF signaling and NMDA-mediated responses in postnatal rats

Hye-Ji Kim, Sang-Chan Jeon, Eun-A Ko, Sung-Cherl Jung
Department of Physiology, School of Medicine, Jeju Natl'. University, Jeju, Korea

Purpose: The high level of blood cortisol is the key factor to identify major depressive disorder (MDD) that is mediated with the dysregulation of brain-derived neurotrophic factor (BNDF) signaling in mammalian brains. However, it is not well known if and how the elevation of cortisol level during prenatal period affects brain functions and induces psychiatric disorders after birth.
Methods: In the present study, we constantly elevated the cortisol level during the prenatal period by an injection of corticosterone (20 mg/kg) per day to maternal rats for 21 consecutive pregnant days until delivery. This procedure significantly elevated cortisol level in both maternal and postnatal pups (CortiPups). Before Morris water maze tests to determine memory functions of pups at postnatal 21st day, we electrophysiologically tested synaptic functions in hippocampal CA1 neurons of CortiPups (postnatal 14~21 days), and then compared to normal pups (NorPups, saline injected).
Results: In results, neuronal excitability was significantly increased and the synaptic function to generate excitatory postsynaptic potentials (EPSCs) was remarkably suppressed in CA1 neurons of CortiPups. Furthermore, the impairment of long-term potentiation (LTP) and behavioral memory deficit were observed in CortiPups. This memory impairment was attributed to the upregulation of synaptic NR2B responses and downregulation of BNDF expression that was mediated by the activation of EF2K and GSK3 signaling cascades.
Conclusions: These results provide direct evidence that maternal cortisol unbalances and neuroendocrine dysregulation during pregnancy may directly impair synaptic development and memory function of pups. This indicates that cortisol may be a key factor to induce learning and memory dysfunctions and behavioral abnormalities that are frequently reported in autism and ADHD.

Keywords: Major depressive disorder, Cortisol, Long-term potentiation, Learning and memory, BDNF, GSK3

P20-02-05

Different action of antioxidant between old and young hippocampus following oxidative injury

KyungHee Lee[1], UnJeng Kim[2], Bae Hwan Lee[2,3]

[1]Division of Health Science, Department of Dental Hygiene, Dongseo University, [2]Department of Physiology, College of Medicine, Yonsei University, [3]Brain Korea 21 Project for Medical Science, College of Medicine, Yonsei University, Korea

Purpose: Ascorbate was known as endogenous antioxidant for neuronal protection in oxidative injury but age-relatively decreased the extracellular concentration and uptake in the brain. Whether chronic treatment of ascorbic acid (AA) is accompanied by age-related neuronal antioxidant activity following oxidative stress are remains unclear. The goal of this study was to investigate the susceptibility of rat hippocampal neurons to oxidative injury following acute and chronic AA administration.
Methods: Using organotypic hippocampal slice cultures (OHSCs) with 6~8 postnatal rats, experimental groups were divided by culture duration (3 w or 9 w). To observe acute and/or chronic effect of AA treatment in aging, OHSCs were once treated with 500 μM AA in cell medium for 24 after the KA treatment. Neuronal cell death was measured by propidium iodide (PI) uptake. Western blot and optical imaging with voltage sensitive dye were performed to observe the antioxidant signals and to examine synaptic changes and/or strength in the OHSCs.
Results: Neuronal cell death was reduced in both 3 w+KA+AA group and the 9 w-daily+KA+AA group, in which chronic AA treatment was applied

for 6 w, compared to the 9 w+KA+AA group. Superoxide dismutase (SOD) expression was significantly increased in older cultures after chronic AA treatment (group 9 w-daily) compared to acute AA treatment (group 9 w). Through optical imaging, the 9 w+KA+AA group showed delayed latencies and decreased signal activity compared to the 3 w+KA+AA group, but 9 w-daily+KA+AA group showed shorter latencies and increased signal activity.

Conclusions: These results suggest that the persistent of antioxidant system by chronic exogenous AA treatment in aging may conserve the action of redox capacity to protect hippocampal neurons against oxidative stress, so that the survived neurons is likely to cause an action of neuronal function and delay the onset of neurodegeneration.

Keywords: Ascorbic acid, Antioxidant, Aging, Organotypic hippocampal slice culture, Neuroprotection, Kainic acid

P20-02-06

Long-Lasting and additive analgesic effects of combined treatment of bee venom acupuncture and venlafaxine on paclitaxel-induced allodynia in mice

Daxian Li[1], Geehoon Chung[2], Seunghui Woo[1], Nari Kim[1], Sun Kwang Kim[1,2]

[1]Department of Science in Korean Medicine, Graduate School, Kyung Hee University, Seoul, [2]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, Korea

Purpose: Paclitaxel, a primary chemotherapeutic agent used to treat numerous solid malignancies, is commonly associated with debilitating peripheral neuropathy. However, a satisfactory gold-standard monotherapy for this neuropathic pain is not currently available. A combination strategy of two or more medications with different properties may achieve more beneficial ects than monotherapy. Thus, we investigated the analgesic effecacies and spinal mechanisms of the combination strategy, including bee venom acupuncture (BVA) and venlafaxine (VLX) against paclitaxel-induced allodynia in mice.

Methods: Four intraperitoneal infusions of paclitaxel on alternating days (2 mg/kg/day) induced cold and mechanical allodynia for at least 1 week as assessed using acetone and the von Frey hair test, respectively.

Results: Co-treatment of BVA (1.0 mg/kg, s.c., ST36) with VLX (40 mg/kg, i.p.) at the medium dose produced a longer-lasting and additive effect than each monotherapy at the highest dose (BVA, 2.5 mg/kg; VLX, 60 mg/kg). Spinal pre-administration of idazoxan (α2-adrenergic receptor antagonist,10 μg), methysergide (mixed 5-HT1/5-HT2 receptor antagonist, 10 μg), or MDL-72222 (5-HT3 receptor antagonist, 10 μg) abolished this analgesia.

Conclusions: These results suggest that the combination therapy with BVA and VLX produces long-lasting and additive analgesic effects on paclitaxel-induced allodynia, via the spinal noradrenergic and serotonergic mechanism, providing a promising clinical strategy.

Keywords: Bee venom acupuncture, Venlafaxine, Combination therapy, Paclitaxel, Allodynia

P20-02-07

The impacts of early social experience on social recognition and its related neural circuits

Gaeun Park[1,2], Changhyeon Ryu[1,2], Soobin Kim[1,2], Yong-Seok Lee[1,2,3], Sang Jeong Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [3]Neuroscience Research Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: It has been well known that social experience in early life causes prolonged deficit in cognitive functions. Previously, social isolation in the postnatal critical period showed altered various medial prefrontal cortical (mPFC) functions including social preference in mice. However, it is still elusive how other aspects of social functions and which downstream targets for the mPFC are affected by the insubstantial social experience in young age.

Methods: In this study, we observed that single isolated mice in their postnatal developmental period showing an impairment in social behavior which was not rescued by following re-socialization period.

Results: Interestingly, we found that the functional connectivity between mPFC and the shell part of nucleus accumbens (NAc shell) is altered after the social isolation. Immunohistochemistry targeting c-fos after a series of social behavior tests showed altered mPFC to NAcSh projecting neuronal activity when encountered by different conspecifics. Metabotropic inhibition of mPFC to NAc shell projecting neuron showed a significant impairment in the social behavior while activation of such neuron in single isolated mice exhibited a rescued social behavior.

Conclusions: This result suggests that early social experience of mice can have a lifelong-lasting impact on their social behavior, which phenomenon critically involved by mPFC-NAc shell circuit.

Keywords: Single isolation, Social behavior, Social memory, Nucleus accumbens

P20-03-01

Higher expression of KCNK10 (TREK-2) K^+ channels and their functional upregulation by lipopolysaccharide treatment in mouse peritoneal B1a cells

Si Won Choi[1], Joohan Woo[2], Kyung Sun Park[3], Juyeon Ko[1], Young Keul Jeon[1], Seong Woo Choi[1,4], Hae Young Yoo[5], Inseong Kho[6], Tae Jin Kim[6], Sung Joon Kim[1,4]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology and Ion Channel Disease Research Center, Dongguk University College of Medicine, Seoul, [3]Wide River Institute of Immunology, Seoul National University College of Medicine, Hongcheon, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, [5]Department of Nursing, Chung-Ang University, Seoul, [6]Department of Immunology, Sungkyunkwan University School of Medicine, Suwon, Korea

Purpose: Innate-like $CD5^+$ B1a cells localized in serous cavities are activated by innate stimuli, such as lipopolysaccharide (LPS), leading to T cell-independent antibody responses. Although ion channels play crucial roles in the homeostasis and activation of immune cells, electrophysiological properties of B1a cells have not been investigated till date. Previously, in the mouse B cell lymphoma cells we found that the voltage-independent two-pore-domain potassium (K2P) channels generate a negative membrane potential and drive Ca^{2+} influx. Here, we newly compared the expression and activities of K2P channels in mouse splenic follicular B (FoB), marginal zone B (MZB), and peritoneal B1a cells.

Methods: Primary B cell isolation and fluorescence activated cell sorting (FACS), Next Generation Sequencing, RT-PCR, Real-time PCR (qPCR), Electrophysiology, Fura-2 fluorimetry, Gene silencing with small interfering RNA (siRNA).

Results: Next-generation sequencing analysis showed higher levels of transcripts for TREK-2 and TWIK-2 in B1a cells than in FoB or MZB cells. Electrophysiological analysis, using patch clamp technique, revealed higher activity of TREK-2 with large unitary conductance in B1a than in FoB or MZB cells. TREK-2 activity was further increased by LPS treatment (>2 h), which was more prominent in B1a than MZB or FoB cells. The cytosolic Ca^{2+} concentration of B cells was decreased by high-K^+-induced depolarization (%), suggesting the basal Ca^{2+} influx to be driven by negative membrane potential. The LPS treatment significantly increased the high-K^+-induced depolarization (%) in B1a, though not in FoB and MZB cells.

Conclusions: Our study was the first to compare the K2P channels in mouse primary B cell subsets, elucidating the functional upregulation of TREK-2 and augmentation of Ca^{2+} influx by the stimulation of Toll-like receptor 4 using LPS in B1a cells.

Keywords: B1a, FoB, MZB, TREK-2, Lipopolysaccharide, Ca^{2+} influx

P20-03-02

Inter-spike mitochondrial Ca^{2+} release enhances high frequency synaptic transmission

Che Ho Yang[1], Kyu-Hee Lee[1,3], Won-Kyung Ho[1,2], Suk-Ho Lee[1,2]

[1]Department of Physiology, Seoul National University Medical Research Center, Cell Physiology Lab., Seoul National University College of Medicine and Neuroscience Research Institute, Seoul, [2]Department of Brain and Cognitive Science, Seoul National University, [3]Center for Cognition and Sociality, Institute for Basic Science, Daejeon, Korea

Purpose: Ca^{2+} sequestered in mitochondria is released back to cytosol primarily through mitochondrial Na/Ca exchanger (mNCX) in excitable cells. It is well established that the fast decay phase of a HFS-induced presynaptic Ca^{2+} transient (CaT) is followed by mitochondrial Ca^{2+} release (MCR) via mNCX, leading to temporary elevation of resting $[Ca^{2+}]$ and post-tetanic potentiation. It has not been previously examined, however, whether MCR via mNCX regulates residual calcium at inter-spike intervals during HFS and thus short-term plasticity (STP) of synaptic transmission.
Methods: Transverse brainstem or hippocampal slices from 7 to 20 days old (P7-9, immature pre-hearing onset; P14-20, mature post-hearing onset) Sprague Dawley rats or 4 to 8 weeks old C57BL/6 wild-type / calbindin knock-out male mice were prepared, respectively. Whole-cell patch-clamp recordings were made from calyx of Held or hippocampal mossy fiber to CA3 pyramidal cell (MF-CA3) synapses. Fiber stimulation, paired recordings, presynaptic cytosolic, mitochondrial, and confocal line scan methods used for Ca^{2+} imagings. Simulation of 3D buffered diffusion of Ca^{2+}. Data are presented as mean±SD.
Results: 2 μM TPP+ (mNCX blocker) reduced STF and EPSCss during HFS at mature calyx synapses under 1.2 mM $[Ca^{2+}]_o$, but not at immature calyx or at 2 mM $[Ca^{2+}]_o$. Inhibitory effects of TPP+ were stronger at synapses with morphologically complex calyces harbouring many swellings and at 32 degC than at simple calyx synapses and at room temperature. Mitochondrial $[Ca^{2+}]$ during HFS was increased by TPP^+ at mature calyces under 1.2 mM $[Ca^{2+}]_o$, and further enhanced at 32°C, but not under 2 mM or at immature calyces. The intra-train MCR enhanced vesicular release probability without altering global presynaptic $[Ca^{2+}]$.
Conclusions: MCR during HFS elevates local $[Ca^{2+}]$ near synaptic sites at interspike intervals to enhance STF and to support stable synaptic transmission under physiological $[Ca^{2+}]_o$.

Keywords: Presynaptic, Mitochondria, Calyx of held, Short-term facilitation, Residual calcium

P20-03-03

Effects of PIP2 and KCNQ activators on the activity of novel KCNQ4 variant channels

Il Soon Choi[1], Hyun Been Choi[1], Heon Yung Gee[2], Sang-Yeon Lee[3], Byung Yoon Choi[3], Tong Mook Kang[1]

[1]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, [2]Department of Pharmacology, Brain Korea 21 PLUS Project for Medical Sciences, Yonsei University College of Medicine, Seoul, [3]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea

Purpose: KCNQ4 (K_v7.4) encodes a voltage-gated potassium channel protein and is the causative gene for DFNA2 hearing loss when mutated. Herein, we tested mechanism-based pharmacological applications to functionally rescue impaired KCNQ4 channels related to novel DFNA2 variants.
Methods: Novel KCNQ4 variants (two pore-mutants and a C-terminus-mutant) were identified, and they were expressed in HEK293T cells either in homomeric or heteromeric setting. The activating effects of the increased PIP2 concentration and KCNQ activators (retigabine, zinc pyrithione, and ML213) on the variant channels were monitored. By whole-cell patch-clamp recording, the increase of channel conductance and negative shift of the half-activation voltage (V0.5) by the activators were compared under the expression of PIP2-generating enzyme, PIP5K.
Results: Both in homomeric and heteromeric setting, the variants in the pore region were non-rescuable by KCNQ activators, PIP5K expression, or a combination of both. By contrast, forced heteromeric state of channel (concatemer) of the C-terminal mutant was partially recovered by KCNQ4 openers, PIP5K, or a combination of both.
Conclusions: Our results shed light on the potential for customized pharmacological approaches for KCNQ4 variants in clinical practice.

Keywords: KCNQ4, Variants, Hearing loss, PIP2, KCNQ activator

P20-03-04

Non-selective cation currents mediated by Cx43 hemichannel-P2X4 receptor signaling pathway in rat atrial myocytes under shear stress

Sun-Hee Woo, Min-Jeong Son, Joon-Chul Kim, Anh TV Vu

Pathophysiology Lab., Chugnam National University, College of Pharmacy, Daejeon, Korea

Purpose: Cardiac myocytes are exposed to shear stress under physiological conditions and hemodynamic disturbances. Shear stress has been thought to trigger two distinct global Ca^{2+} waves in atrial myocytes through autocrine activation of P2X4 receptor (P2X4R) and P2Y1-receptor (P2Y1R) via connexin 43 (Cx43)-mediated ATP release. In this study, we examined whether such shear stress-triggered Cx43-ATP-P2 receptor signaling generates depolarizing currents in atrial myocytes.
Methods: Whole-cell patch clamp and dye-flux assays were used with genetic and pharmacological interventions in rat atrial myocytes and HL-1 cells.
Results: We found shear stress activated a non-selective inward cation (Cs^+) current ($I_{S,Cs}$) at resting potential which was eliminated by Cx43 blockade using siRNA, antibodies, carbenoxolone or La^{3+}. $I_{S,Cs}$ was enhanced by removal of external Ca^{2+}, whereas internal Ca^{2+} buffering suppressed $I_{S,Cs}$. Hemichannel-mediated dye (calcein red-orange) flux was induced by shear stress. This response was augmented by removing external Ca^{2+} but was not affected by pannexin blocker (probenecid). The shear-activated current was significantly reduced when Cs^+ was replaced with $NMDG^+$, and this was enhanced by quinine and suppressed by La^{3+}. P2X4R antagonists (5-BDBD and PSB-12054), P2XR-blocker (iso-PPADS) and anti-P2X4R antibodies each blocked $I_{S,Cs}$ by approximately 50%. About 25% of $I_{S,Cs}$ was suppressed by P2Y1R inhibitor (MRS2179) and/or transient receptor potential melastatin 4 blocker (9-phenanthrol). P2X4R, but not P2X5R, was co-localized with Cx43 at the cell ends. Our data suggest that shear stress triggers Cx43 hemichannels to gate P2X4Rs in their vicinity. This signaling may play an important role in membrane depolarization in resting atrial myocytes under shear stress.
Conclusions: Our data suggest that shear stress triggers Cx43 hemichannels to gate P2X4Rs in their vicinity. This signaling may play an important role in membrane depolarization in resting atrial myocytes under shear stress.

Keywords: Shear stress, Atrial myocytes, Non-selective cation current, P2X4 receptor, Cx43 hemichannel

P20-03-05

Temperature-dependent facilitation of the voltage-dependent activation of calcium homeostasis modulator 1 ion channel

Young Keul Jeon[1], Si Won Choi[1], Jae Won Kwon[1], Seong Woo Choi[1], Sang Jeong Kim[1,3,4], Sung Joon Kim[1,3,4]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology, Dongguk University College of Medicine, Gyeongju, [3]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: Calcium homeostasis modulator 1 (hCALHM1, calhm1) is a voltage-gated channel showing unselective permeability to various ions, including Ca^{2+} and ATP^{4-}. Its activation is exceedingly slow and requires long periods of strong depolarization, hampering its electrophysiological study.
Methods: Here, we examined whether physiological temperature facilitates the activation of the calhm1 current (Icalhm1).
Results: In a whole-cell patch clamp experiment with HEK293 cells expressing calhm1, sustained depolarizations (>0 mV, 1-5 s) induced outwardly rectifying Icalhm1. The amplitude and activation speed of Icalhm1 was markedly increased when the bath temperature was raised from 27°C to 37°C and 42°C, which also confirmed in the inside-out patch configuration. A similar thermosensitivity was observed in HEK293 cells overexpressing calhm2 and hCALHMs. The half-activation voltage (V1/2) of Icalhm1 was shifted to the left by raising the temperature. Previous studies have found that the voltage-dependence of calhm1 is affected by extracellular Ca^{2+} ($[Ca^{2+}]_e$). A similar temperature-dependent shift of V1/2 was observed at 2 or 0.5 mM $[Ca^{2+}]_e$, indicating separate regulatory mechanisms. Recent structural studies have suggested the critical role of the cytoplasmic C-terminal in the oligomerization of calhm1. Consistently, an extensive deletion of Ct up to the first helix (CH1) fully impaired calhm1 activity. However, incremental deletions up to the second C-helix (CH2) affected neither voltage-dependent activation nor thermosensitivity in Icalhm1.
Conclusions: Since calhm1 is highly expressed in the brain and gustatory cells, the prominent thermosensitivity of CALHM should be taken into account when interpreting its physiological roles.

Keywords: Calcium homeostasis modulator 1 (calhm1), Thermosensitivity, Ion channel, Voltage-dependent activation, Patch clamp study

P20-03-06

Identification of extracellular disulfide bonds essential for proper folding and function of calcium homemostasis modulator channel

Young Keul Jeon[1,2], Jae Won Kwon[1,2], Sung Joon Kim[1,2,3]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [3]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: Calcium homeostasis modulator 1 (CALHM1) was recently identified as a physiologically important plasma membrane ion channel, showing a slow activation and poor ionic selectivity. Amino acid sequence alignment of CALHM family revealed four absolutely conserved cysteine residues in the extracellular domain, suggesting possible disulfide bonds. Recent studies of CALHM1 using cryo-EM suggested that there are two intramolecular bindings including C42-C127 and C44-C161.
Methods: Here, we investigated the role of disulfide bonds using electrophysiological and molecular technique.
Results: Replacement of these cysteine residues in the CALHM1 with either serine or alanine abolished CALHM1 current (ICALHM1). We also confirmed that the mutated CALHM1 proteins were not trafficked to the membrane, using confocal imaging, suggesting the disulfide bonds are essential for proper folding and membrane trafficking. As disulfide bond also provides a structural stability of protein and regulates function of ion channel, we recorded ICALHM1 with acute treatment of reducing agent (tris(2-carboxyethyl)phosphine, TCEP). Surprisingly, the amplitude of ICALHM1 was increased with 2 mM TCEP, and the speed of activation was significantly increased. The half-activation voltage (V1/2) of ICALHM1 was also shifted to the left by 2 mM TCEP.
Conclusions: Taken together, these results suggest that intramolecular two disulfide bonds exist between four cysteine residues and these cross-links might be required for proper channel folding. Furthermore, the reducing of disulfide bonds modified the electrophysiological properties in properly expressed channel.

Keywords: Calcium homemostasis modulator channel, Disulfide bonds, Protein folding, Ion channel, Trafficking

P20-03-07

The inhibitory effect of the tricyclic antidepressant imipramine on voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells

Jin Ryeol An, Won Sun Park

Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Imipramine, a tricyclic antidepressant, is used in the treatment of depressive disorders. However, the effect of imipramine on vascular ion channels is unclear.
Methods: We examined the effect of imipramine on voltage-dependent K^+ (K_v) channels in freshly isolated rabbit coronary arterial smooth muscle cells using a patch-clamp technique.
Results: K_v channels were inhibited by imipramine in a concentration-dependent manner, with an IC_{50} value of 5.55 ± 1.24 μM and a Hill coefficient of 0.73 ± 0.1. Application of imipramine shifted the steady-state activation curve in the positive direction, indicating that imipramine-induced inhibition of K_v channels was mediated by influencing the voltage sensors of the channels. The recovery time constants from K_v-channel inactivation were increased in the presence of imipramine. Furthermore, application of train pulses (of 1 or 2 Hz) progressively augmented the imipramine-induced inhibition of K_v channels, suggesting that the inhibitory effect of imipramine is use (state)-dependent. The magnitude of K_v current inhibition by imipramine was similar during the first, second, and third depolarizing pulses. These results indicate that imipramine-induced inhibition of K_v channels mainly occurs in the closed state. The imipramine-mediated inhibition of K_v channels was associated with the $K_v1.5$ channel, not the $K_v2.1$ or K_v7 channel. Inhibition of K_v channels by imipramine caused vasoconstriction.
Conclusions: We conclude that imipramine inhibits vascular K_v channels in a concentration- and use- (closed state)-dependent manner by changing their gating properties regardless of its own function.

Keywords: Imipramine, Voltage-dependent K^+ channel, Coronary arerial smooth muscle cells

P20-03-08

Dual regulatory effects of $PI(4,5)P_2$ on TREK-2 K^+ channel through antagonizing interaction between the alkaline residues (K^{330} and $R^{355-357}$) in the cytosolic C-terminal helix

Sung Eun Kim[1], Myoung-Hwan Kim[2], Joohan Woo[3], Sung Joon Kim[4]

[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Physiology, Seoul National University College of Medicine, [3]Department of Physiology, Dongguk University College of Medicine, [4]Department of Physiology, Seoul National University College of Medicine, Korea

Purpose: To further elucidate the role of putative bent conformation at G^{334} through various mutation forms.

Methods: cDNAs of TREK-2 and point mutations were transfected into HEK293T cells using a Turbofect transfection reagent. Using inside-out patch clamp technique.
Results: The combined mutation of G^{334} with K^{330} or with $R^{355-357}$ induces changes in the basal activity and the responses to the acidic pHi and ATP, which were the same as the changed properties of $K^{330}A$ and $R^{355-357}A$.
Then to find a suspicious hidden role of more distal consecutive R377-9 in the Ct of hTREK-2. Our present study indicates that R^{377-9} may not play a significant role even after neutralizing the R^{377-9}.
Conclusions: The present study of hTREK-2 and its various mutants could provide further, although indirect, evidence supporting our model of the dual regulation by PIP_2 via closely located cationic residues. The tug-of-war or see-saw model of Ct might be helpful to understand the complex responses of TREK channels since the allosteric interaction between proximal Ct and the fourth transmembrane segment is critical for the gating of TREK channels.

Keywords: K2P, TREK-2, C-terminal, Phosphatidylinositol 4,5- bisphosphate, Alkaine residues

P20-03-09

Electrophysiological assessment of molecular interaction between TRPC4 channel and Exo70

Christine Haewon Park, Insuk So
Department of Physiology, Seoul National University College of Medicine, Korea

Purpose: TRPC4 (Canonical transient receptor potential 4) channels are nonselective, calcium-permeable cation channels that are implicated in regulation of various physiological processes in mammalian cells. Although many mechanisms that regulate its activity have been proposed, the trafficking process that controls the expression of these channels at plasma membrane remain elusive. Exo70 is a crucial component of the exocyst complex and its function is to deliver and tether different types of vesicles to the membrane before SNARE-mediated membrane fusion.
Methods: From our yeast-two-hybrid screen, Exo70 has been identified as a candidate molecule interacting with the TRPC4 channel, and its regulatory role on the channel has been examined using whole-cell patch clamp method. Various loss-of-function and gain-of-function mutants have been generated using site-directed mutagenesis, and these mutants were co-expressed with TRPC4 channels to assess change in channel activity in HEK293 cells.
Results: However, the electrophysiology results show that none of these mutants had a significant effect on TRPC4 currents. TRPC4 current amplitudes showed no change when co-expressed with wild type or mutant Exo70. Moreover, the expression patterns of Exo70 and TRPC4 channels examined by fluorescence microscopy on a cellular scale showed to be opposite from each other, further questioning the interaction between the two molecules.
Conclusions: Our proposed mechanism relating Exo70-mediated trafficking process and TRPC4 channel expression and activity thus may have several missing links in between that need further explanation.

Keywords: TRPC4, Exo70, Membrane trafficking

P20-03-10

Cell cycle-associated SK4 activity in head and neck squamous cell carcinoma cells: Role in cell proliferation and its potential clinical applicability

Ji Young Kim[1], Young Keul Jeon[1], Joo Hyun Nam[2,3], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Physiology, Dongguk University College of Medicine, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Korea

Purpose: Accumulating data suggest that potassium channels are important in cancer cell cycle progression, proliferation, and metastasis. In our previous study, we determined that small conductance calcium-activated potassium channel 4 (SK4) is highly expressed in head and neck squamous cell carcinoma (HNSCC). Till date, the physiological role of the SK4 channel in controlling the HNSCC cell cycle and proliferation remains elusive; nevertheless, it can provide important information related to the development of anti-cancer therapy by targeting SK4.
Methods: In this study, using electrophysiology, biochemistry, and calcium imaging, we revealed a correlation between cell cycle and functional expression of SK4 in the HNSCC cell line (SNU-1076).
Results: SK4 current (ISK4) density increased in cells pharmacologically synchronized in G_0/G_1 phase compared with those in G_2/M phase. The change in ISK4 by cell cycle was also related to the cell volume. Potassium channels generally regulate resting membrane potential (RMP) and intracellular calcium concentration ($[Ca^{2+}]_i$). In the cells synchronized in G_0/G_1 phase, basal $[Ca^{2+}]_i$ was higher than that in G_2/M cells and also showed a oscillating pattern of RMP. Blocking SK4 channels with a specific blocker, TRAM-34, induced depolarization of RMP in G_0/G_1 cells. The effects of SK4 activity on proliferation rate were investigated employing an SK4 agonist and an antagonist in the HNSCC cell line. Blocking SK4 with TRAM-34 dose-dependently inhibited cell proliferation whereas using an SK4 agonist, 1-EBIO, accelerated cell proliferation rate. Interestingly, the combined administration of cisplatin with K^+ channel modulators showed opposite effects on cell proliferation in the HNSCC cell line. Furthermore, using the cBioportal Cancer Genomics database, we found a tremendous up-regulation of SK4 mRNA in ~10% of HNSCC patients, which was associated with a poor survival rate among HNSCC patients.
Conclusions: The modulation of SK4 channel activity may useful as a therapeutic option for patients with HNSCC with upregulated SK4 channel expression

Keywords: SK4, Proliferation, Head and neck squamous cell carcinoma, Cell cycle, Intracellular calcium concentration

P20-03-11

NALCN channel regulates burst intensities of dopaminergic neurons in the substantia nigra pars compacta

Suyun Hahn, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, Korea

Purpose: Dopaminergic (DA) pacemaker neurons in the substantia nigra pars compacta (SNc) fire high-frequency bursts in response to unexpected rewards or sensory stimuli associated with primary rewards, resulting in a phasic increase of DA in target brain areas. Burst firing is elicited by glutamatergic stimulation and critically depends on activation of NMDA receptors. Burst firing in most central neurons occurs with plateau potentials but burst activities of DA neurons do not accompany plateau potentials. Recently, sodium leak channel (NALCN) is known to control the resting membrane potential (RMP) and neuronal excitability in various neurons. However, despite the potential importance of persistent Na^+ leak currents in action potential generation and excitability in many neurons, little is known about the roles of NALCN in burst activities of DA neurons. Therefore, we have investigated whether NALCN channels contribute to burst firings of DA neurons.
Results: We observed that inhibition of NALCN channels suppresses background Na^+ leak current and hyperpolarizes membrane potential in the DA neurons that express NALCN endogenously. NALCN channel blockade completely inhibited spontaneous firings which can be revived by injecting a leak-like current. When caged glutamate was locally uncaged at dendritic locations of the DA neurons, burst firing was evoked. NALCN channel inhibition attenuated the glutamate-evoked burst firing and concurrently produced a plateau potential which is normally not seen in DA neurons. In addition, after NALCN channel inhibition, although spontaneous firing was reinstated by injecting a leak-like current, glutamate stimulation attenuated or often failed to evoke burst firings.
Conclusions: These results suggest that NALCN channels play an important role in generation and regulation of burst discharges in SNc DA neurons.

Keywords: Burst firing, Dopaminergic neuron, NALCN channel, Plateau potential

P20-03-12

The role of lysosomal Ca^{2+} channels in direct cell death *via* obinutuzumab binding

Jeong Ryeol Kim, Narae Jin, Sun Hyung Kwon, Donghyuk Lee, Joo Young Kim

Department of Pharmacology and BK21 Plus, Yonsei University College of Medicine, Korea

Purpose: Obinutuzumab is humanized anti-CD20 antibody used to non-hodgkin's lymphoma and chronic lymphocytic leukemia patient to deplete malignant B cells. Obinutuzumab induced cell death via binding and lysosomal rupture is known to be essential for this cell death pathway, but the molecular mechanism of how lysosomal rupture occurs is not known. In this study, we firstly elucidated early phenomena associated with the change of lysosomes after obinutuzumab binding, and investigated the role of TRPML2, a lysosomal Ca^{2+} channel, in this direct cell death process.
Methods: TRPML2, a lysosomal Ca^{2+} channel which is important in lysosomal fusion, fission, and trafficking is mainly expressed in Ramos cell. We measured the change of TRPML2 mediated lysosomal Ca^{2+} release by obinutuzumab binding using GCaMP fused with TRPML2.
Results: We found that obinutuzumab binding induced lysosome swellings before rupture. Not only the swelling and rupture, but also the direct cell death by obinutuzumab was significantly reduced by BAPTA-AM, the endosomal fusion inhibitor. The ML-SA1,TRPML2 agonist, induced Ca^{2+} release through TRPML2 was inhibited after 5 minutes after obinutuzumab binding and this phenomenon was also proved by measuring the cytosolic Ca^{2+} increase using Fluo-4 and measuring the lysosomal Ca^{2+} decrease using Oregon green BAPTA-dextran. Gold labeled obinutuzumab was found in vesicular structure in EM 5 minutes after obinutuzumab binding, but rituximab was remained to the surface. Interestingly, inhibition of ML-SA1 induced TRPML2 Ca^{2+} release by obinutuzumab was abolished by filipin, an inhibitor of caveolin dependent endocytosis. Furthermore, treatment of sphingomyelinase, an inhibitor of TRPML2, reversed the inhibition of ML-SA1 induced TRPML2 Ca^{2+} release by obinutuzumab.
Conclusions: In this study, we suggest that obinutuzumab induced lysosome swelling is concomitant with TRPML2 inhibition. We claim that inhibition of TRPML2 function by obinutuzumab binding was mediated by caveolin mediated endocytosis which bring sphingomyelin from the plasma membrane through obinutuzumab binding induced endocytosis.

Keywords: Obinutuzumab, Cell death by antibody binding, Lysosome rupture, TRPML2, Endocytosis

P20-03-13

Regulation of transient receptor potential canonical 4 activity by phospholipase C-δ1

Juyeon Ko, Jongyun Myeong, Misun Kwak, Insuk So

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

Purpose: Transient receptor potential canonical 4, 5 channels are non-selective calcium-permeable cation channels that maintained by phosphoinositol 4,5-bispphosphate ($PI(4,5)P_2$) and the channel inactivation occurs due to its hydrolysis. Phospholipase C (PLC) is an enzyme that cleaves phospholipids and the PLCδ subtype is the most calcium-sensitive form among the isozymes that stimulated by physiological Ca^{2+} concentration. In this article, we set out to determine the interaction between TRPC and PLCδ, and to identify the regulation mechanism of TRPC4 channel by the Ca^{2+}, PLCδ, and $PI(4,5)P_2$ signaling cascade.
Methods: In order to identify the interaction between ion channel and PLCδ protein, we implied co-immunoprecipitation and Foster Resonance Energy Transfer imaging. We evaluated the activity of channels with electrophysiological recordings in HEK293 cells expressing TRPC channels.
Results: The TRPC4 directly interacts with PLCδ1 but TRPC5 not. The Ca^{2+} through opened channel in physiological intracellular calcium conditions promotes the activation of PLCδ1, which subsequently decreases PIP2 level. By comparison the TRPC4 activity with or without PLCδ1 using differently $[Ca^{2+}]i$ buffered solution, we demonstrated that PLCδ1 functions in conditions with physiological intracellular calcium concentration. The negative regulation effect of PLCδ1 on TRPC4 helps to elucidate the roles of each PIP2 binding residues whether they are in channel maintenance or inhibition of channel activity.
Conclusions: The TRPC4-bound PLCδ1 is activated by channel-mediated calcium increase, thereby reduces PIP2 level. This reduction in PIP2 level promotes reduction of TRPC4 currents. Resultantly, the TRPC4 current is tightly regulated by PLCδ1.

Keywords: Transient receptor potential channels, Phospholipase C, Calcium, Phosphoinositide

P20-03-14

The agonistic action of URO-K10 on $K_v7.4$ and 7.5 channels is attenuated by co-expression of KCNE4 ancillary subunit

Jung Lee[1], Christine Haewon Park[1], Insuk So[1], Jinsung Kim[1], Hana Kang[1], Juyeon Ko[1], Suhan Cho[1], JooHan Woo[3], Meree Chae[2], Sungwon Lee[2], Sung Joon Kim[1]

[1]Department of Physiology, Seoul National University of College of Medicine, [2]Department of Urology, Samsung Medical Center, [3]Department of Physiology, Dongguk University College of Medicine, Korea

Purpose: We examined the electrophysiological characteristics of overexpressed KCNQ4, 5, channels in Human embryonic kidney 293 (HEK293) cells with a recently developed KCNQ-specific agonist named URO-K10. As the ancillary subunit KNCE4 has been known to modulate the expression and activity of channels formed by KCNQ subunits, we also examined whether the interaction between KCNQ4, and KCNE4 alters the agonistic effect of URO-K10.
Methods: HEK293 cells were transfected with FuGENE 6 and proceeded for whole-cell and single-channel current recordings. Interaction between KCNQ4, 5 and KCNE4 were found through FRET and co-immunoprecipitation. Isometric tension of mesenteric vascular smooth muscle was also measured with a dual-wire multi-channel myograph system.
Results: URO-K10 not only increased the open probability of KCNQ4 channel but also increased slope conductance of the channel. The overall effect of the drug in whole-cell configuration was to increase maximal whole-cell conductance, to prolongate the activation process, and left-shift of the activation curve. The agonistic action of the drug, however, was highly attenuated by the co-expression of one of the beta ancillary subunits of KCNQ family, KCNE4. Strong in vitro interaction between KCNQ4, 5 and KCNE4 were found through FRET and co-immunoprecipitation. Although the expression levels of both KCNQ4 and KCNE4 are high in mesenteric arterial smooth muscle cells, we found that 1uM of the agonist was sufficient to almost completely relax phenylephrine-induced contraction of the muscle strip
Conclusions: Given that genuine purpose of URO-K10 was to elicit as large potassium current across the membrane as possible through KCNQ4, 5 channels, the co-existence of KCNE4 in the membrane could be one of the major obstacles. However, in our hands, 1uM URO-K10 almost completely relaxed phenylephrine-induced mesenteric artery vasoconstriction, and this assures similar dilative effect on corpus cavernosum where expression of both KCNQ4 and KCNE4 are as significant as mesenteric vascular smooth muscle cells

Keywords: KCNQ4, KCNQ5, KCNE4

P20-03-15

Properties of the chimeric TRPM4 channel as its trafficking marker

Eun Mi Hwang[2], Eun-Hye Byeon[1], Seung-Chan Kim[2], Dawon Kang[1], Dong Kun Lee[1], Jaehee Han[1], Seong-Geun Hong[1]

[1]Department of Physiology, Gyeongsang National University College of Medicine, Jinju, [2]Brain Science Institute, KIST, Seoul, Korea

Purpose: TRPM4 channels play not only as a nonselective cation channel but also as one of critical regulators in cellular functions, such as cell adhesions or proliferations. These functions have documented to be closely related to TRPM4 expression in the cell membrane. Despite the increasing demand to investigate the TRPM4 function, efficient tools to show the mechanism of membrane trafficking of TRPM4 are limited. Here we introduce a novel, genetically modified chimera as a TRPM4 trafficking marker sharing with its channel properties.
Methods: We generated the chimeric TRPM4 inserted hemagglutinin (TRPM4-HA) in the extracellular loop located between putative 5th and 6th transmembrane domains. GFP-conjugated TRPM4 (GFP-TRPM4) and GFP were applied as control and negative control, respectively. Analyses for trafficking and expression of TRPM4-HA were performed by patch experiments, immunolabeling, and Western blots in HEK 293 cells stably overexpressed with TRPM4-HA, GFP-TRPM4, or GFP alone.
Results: Western blot and immunocytochemical analyses showed that TRPM4-HA was localized to the cell membrane. Direct immunolabeling was performed without membrane permeablization process. In TRPM4-HA expressed HEK293 cells, the currents produced from whole-cell and inside-out patches were Ca^{2+}-sensitive and rapidly desensitized. 9-phenanthrol, a specific TRPM4 blocker, remarkably reduced the currents. The currents were abolished in the absence of sodium replaced by the impermeable cation NMDG. Both current density and the outwardly rectifying current-voltage (I-V) relationship curve shape were similar to those of the GFP-TRPM4 expressing cells. All these findings showed that TRPM4-HA is likely to share the channel properties with those of typical TRPM4.
Conclusions: These results demonstrate that the TRPM4-HA can be a novel and efficient biomarker to evaluate TRPM4 trafficking as a functional channel in various naïve cells. However, further experiments is needed to see if TRPM4-HA can act like TRPM4 with regard to cellular function.

Keywords: TRPM4, Hemaggultinin, Trafficking tag, HEK 293

P20-03-16

Soluble αKlotho downregulates Orai1-mediated store-operated Ca^{2+} entry and tumor cell migration *via* PI3K-dependent signaling

Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Bao TN Dang[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2]

Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

Purpose: αKlotho is a type 1 transmembrane anti-aging protein. αKlotho-deficient mice have premature aging phenotypes and imbalance of ion homeostasis including Ca^{2+} and phosphate. Soluble αKlotho is known to regulate multiple ion channels and growth factor-mediated phosphoinositide-3-kinase (PI3K) signaling. Store-operated Ca^{2+} entry (SOCE) mediated by pore-forming subunit Orai1 and ER Ca^{2+} sensor STIM1 is a ubiquitous Ca^{2+} influx mechanism and has been implicated in multiple diseases. However, it is currently unknown whether soluble αKlotho regulates Orai1-mediated SOCE via PI3K-dependent signaling.
Results: Among Klotho family, αKlotho downregulates SOCE while βKlotho or γKlotho has no effect on SOCE. Soluble αKlotho suppresses serum-stimulated SOCE and Ca^{2+} release-activated Ca^{2+} (CRAC) channel currents. Serum increases cell-surface abundance of Orai1 via stimulating vesicular exocytosis of the channel. The serum-stimulated SOCE and cell-surface abundance of Orai1 are inhibited by preincubation of αKlotho protein or PI3K inhibitors. Moreover, the inhibition of SOCE and cell-surface abundance of Orai1 by pretreatment of brefeldin A or tetanus toxin or PI3K inhibitors prevents further inhibition by αKlotho. Functionally, we further show that soluble αKlotho ameliorates serum-stimulated SOCE and cell migration in breast and lung cancer cells.
Conclusions: These results demonstrate that soluble αKlotho downregulates SOCE by inhibiting PI3K-driven vesicular exocytosis of Orai1 channel and contributes to suppressing SOCE-mediated tumor cell migration.

Keywords: SOCE, STIM1, FGF23, CRAC channel

P20-03-17

Roles of metabotropic glutamate receptors in synaptically-induced Ca^{2+}-spikes in cultured rat hippocampal neurons

Ji Seon Yang[1,2], Sujeong Jeon[1,2], Hyun-Jong Jang[1,2], Shin Hee Yoon[1,2]

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, [2]Catholic Neuroscience Institute, The Catholic University of Korea, Korea

Purpose: Group 1 metabotropic glutamate receptors (mGluRs) can positively affect postsynaptic neuronal excitability and epileptogenesis. The objective of the present study was to determine whether group 1 mGluRs might be involved in synaptically-induced intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) spikes and neuronal cell death.
Methods: Intracellular free Ca^{2+} spikes and neuronal cell death were induced by 0.1 mM Mg^{2+} and 10 μM glycine in cultured rat hippocampal neurons from embryonic day 17 fetal Sprague-Dawley rats using imaging methods for Ca^{2+} and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays for cell survival.
Results: Reduction of extracellular Mg^{2+} concentration ($[Mg^{2+}]_o$) to 0.1 mM induced repetitive $[Ca^{2+}]_i$ spikes within 30 s at day 11.5. The mGluR5 antagonist 6-Methyl-2-(phenylethynyl) pyridine (MPEP) almost completely inhibited the $[Ca^{2+}]_i$ spikes, but the mGluR1 antagonist LY367385 did not. The group 1 mGluRs agonist, 3,5-dihydroxyphenylglycine, significantly increased the $[Ca^{2+}]_i$ spikes. The phospholipase C inhibitor U73122 significantly inhibited the $[Ca^{2+}]_i$ spikes. The IP_3 receptor antagonist 2-aminoethoxydiphenyl borate or the ryanodine receptor antagonist TMB-8 significantly inhibited the $[Ca^{2+}]_i$ spikes. PKCε translocation inhibitor peptide significantly inhibited the $[Ca^{2+}]_i$ spikes, whereas the PKCα inhibitor Gö6983 or the atypical PKCζ pseudosubstrate inhibitor did not. The diacylglycerol analogue 1-oleoyl-2-acetyl-sn-glycerol significantly increased the $[Ca^{2+}]_i$ spikes. The TRPC channel inhibitors SKF96365 and flufenamic acid significantly inhibited the $[Ca^{2+}]_i$ spikes. The mGluR5 antagonist MPEP significantly increased the neuronal cell survival, but mGluR1 antagonist LY367385 did not.
Conclusions: These results suggest that mGluR5 is involved in synaptically-induced $[Ca^{2+}]_i$ spikes by releasing Ca^{2+} from IP_3 and ryanodine-sensitive intracellular stores and activating PKCε and TRPC channels, which may be involved in neuronal cell death.

Keywords: Hippocampal neurons, IP_3 receptor, Low Mg^{2+}, Metabotropic glutamate receptor, Ryanodine receptor, Synaptically-induced $[Ca^{2+}]_i$ spike

P20-03-18

Cerebellar output networks for fear learning and memory

Kyoung-Doo Hwang[1,2], Hyun-Hee Ryu[1], Hyun Geun Shim[1,2], Sang Jeong Kim[1,2], Yong-Seok Lee[1,2]

[1]Department of Physiology, [2]Department of Biomedical Science, Seoul National University College of Medicine, Seoul, Korea

Purpose: To understand how the cerebellar output from the deep cerebellar nuclei (DCN) regulates the fear learning and memory
Methods: We used the optogenetic method to manipulate the neural activity of the DCN. We also used the fiber photometry system to monitor calci-

um responses in the brainstem-projecting DCN.
Results: Optogenetic suppression of the DCN-brainstem circuit blocked the auditory cued fear memory retrieval without affecting either contextual fear memory retrieval or innate fear response whereas optogenetic activation of the same circuit induced the freezing response after fear conditioning. Moreover, DCN-brainstem circuit showed plastic changes after cued fear conditioning.
Conclusions: Cerebello-brainstem circuit is critically involved in processing sensory cued fear memory.

Keywords: Deep cerebellar nuclei, Brainstem, Fear memory, Optogenetics, Plasticity

P20-03-19

Tricyclic antidepressants regulate abnormal colonic motility like diarrhea and constipation through TRPC4/C5 channel depending on the opioid receptor

Byeongseok Jeong[1], Insuk So[2], Chansik Hong[1]

[1]Department of Physiology, Chosun University School of Medicine, Gwangju, [2]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

Purpose: Tricyclic antidepressants (TCAs) have been used to treat depression for ages, and now, they are used as treatments for other diseases such as chronic pain, PTSD, Parkinson's disease. Particularly when considering whether selective serotonin reuptake inhibitors (SSRIs) are first-line for treating depression, TCAs have recently been shown to have clinical efficacy in treating irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). However, molecular mechanism of TCA action in gastrointestinal (GI) tract remains poorly understood. Transient receptor potential channel canonical subtype (TRPC) members 4 and 5, which are nonselective cation channels, are important regulators of electrical excitability in both GI smooth muscle and neurons. In our previous results, TRPC4/C5 are activated to Gi-coupled receptor such as muscarinic acetylcholine receptors and opioid receptors. A recently study reports that opioid receptors, which have important roles in GI motility, are stimulated by TCAs. Herein, we investigated whether TCA modulates TRPC4/C5 activity depending on the opioid receptor, and consequently alters colonic motility through TRPC4/C5.
Results: On HEK293 cells overexpressed with TRPC4β or TRPC5, we recorded the current using the whole-cell patch clamp technique. By perfusion with amitriptyline (AMI), desipramine (DES), and imipramine (IMI), TRPC4β/C5 currents were dose-dependently inhibited with IC_{50} values of 2.88 μM, 10.31 μM, and 11.68 μM, respectively. The short (5 min) or long-term (16 hr) incubation with TCAs did not show any change in the expression level of TRPC4β/C5 channels on surface membrane. Interestingly, when co-expressed with OR, TCAs significantly increased the inward current of TRPC4β/C5 channels and the influx of intracellular calcium through the channels. To clarify whether TCA directly stimulates OR, we measured cAMP levels using ELISA. The pretreatment of DES decreased the cAMP level in μOR-expressing HEK cell. Besides, μOR-dependent TRPC5 activation by TCA was completely suppressed by co-expression of dominant-negative mutant (G203T) of $G\alpha_{i2}$ and pretreatment with pertussis toxin. Next, we found an active residue (D147) of μOR with TCA using protein-ligand docking analysis. The mutation of aspartic acid-147 to arginine significantly attenuated TRPC5 activation by TCA. To apply this regulatory mechanism of TRPC4/C5 by TCA to abnormal GI motility, we measured contraction-driven motility in human colon. IMI apparently reduced the amplitude and frequency of the colon smooth muscle spontaneous contractility, while a TRPC4/C5 agonist, englerin A increased the motility.
Conclusions: Taken together, TCA directly inhibits an activity of TRPC4/C5, in contract, and it could increase the channel activity in presence of opioid receptors. We suggest two evidences on the TRPC4/C5 regulation of TCA in diarrhea and constipation. First, TCA action on IBS might result from the inhibition of both TRPC5 in enteric neuron and TRPC4 in smooth muscle. Second, TCA might have an action on diarrhea by TRPC4 hyperactivation through OR. These findings could provide insights into potential mechanisms underlying therapeutic and side effects of TCA in abnormal GI motility.

Keywords: TRPC, TCA, Colon motility, IBD, IBS

Acknowledgement: This study was supported by grants from the National Research Foundation of Korea, which is funded by the Ministry of Education (2015R1A6A3A04058395) and Ministry of Science and ICT (2020R1F1A1050018) of the Korean government.

P20-03-20

Distinct shear-induced Ca^{2+} signaling in the left and right atrial myocytes: role of P2 receptor context

Joon-Chul Kim[1,2], Qui Anh Le[1], Kyeong-Hee Kim[1], Anh Thi Van Vu[1], Sun-Hee Woo[1]

[1]Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, [2]Department of New Drug Development, NEXEL Co. Ltd., Seoul, Korea

Purpose: Atrial myocytes are continuously exposed to shear stress during cardiac cycles. Previous reports have shown that shear stress induces two different types of global Ca^{2+} signaling in atrial myocytes-longitudinal Ca^{2+} waves (L-waves) and action potential-involved transverse waves (T-waves), and suggested an underlying role of the autocrine activation of P2 receptors. We explored the correlations between ATP release and Ca^{2+} wave generation in atrial myocytes and investigated why the cells develop two Ca^{2+}-wave types during the same shear force.
Methods: We examined whether ATP release correlates with different shear-stress (~16 dyn/cm^2)-mediated Ca^{2+} signaling by simultaneous measurement of local Ca^{2+} and ATP release in individual atrial myocytes using two-dimensional confocal imaging and sniffer patch techniques, respectively. Functional P2X7-receptor-expressing HEK293 cells were established as sniffer cells, which generated currents in real time in response to ATP released from a closely positioned atrial myocyte.
Results: Both shear-stress-induced L- and T-waves were preceded by sniffer currents with no difference in the current magnitude. Left atrial (LA) myocytes had two- to three-fold larger sniffer currents than right atrial (RA) cells, as was confirmed by ATP chemiluminescence assay. Shear-stress-induced ATP release was eliminated by connexin (Cx) 43 hemichannel inhibition using La3+, Gap19, or knock-down of Cx43 expression. The level of phosphorylated Cx43 at Ser386 (p-Cx43Ser368), but not total Cx43, was higher in LA versus RA myocytes. Most LA cells (~70%) developed L-waves, whereas most RA myocytes (~80%) presented T-waves. Shear-stress-induced T-waves were completely removed by inhibition of P2X4R, which were most abundant in rat atrial cells. Expression of P2X4R was higher in RA than LA myocytes, whereas expression of P2Y1R, the mediator of L-waves, was higher in LA than RA myocytes.
Conclusions: ATP release mainly triggers L-waves in LA myocytes and T-waves in RA myocytes under the same shear force, partly because of the differential expression of P2Y1R and P2X4R between LA and RA myocytes. Higher ATP release in LA myocytes under shear stress may not contribute to determination of the wave pattern.

Keywords: Shear stress, Atrial myocyte, Purinergic receptor, Ca^{2+} signaling

P20-03-21

Inhibition of voltage-dependent K^+ channels by tricyclic antidepressant protriptyline in coronary arterial smooth muscle cells

Jin Ryeol An, Won Sun Park

Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Protriptyline, a tricyclic antidepressant, is used in the treatment of depressive disorders. However, the effect of protriptyline on vascular ion channels is unclear.
Methods: We examined the effect of protriptyline on voltage-dependent K^+ (K_v) channels in freshly isolated rabbit coronary arterial smooth muscle

cells using a patch-clamp technique.
Results: Protriptyline inhibited the vascular K_v current in a concentration-dependent manner, with an IC_{50} value of 5.05 ± 0.97 μM and a Hill coefficient of 0.73 ± 0.04. Protriptyline did not affect the steady-state activation kinetics. However, the drug shifted the steady-state inactivation curve to the left, suggesting that protriptyline inhibited the K_v channels by changing their voltage sensitivity. Application of 20 repetitive train pulses (1 or 2 Hz) progressively increased the protriptyline-induced inhibition of the K_v current, suggesting that protriptyline inhibited K_v channels in a use (state)-dependent manner. The extent of K_v current inhibition by protriptyline was similar during the first, second, and third step pulses. These results suggest that protriptyline-induced inhibition of the K_v current mainly occurs principally in the closed-state. The increase in the inactivation recovery time constant in the presence of protriptyline also supported use (state)-dependent inhibition of K_v channels by the drug. In the presence of the K_v1.5 inhibitor DPO-1, protriptyline did not induce further inhibition of the K_v channels. However, pretreatment with a K_v2.1 or K_v7 inhibitor (gungxitoxin or linopirdine) induced the further inhibition of K_v current to a similar to that observed with protriptyline alone.
Conclusions: We conclude that protriptyline inhibited the vascular K_v channels in a concentration- and use (state, mainly closed state)-dependent manner by changing their gating properties regardless of its own function. Furthermore, protriptyline-induced inhibition of K_v channels mainly involves the K_v1.5 subtype.

Keywords: Protriptyline, Voltage-dependent K^+ channels, Coronary artery

P20-03-22

The direct effect of oxybutynin on voltage-gated K^+ channels in coronary arterial smooth muscle cells

Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Oxybutynin is an anticholinergic drug used to treat overactive bladder. The adverse effects of oxybutynin on vascular ion channels, specifically voltage-dependent K^+ (K_v) channels have not been investigated.
Methods: We examined the effect of oxybutynin on voltage-dependent K^+ (K_v) channels in freshly isolated rabbit coronary arterial smooth muscle cells using a patch-clamp technique.
Results: Oxybutynin inhibited vascular K_v channels in a concentration-dependent manner, with an IC_{50} value of 11.51 ± 0.38 μM and a Hill coefficient (n) of 2.25 ± 0.12. Application of oxybutynin shifted the activation curve to the right and the inactivation curve to the left. Pretreatment with the K_v1.5 subtype inhibitor DPO-1 and the K_v2.1 subtype inhibitor guangxitoxin suppressed the oxybutynin-induced inhibition of the K_v current. However, application of the K_v7 subtype inhibitor linopirdine did not affect the inhibition by oxybutynin of the K_v current. The anticholinergic drug atropine did not inhibit the K_v current nor influence oxybutynin-induced inhibition of the K_v current.
Conclusions: We concluded that oxybutynin inhibited the vascular K_v current in a concentration-dependent manner by influencing the steady-state activation and inactivation curves independent of its anticholinergic effect.

Keywords: Oxybutynin, Voltage-gated K^+ channels, Coronary artery

P20-03-23

The inhibitory effect of iloperidone on voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells

Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Iloperidone, a second-generation atypical antipsychotic drug, is widely used in treatment of schizophrenia. However, the side effects of iloperidone on vascular K^+ channels remain to be determined.
Methods: We explored the effect of iloperidone on voltage-dependent K^+ (K_v) channels in rabbit coronary arterial smooth muscle cells using the whole-cell patch-clamp technique.
Results: Iloperidone inhibited vascular K_v channels in a concentration-dependent manner with an IC_{50} of 2.11 ± 0.5 μM and a Hill coefficient of 0.68 ± 0.03. Iloperidone had no effect on the steady-state inactivation kinetics. However, it shifted the steady-state activation curve to the right, indicating that iloperidone inhibited K_v channels by influencing the voltage sensors. Application of 20 repetitive depolarizing pulses (1 and 2 Hz) progressively increased the inhibition of the K_v current in the presence of iloperidone. Furthermore, iloperidone increased the recovery time constant from K_v channel inactivation, suggesting that iloperidone-induced inhibition of K_v channels is use (state)-dependent. Pretreatment with a K_v1.5 inhibitor (DPO-1) inhibited the K_v current to a level similar to that with iloperidone alone. However, pretreatment with a K_v2.1 or K_v7.X inhibitor (guangxitoxin or linopirdine) did not affect the inhibitory effect of iloperidone on K_v channels.
Conclusions: Iloperidone directly inhibits K_v channels in a concentration- and use (state)-dependent manner independently of its antagonism of serotonin and dopamine receptors. Furthermore, the primary target of iloperidone is the K_v1.5 subtype.

Keywords: Iloperidone, Voltage-dependent K^+ channel, Coronary arterial smooth muscle cell

P20-03-24

Inhibition of voltage-dependent K^+ channels by ziprasidone in rabbit coronary arterial smooth muscle cells

Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Ziprasidone, a second-generation atypical antipsychotic drug, is widely used in treatment of schizophrenia. However, the side effects of ziprasidone on vascular K^+ channels remain to be determined.
Methods: We explored the effect of ziprasidone on voltage-dependent K^+ (K_v) channels in rabbit coronary arterial smooth muscle cells using the whole-cell patch-clamp technique.
Results: Ziprasidone dose-dependently inhibited K_v channels with an IC_{50} value of 0.39 ± 0.06 μM and a Hill coefficient of 0.62 ± 0.03. Although ziprasidone had no effect on the steady-state inactivation kinetics of the K_v channels, the steady-state activation curve shifted towards a more positive potential. These results suggest that ziprasidone inhibits K_v channels by targeting their voltage sensors. The recovery time constant of K_v channel inactivation was increased in the presence of ziprasidone. Furthermore, application of train steps (of 1 and 2 Hz) in the presence of ziprasidone led to a progressive increase in the blockade of K_v currents, suggesting that ziprasidone-induced inhibition of K_v channels is use (state)-dependent. Pretreatment with K_v1.5, K_v2.1, and K_v7 subtype inhibitors partially suppressed the ziprasidone-induced inhibition of K_v currents.
Conclusions: These results suggest that ziprasidone inhibits vascular K_v channels through its effect on gating properties. The K_v channel-inhibiting action of ziprasidone is concentration- and use (state)-dependent.

Keywords: Atypical antipsychotics, Ziprasione, Voltage-dependent K^+ channel, Smooth muscle cell

P20-03-25

The anti-cholinergic drug tolterodine blocks voltage-dependent K^+ channels in rabbit coronary arterial smooth muscle cells

Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: Anti-cholinergic drug tolterodine is used in the treatment of overactive bladder disorders. However, the effect of tolterodine on vascular ion channels is unclear.
Methods: We explored the effect of the tolterodine on voltage-dependent K^+ (K_v) channels using the patch clamp technique in coronary arterial smooth muscle cells freshly isolated from rabbits.
Results: Tolterodine inhibited K_v channels in a concentration-dependent manner, with an IC_{50} of 1.71 ± 0.33 μM and Hill coefficient of 0.69 ± 0.03. Tolterodine accelerated the decay rate of K_v channel inactivation. The apparent rate constants of association and dissociation for tolterodine were 1.79 ± 0.13 $\mu M^{-1}s^{-1}$, and 3.13 ± 0.96 s^{-1}, respectively. Although 3 μM tolterodine had no effect on the steady-state activation of the K_v current, it shifted the steady-state inactivation curve towards a negative potential. Application of consecutive train steps (1 or 2 Hz) progressively decreased the K_v current and promoted its inhibition. Furthermore, the recovery time constant was augmented in the presence of tolterodine, indicating that tolterodine-induced K_v channel blockade is use (state) dependent. Pretreatment with inhibitors of the $K_v1.5$, $K_v2.1$, and K_v7 subtypes (DPO-1, guangxitoxin, and linopirdine) partially reduced the inhibitory effect of tolterodine on K_v channels. The alternative muscarinic receptor antagonist atropine did not inhibit the K_v current nor influence tolterodine-induced inhibition of the K_v current. Tolterodine induced vasoconstriction and membrane depolarization.
Conclusions: We conclude that tolterodine inhibits K_v channels in concentration-, time-, and use (state)-dependent manners, irrespective of its antagonism of muscarinic receptors.

Keywords: Tolterodine, Voltage-gated K^+ channels, Coronary artery

P20-03-26

Hydrogen peroxide (H_2O_2) suppresses the gonadotropin-releasing hormone neurons excitability *via* ATP-sensitive potassium channels

Santosh Rijal, Soo Joung Park, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Jeonbuk National University, Jeonju, Korea

Purpose: Reactive oxygen species (ROS), at lower cellular levels has emerged as potent signaling molecules in the central nervous system, where neurons can sense, convey and convert ROS signals into relevant intracellular signals, including synaptic plasticity. ROS generated due to endogenous and exogenous factors is reported to alter the reproductive functions, reduce the gonadal hormones, and interfere in the cross-talk between the hypothalamic-pituitary-gonadal (HPG) axis and other endocrine axis which eventually affects fertility. Gonadotropin-releasing hormone (GnRH) neurons are the central regulator of the HPG axis that plays a pivotal role in the regulation of reproductive physiology and release of gonadotropins in mammals. In the hypothalamic region, ROS is found to involve in the regulation of energy metabolism and food intake but the ROS effect on the hypothalamic regulation of fertility does not exist. Therefore, in this study, we used hydrogen peroxide (H_2O_2) as a source of ROS and its influence on the hypothalamic GnRH neuronal excitability and membrane properties was assessed via electrophysiological approach.
Methods: Whole-cell current-clamp technique was conducted to find out the effect of H_2O_2 on neuronal excitability and membrane properties of GnRH neurons distributed on hypothalamic pre-optic area of brain slices.
Results: Our result suggests that H_2O_2 can modulate the intrinsic properties and induce membrane hyperpolarization in the majority of GnRH neurons. Furthermore, H_2O_2 induce hyperpolarization response tended to increase with increased concentration and persisted in the presence of tetrodotoxin (a voltage-gated Na^+ channel blocker) and amino acids receptors blocker. However, the hyperpolarization mediated by H_2O_2 was completely blocked by ATP-sensitive potassium channel blockers.
Conclusions: H_2O_2 can regulate the GnRH neurons by suppressing the excitation via KATP channels and has an impact on the hypothalamic regulation of fertility.

Keywords: Reactive oxygen species, Gonadotropin-releasing hormone neurons, Hydrogen peroxide, Whole-cell current-clamp technique, KATP channels

P20-03-27

Thermal stimulation triggers TRPV3-mediated Ca^{2+} influx in keratinocytes from patients with atopic dermatitis

Sohyun Kim[1], Seong Hoon Seo[2], Sang Eun Lee[2], Seungsoo Chung[1]
[1]Department of Physiology, Brain Korea 21 Plus Project for Medical Science, Yonsei University College of Medicine, Seoul, [2]Department of Dermatology, Gangnam Severance Hospital, Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea

Purpose: Epidermal dysregulation of Ca^{2+} signaling has been regarded as one of the hallmarks of abnormal skin functions. The contribution of TRPV3-mediated Ca^{2+} signaling in the development of thermo-sensitive hypersensitivity in atopic dermatitis (AD) is yet unknown. The aim of this study was to investigate the functional role of TRPV3 in the keratinocytes from AD patients.
Methods: Keratinocytes were loaded with Fura-2-acetoxymethyl (AM) ester. We conducted the measurements of calcium influx of keratinocytes from normal and AD tissues after sequential thermal stimulation or application of a chemical cocktail of TRPV3 agonists (i.e. 200 μM 2-APB and 500 μM carvacrol). All calcium measurements were performed at 22°C or 37°C using a temperature controller.
Results: To investigate the function of thermo-sensitive TRPV3 channels, we firstly examined the Ca^{2+} influx in keratinocytes with thermal stimulation only. We explored the heat activation profiles of TRPV3 in both normal and AD keratinocytes. AD keratinocytes were activated more with higher levels of calcium influx than normal keratinocytes when a thermal stimulation of 37°C was applied, and this was statistically validated to be significant. The average physiological body temperature was sufficient to activate TRPV3 in AD keratinocytes to cause hypersensitivity, whereas TRPV3 in normal keratinocytes showed an almost quiescent activity at 37°C. Functionally, we found that the calcium influx via TRPV3 channels in AD keratinocytes was more significantly augmented than normal keratinocytes when evoked with chemical agonists at 22°C.
Conclusions: This study has highlighted an important pathophysiological role of hyperactive TRPV3-mediated intracellular Ca^{2+} signaling in AD. We have shown that TRPV3 is highly upregulated in AD keratinocytes when compared with normal keratinocytes. We also demonstrated that AD keratinocytes displayed the overexpression TRPV3, which subsequently led to hyperactive channel functions with abnormally increased calcium influx. As our calcium imaging data has shown, the altered expression of TRPV3 may promote more aggressive AD subtypes with other associated complications such as heat-induced psoriasis and skin hypersensitivity via the abnormal downstream molecular pathway of TRPV3. In summary, this study proposed that the aberrant Ca^{2+} signaling may be implicated in the development of AD.

Keywords: Atopic dermatitis, Keratinocyte, Transient receptor potential vanilloid, Intracellular calcium

Acknowledgement: This study was supported by the Technology Innovation Program (or Industrial Strategic Technology Development Program-Development of Core Industrial Technology) (20003822, Development of Navigation System Technologies of MicroNano Robots with Drug for Brain Disease Therapy) funded by the Ministry of Trade, Industry & Energy (MOTIE, Kora).

P20-03-28

Oxidative stress by Ca^{2+} overload is critical for phosphate-induced vascular calcification

Nhung Thi Nguyen[1,2], Tuyet Thi Nguyen[3], Dat Da Ly[1,2], In-Kyu Lee[4], Seung-Kuy Cha[1,2], Kyu-Sang Park[1,2]

[1]Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea, [3]Internal Medicine Residency Program, College of Health Sciences, VinUniversity, Hanoi, Vietnam, [4]Department of Internal Medicine, School of Medicine, Kyungpook National University, Daegu, Korea

Purpose: Hyperphosphatemia is the primary risk factor for vascular calcification, which is closely associated with cardiovascular morbidity and mortality. Recent evidence showed that oxidative stress by high inorganic phosphate (Pi) mediates calcific changes in vascular smooth muscle cells (VSMCs). However, intracellular signalings responsible for Pi-induced oxidative stress remain unclear. Here, we investigated molecular mechanisms of Pi-induced oxidative stress related with intracellular Ca^{2+} ($[Ca^{2+}]_i$) disturbance, which is critical for calcification of VSMCs
Methods: VSMCs isolated from rat thoracic aorta or A7r5 cells were incubated with high Pi-containing medium.
Results: Extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin were activated by high Pi that was required for vascular calcification. High Pi upregulated expressions of type III sodium-phosphate cotransporters, PiT-1 and -2, and stimulated their trafficking to the plasma membrane. Interestingly, high Pi increased $[Ca^{2+}]_i$ exclusively dependent on extracellular Na^+ and Ca^{2+} as well as PiT-1/2 abundance. Furthermore, high Pi induced plasma membrane depolarization mediated by PiT-1/2. Pretreatment with verapamil, as a voltage-gated Ca^{2+} channel (VGCC) blocker, inhibited Pi-induced $[Ca^{2+}]_i$ elevation, oxidative stress, ERK activation and osteogenic differentiation. These protective effects were reiterated by extracellular Ca^{2+} free condition, intracellular Ca^{2+} chelation or suppression of oxidative stress. Mitochondrial superoxide scavenger also effectively abrogated ERK activation and osteogenic differentiation of VSMCs by high Pi.
Conclusions: Taken together, we suggest that high Pi activates depolarization-triggered Ca^{2+} influx via VGCC, and subsequent $[Ca^{2+}]_i$ increase elicits oxidative stress and osteogenic differentiation. PiT-1/2 mediates Pi-induced $[Ca^{2+}]_i$ overload and oxidative stress, but in turn, PiT-1/2 is upregulated by consequences of these alterations.

Keywords: Hyperphosphatemia, Oxidative stress, Calcium overload, Voltage-gated calcium channel, Type III sodium-phosphate cotransporters, Vascular calcification

P20-03-29

Effects on tonic GABAA inhibition and memory by pharmacological modulation of STING

Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Hyun Jin Shin[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]

[1]Department of Medical Sciences, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, Chungnam National University, School of Medicine, Korea

Purpose: STING (stimulator of interferon genes) is a protein, plays a central role in innate immunity. Role of STING and STING activated pathways have been studied in systemic inflammation, infection and cancer but its role in the CNS remains unclear. We previously identified that genetic deletion STING caused memory deficits at least in part by, increase tonic GABAA inhibition in dentate gyrus granular cells (DGGCs) with decreased expression of STING signalling molecules (STING-TBK1-IRF3) and GATs. Here we hypothesized that memory and tonic GABAA inhibition can also be modulated pharmacologically through STING signalling pathway.
Methods: The C57BL/6 J-(WT) and/or heterozygous STING ($STING^{+/-}$) mice were subjected to systemic administration (via i.p injection) of H-151 (a STING inhibitor for 12 days) and cGAMP (a STING agonist for 14 days), and effects were analysed by using behaviour test, electrophysiological recording and molecular techniques
Results: Systemic injection of H-151 in WT mice mimic the STING knockout effect in terms of both memory and tonic GABAA inhibition along with expression of STING signalling molecules and GATs. Conversely, cGAMP caused significant improvement on memory with reduced tonic GABAA inhibition in $STING^{+/-}$ mice. However the expression of STING signalling molecules and GATs were consistently increased in both WT and $STING^{+/-}$ mice after cGAMP injection.
Conclusions: Our results implicates that pharmacological modulation of STING can regulates tonic GABAA inhibition in DGGCs and subsequently modify the cognitive function in mice.

Keywords: STING, GATs, Memory, Tonic GABAA inhibition, DGGCs

P20-03-30

GluN2D containing NMDA receptors activity in hippocampal GABAergic interneurons modulate development of Status Epilepticus

Ramesh Sharma[1,2,3], Chiranjivi Neupane[1,2,3], Hyun Jin Shin[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]

[1]Department of Medical Sciences, [2]Department of Physiology, [3]Department of BK21Plus CNU Integrative Biomedical Education Initiative, Chungnam National University, School of Medicine, Korea

Purpose: To investigate the involvement of a GluN2D subunit containing NMDA receptors in pilocarpine induced status epilepticus mice.
Methods: Pilocarpine induced status epilepticus were developed by intraperitoneal injection of 180-200 mg/kg pilocarpine in mice. Initially, hippocampal slices were obtained from GAD-67-GFP transgenic ICR mice and C57BL/6-WT mice with or without pilocarpine injection for the electrophysiological recording. Tonic current was measured in voltage current mode. Electroshock induced seizure activity was recorded after electroconvulsions were produced by a current (5-6 mA, 60 Hz, 0.2 s stimulus duration) delivered via ear-clip electrodes in C57BL/6- WT and GluN2D KO mice. Spontaneous seizure was recorded using continuous video recording and were analyzed manually. Frozen brain sections were immunostained to determine pilocarpine induced changes in neurons and glia.
Results: Our experiments explore that Mg^{++} resistant tonic NMDA current, (sensitive to PPDA, a GluN2C/2D antagonist) was generated in GABAergic interneurons in CA1 subfield in pilocarpine induced epileptic mice but not in control mice. Furthermore, we examined the role of GluN2D in epilepsy progression after pilocarpine injection. Interestingly, transauricular electroshock-induced seizure activity was increased in the pilocarpine injected GluN2D knockout (KO) mice compared to the wild type (WT) mice. In addition, the KO mice showed spontaneous recurrent seizure in chronic stage, starting from the third weeks of pilocarpine injection. Along with spontaneous seizure, the KO mice showed progressive death of hippocampal pyramidal neurons and significant changes in neuroglia including astrogliosis and microgliosis in chronic stage compared to the WT mice.
Conclusions: Our results demonstrated that the GluN2D mediated Mg^{++} resistant tonic NMDA current is generated in GABAergic interneurons in pilocarpine induced epileptic hippocampi and Glu2D deficiency facilitates changes in neuronal and glial cells during epilepsy progression.

Keywords: Status epilepticus, NMDARs, GluN2D, GABAergic interneurons

P20-03-31

GABA- and glycine-mimetic responses of linalool on the substantia gelatinosa of the trigeminal subnucleus caudalis in mice

Seon Hui Jang, Soo Joung Park, Seong Kyu Han

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

Purpose: Substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) are involved in transmission of the orofacial nociceptive inputs through the thin myelinated Aδ and unmyelinated C primary afferent fibers. Linalool, a major odorous constituent in essential oils extracted from lavender, has been proven to have a wide range of physiological effects on humans including pain control, but to date, the orofacial antinociceptive mechanism of linalool has not yet been completely clarified at the level of central nervous system.
Methods: To examine the direct action of linalool and to identify the receptors involved, whole-cell patch-clamp technique was performed on the SG neurons of the Vc in juvenile mice.
Results: In the voltage-clamp mode, the nondesensitized and repeatable linalool-induced inward currents preserved in the presence of tetrodotoxin, a voltage-gated Na^+ channel blocker, 6-cyano-7-nitro-quinoxaline-2,3-dione, a non-N-methyl-D-aspartic acid (NMDA) glutamate receptor antagonist, and DL-2-amino-5-phosphonopentanoic acid, an NMDA receptor antagonist. However, linalool-induced inward currents were partly suppressed by picrotoxin, a GABAA receptor antagonist, or strychnine, a glycine receptor antagonist. These responses were almost blocked in the presence of both picrotoxin and strychnine. It was also experienced that linalool exhibited potentiation effect with GABA- and glycine-induced inward currents.
Conclusions: Taken together, we propose that linalool has GABA- and glycine-mimetic effects and this agent might be a promising treatment for orofacial pain ailments by activation of inhibitory neurotransmission on the SG area of the Vc.

Keywords: Linalool, Substantial gelatinosa, Whole-cell patch-clamp techniques, Orofacial pain

P20-03-32

Pathway-specific cholinergic modulation of synaptic plasticity in rat primary visual cortex *in vivo*

Kayoung Joo[1], Kwang-Hyun Cho[1], Duck-Joo Rhie[2]

[1]Department of Physiology, College of Medicine, The Catholic University, [2]Department of Physiology, Catholic Neuroscience Institute, College of Medicine, The Catholic University, Korea

Purpose: Acetylcholine is an important neuromodulator for regulation of visual attention, plasticity, and perceptual learning. Acetylcholine is released in the visual cortex from cholinergic projections of the basal forebrain, where may differentially modulate synaptic transmission between sensory and associative inputs depending on the brain state. Previously, we demonstrated that cholinergic stimulation differentially modulated long-term synaptic plasticity in acute slice preparation, depending on cortical layers in layer 2/3 pyramidal neurons of the primary visual cortex (V1). However, little is known about layer-specific cholinergic modulation of synaptic transmission in vivo. The aim of this study was to know pathway-specific cholinergic modulation of synaptic transmission evoked by the lateral geniculate nucleus (LGN) and contralateral V1 (cV1).
Methods: We stimulated the basal forebrain with optical stimulation to increase acetylcholine level in V1 and recorded field potentials (FPs) evoked by electrical stimulation of either LGN or cV1 using a 16-channel multi-electrode array. Channelrhodopsin-2 (ChR2) was delivered by AAV-viral vector to the basal forebrain of rats at age of 3 weeks. After 2 weeks, optical stimulation (480 nm) was applied to the basal forebrain. Viral expression was monitored by fluorescence imaging of reporter proteins. Tungsten electrodes were positioned in the LGN for activation of sensory inputs and cV1 for activation of associative inputs.
Results: Upon optical stimulation of ChR2-expressed basal forebrain, the amplitude of FPs evoked by activation of the LGN increased in layers 1, 2/3, and 4 of V1, while FPs evoked by stimulation of cV1 decreased in layers 1 and 2/3. Moreover, muscarinic and nicotinic receptors exhibited differential effects depending on inputs. The amplitude of LGN-FPs decreased to control levels by the muscarinic antagonist scopolamine (5-20 mg/kg, i.p.) but not by the nicotinic antagonist mecamylamine (5-20 mg/kg). The amplitude of cV1-FP increased to control values by mecamylamine, but not by scopolamine.
Conclusions: Therefore, these results indicated that cholinergic modulation of synaptic transmission differs depending on inputs to V1, which might be important for the information processing balance depending on the brain state.

Keywords: Acetylcholine, Muscarinic receptors, Nicotinic receptors, Basal forebrain, LGN, Visual cortex

Acknowledgement: Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).

P20-03-33

Enhanced tonic NMDA current in supraoptic neucleus neurons of DOCA-salt hypertension model

Hyun Jin Shin[1,2,3], Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Su Eun Park[1,2,3], Jin Bong Park[1,2,3]

[1]Department of Medical Sciences, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, School of Medicine and Brain Research Institute, Chungnam National University, School of Medicine, Korea

Purpose: NMDA receptors (NMDARs) with increased glutamate tone play a critical role in modulating hypothalamic neurosecretory function during disease states. Hypertensive patients show elevated circulating vasopressin (VP), which is in agreement with enhanced excitability of magnocellular neurosecretory cell (MNCs) in hypothalamic paraventricular nucleus and supraoptic nucleus (SON) in animal models of hypertension. However, the contribution of VP to the process of hypertension has been examined in a number of models but roles of tonic NMDA current in hypertension remains to be explored. In this study we demonstrate that tonic NMDA currents in SON MNCs contributing to neurohumoral activation in deoxycorticosterone acetate (DOCA)-salt hypertension rats.
Methods: All rats were uninephrectomised and kept for 7-days recovery. After 7-days, DOCA is implanted sub-dermally and animals were assigned as DOCA-salt group provided 0.8% NaCl and 0.2% KCl in tap water and DOCA-H_2O group provided free access of normal tap water. On the 6th day after DOCA implantation mice were kept in metabolic cage for 24 hour urine collection to measure the metabolic rate indicators. After 24 hour, water intake, urine output and urine osmolality were measured. Before the decapitation of rats for electrophysiology systolic blood pressure was measured by using tail cuff method.
Results: Blood pressure started to increase at 2-week and reached maximum at 4-week DOCA-salt model. In SON MNCs, tonic NMDA current (INMDA) significantly increased in DOCA-salt rats of 1-week model, decreased at 2 week,and returned to basal level at 4-week groups. While there is no difference in blood pressure, urine osmolality between 1-week DOCA-salt and DOCA-H_2O groups, water intake and urine volume was significantly in 1-week DOCA-salt than DOCA-H_2O groups. INMDA of SON MNCs was blocked by NR2A antagonist (PEAQX) but not by NR2B antagonist (ifenprodil) and NR2C/D antagonist (PPDA) in both 1-week DOCA-salt and DOCA-H_2O groups. Interestingly, INMDA difference between the groups was not observed with increased glutamate concentration in extracellular recording solution. The increased glutamate tone was mediated by the reduced transporter tone of DOCA-salt.
Conclusions: Overall our results suggested that increased concentration of extracellular glutamate activated more NR2A-containig NMDARs in SON NMCs of 1-week DOCA-salt model.

Keywords: NMDA, DOCA, SON NMCs, Glutamate trnsporter

P20-03-34

Direct calcium binding at S2-S3 linker modifies gating of TRPC4, 5 channels

Jin Hyeong Kim, Jinsung Kim, Juyeon Ko, Insuk So
Department of Physiology, Seoul National University College of Medicine, Korea

Purpose: It is generally acknowledged that activity of TRPC4 and 5 channels are regulated by G-protein signaling such as PIP_2 hydrolysis or $[Ca^{2+}]_i$. Recently, Cryo-EM structure of TRPC4 suggested that calcium ion directly binds to S2-S3 linker sequence. We examined the effect of such binding in over-expressed TRPC4 or TRPC5 channels, and tried to suggest a plausible structural mechanism for such an effect.
Methods: HEK293 cells were cultured in 12-well plate and mTRPC4β-EYFP or EYFP-mTRPC5 channels were transiently transfected using FuGENE6 after 24 hrs. Cells were trypsinized and transferred to recording chamber the next day. Ramp pulses from -100 mV to +100 mV in 500ms were applied in every 10 s. Holding potential of -60 mV was applied in between. All mutagenesis was conducted using QuikChange Mutagnenesis kit (Agilent Technologies). PyMol software (Schrodinger) was used for molecular visualization.
Results: Amino acids constituting the Direct Calcium Binding Domain (DCaBD) - EQYND in TRPC4β and EEYND in TRPC5 - were singularly substituted to alanine. In TRPC4β, all 5 mutant channels showed typical double-rectifying response when 100nM of Englerin-A, a TRPC4, 5-selective activator, was applied in extracellular solution. Mutated TRPC5 channels also showed similar whole-cell current in response to Englerin-A. Interestingly, however, inward current was more severely reduced in mTRPC4βY429A and mTRPC4bβN435A channels. Intracellular dialysis of GTPγS or over-expression of gain-of-function mutant of Gαi2 (Gαi2Q205L) could not elicit TRPC4 or 5 like current in 9 mutants. Activation of muscarinic acetylcholine receptor subtype 3 (mAChR3) signaling by extracellular carbachol could not activate 9 mutant channels. Notably, EYFP-mTRPC5E421A could be activated by GTPγS, Gαi2Q205L, and mAChR3 signaling. All mutants showed similar plasma membrane expression level comparable to wild-type. Fluorescence pattern was also similar.
Conclusions: According to Cryo-EM structures, S2-S3 linker resides near TRP helix. Any structural perturbation of S2-S3 linker may change pore contour by moving TRP-helix, hence S6 and Pore Helix.

Keywords: Structure-function relationship, TRPC4, TRPC5, Calcium binding

P20-03-35

TRPML3-GATE16 interaction regulates both early and late autophagy by the multimerization of TRPML channels

Areum Choi, Suzi Choi, So Woon Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

Purpose: We have previously shown that the intracellular Ca^{2+} channel TRPML3 interacts with mammalian ATG8 homologue, GATE16 in autophagy. However, the exact mechanism by which the TRPML3-GATE16 interaction regulates autophagy is not known. GATE16 is originally known to be involved in autophagosome formation, but recent studies showed that it also plays an important role in autophagosome-lysosome fusion. Our preliminary data suggest that TRPML3 homomer provides Ca^{2+} in autophagosome formation and TRPML1/3 heteromer in autophagosome-lysosome fusion. Thus, we investigated whether TRPML3-GATE16 interaction plays a different role in each autophagy step by the multimerization of TRPML channels.
Methods: Confocal microscopy was used to image the fluorescence tagged proteins. Co-immunoprecipitation and GST pull down assay were employed to show the interaction between proteins. To monitor autophagic flux, imaging of mRFP-GFP-LC3 and LC3 western blot analysis were conducted.
Results: We found that GATE16 colocalizes and interacts with both TRPML3 homomer and TRPML1/3 heteromer and identified that Cys50 in N-terminus of TRPML3 is responsible for the binding to GATE16. Inhibition of the interaction by Cys50 to Ala mutation did not affect the colocalization between GATE16 and the channels and channel properties. However, the mutation greatly decreased autophagosome formation in cells expressing TRPML3 homomer, but not TRPML1/3 heteromer. By contrast, in cells expressing TRPML1/3, the mutation markedly reduced autophagosome-lysosome fusion and autophagic degradation. These results suggest that the interaction of GATE16-TRPML3 homomer functions in the early stage of autophagy to increase autophagosome formation, while that of GATE16-TRPML1/3 heteromer acts at the late stage of autophagy to promote autophagosome-lysosome fusion.
Conclusions: In conclusion, TRPML3-GATE16 interaction functions differently at different stages of autophagy by the multimerization of TRPML channels.

Keywords: GATE16, TRPML3, TRPML1/3, Autophagy

P20-03-36

SYT5 is the Ca^{2+} sensor of autophagosome-lysosome fusion

Seokwoo Hong, Suzi Choi, So Woon Kim, Hyun Jin Kim
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

Purpose: Autophagy is an intracellular degradation pathway that delivers cytoplasmic contents to the lysosome, which requires numerous fusion events in a Ca^{2+} dependent way. Our preliminary data suggest that TRPML3 homomer provides Ca^{2+} in autophagosome formation and TRPML1/3 heteromer in autophagosome-lysosome fusion. However, the molecular mechanism of how the Ca^{2+} triggers fusion between vesicles and organelles at each stage of autophagy is still unclear. Therefore, we searched a Ca^{2+} sensor involved in autophagy and investigated the role of the Ca^{2+} sensor in association with TRPML channels.
Methods: Split-ubiquitin membrane yeast-two hybrid system was obtained to screen TRPML3 interacting proteins. Co-IP and GST pull-down assay were used to determine the interaction between proteins. TRPML3- and TRPML1/3-GCaMP6 were used as specific reporters of early or late stage of autophagy. Subcellular localization was assessed by various organellar marker proteins. Autophagy was monitored using the tandem-fluorescence LC3B and LC3 western blot analysis. Protein-lipid overlay assay and PIP bead binding assay were used to investigate the protein-lipid interaction.
Results: We identified a Ca^{2+} sensor SYT5 as a TRPML3 interacting protein based on a yeast-two hybrid screening. Co-IP and GST pull-down assay revealed that SYT5 interacts with both TRPML3 homomer and TRPML1/3 heteromer via its C2B domain. However, the experiments using TRPML3- and TRPML1/3-GCaMP6 showed that SYT5 functionally colocalized only with TRPML1/3 heteromer, but not with TRPML3 homomer. Moreover, SYT5 was mainly localized on the lysosomes and the autolysosomes, which is similar to TRPML1/3. In autophagy, SYT5 promoted autophagic flux, mainly by increasing autophagosome-lysosome fusion just as TRPML1/3 does. Importantly, SYT5 specifically interacted with PI4P that functions upstream of TRPML1/3. In addition, inhibition of PI4P binding to SYT5 reduced the interaction of SYT5 with TRPML1/3 and the number of autolysosomes, suggesting that SYT5 works in a complex with PI4P-TRPML1/3 in autophagosome-lysosome fusion.
Conclusions: Taken together, our data suggest that the Ca^{2+} sensor SYT5 triggers autophagosome lysosome fusion by functional interaction with TRPML1/3 in a PI4P-dependent manner.

Keywords: Autophagy, SYT5, TRPML1/3, PI4P

P20-04-01

Inhibition of excessive autophagy ameliorates mesenteric artery dysfunction of angiotensin II-induced hypertensive mice

Youngin Kwon, Soo-Kyoung Choi, Chae Eun Haam, Seonhee Byeon, Young-Ho Lee

Department of Physiology, Yonsei University, College of Medicine, Korea

Purpose: Autophagy is an intracellular degradation system that disassembles cytoplasmic components through autophagosomes with lysosomes. Recently, there are increasing interests about involvement of autophagy in cardiovascular diseases including pulmonary hypertension, atherosclerosis and myocardial ischemia. However, the involvement of autophagy in hypertension is not well understood. In the present study, we hypothesized that autophagy contributes to dysfunction of mesenteric arteries in angiotensin II (Ang II)-induced hypertensive mice.
Methods: Immunoblot, immunofluorescence and qRT-PCR were used to measure levels of autophagy-related proteins and mRNA. Blood pressure was measured using tail-cuff method in Ang II-induced hypertensive mice. To inhibit autophagic process, 3-methyladenine (30 mg/kg/day) was intraperitoneally injected.
Results: The Ang II induced increase in expression levels of beclin1 and LC3 II and decrease in expression level of p62 in vascular smooth muscle cells, which were increased by an autophagy inhibitor, chloroquine. The Ang II induced increase in expression levels of beclin1 and LC3 II and decrease in p62, which were reversed by an autophagy inhibitor, 3-methyladenine. Chloroquine and 3-methyladenine dose-dependently dilated arteries pre-contracted by U46619. To determine in vivo role of autophagy in hypertension, we treated Ang II-induced hypertensive mice with 3-methyladenine. Blood pressure was significantly higher in Ang II-treated mice compared with vehicle-treated mice. Interestingly, 3-methyladenine reduced the blood pressure in Ang II-treated mice. Endothelium-dependent relaxation was significantly impaired in Ang II-treated mice, which was recovered by treatment of 3-methyladenine. We measured fluorescence intensity of DAF-FM diacetate, an indicator of nitric oxide, to detect production of nitric oxide. The fluorescence intensity of DAF-FM diacetate was reduced in mesenteric arteries from Ang II-induced hypertensive mice, which was restored by treatment of 3-methyladenine. Expression level of p-eNOS (S1177) was decreased in mesenteric arteries from Ang II-treated mice, which was reversed by treatment of 3-methyladenine.
Conclusions: Autophagy inhibition ameliorates elevated blood pressure in Ang II-induced hypertensive mice, which is associated with improvement of endothelium-dependent relaxation. These results suggest that inhibition of autophagy exerts beneficial effects on dysfunction of mesenteric arteries in hypertension.

Keywords: Autophagy, Hypertension, Angiotensin II, Mesenteric artery, Endothelium-dependent relaxation

P20-04-02

DPP-4 class anti-diabetic drug gemigliptin induces vasodilation via the activation of voltage-dependent K^+ channels and SERCA pumps

Hee Seok Jung, Won Sun Park

Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: This study investigated the vasodilatory effects and acting mechanism of gemigliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor.
Methods: Tests were conducted in aortic rings pre-contracted with phenylephrine.
Results: Gemigliptin induced dose-dependent vasodilation of the aortic smooth muscle. Several pre-treatment groups were used to investigate the mechanism of action. While pre-treatment with large-conductance Ca^{2+}-activated K^+ channel inhibit, ATP-sensitive K^+ channel inhibitor, and inwardly rectifying K^+ channel inhibitor, had no impact on the vasodilatory effect of gemigliptin, pre-treatment with voltage-dependent K^+ (K_v) channel inhibit, effectively attenuated the vasodilatory action of gemigliptin. In addition, pre-treatment with thapsigargin, SERCA pump inhibitor, significantly reduced the vasodilatory effect of gemigliptin. cAMP/PKA-related or cGMP/PKG-related signaling pathway inhibitors did not alter the vasodilatory effect of gemigliptin. Similarly, elimination of the endothelium and pre-treatment with a NO synthase inhibitor did not change the gemigliptin effect.
Conclusions: These findings suggested that gemigliptin induces vasodilation through the activation of K_v channels and SERCA pumps independent of cAMP/PKA-related or cGMP/PKG-related signaling pathways and the endothelium.

Keywords: Gemigliptin, Voltage-dependent K^+ channel, SERCA pump, Aorta

P20-04-03

SGLT2 inhibitor empagliflozin induces vasodilation via activation of PKG and voltage-gated K^+ channels

Mi Seon Seo, Won Sun Park

Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: We investigated the vasodilatory effects of empagliflozin (a sodium-glucose co-transporter 2 inhibitor) and the underlying mechanisms using rabbit aorta.
Methods: Male New Zealand White rabbits were used in the experiment and its arterial tone was measured by using myogragh system.
Results: Empagliflozin induced vasodilation in a concentration-dependent manner independently of the endothelium. Likewise, pretreatment with the nitric oxide synthase inhibitor L-NAME or the SKca inhibitor apamin together with the IKca inhibitor TRAM-34 did not impact the vasodilatory effects of empagliflozin. Pretreatment with the adenylyl cyclase inhibitor SQ22536 or a guanylyl cyclase inhibitor ODQ or a protein kinase A (PKA) inhibitor KT5720 also did not alter the vasodilatory response of empagliflozin. However, the vasodilatory effects of empagliflozin were significantly reduced by pretreatment with the protein kinase G (PKG) inhibitor KT5823. Although application of the ATP-sensitive K^+ (KATP) channel inhibitor glibenclamide, large-conductance Ca^{2+}-activated K^+ (BKCa) channel inhibitor paxilline, or inwardly rectifying K^+ (Kir) channel inhibitor Ba^{2+} did not impact the vasodilatory effects of empagliflozin, pretreatment with the voltage-dependent K^+ (K_v) channel inhibitor 4-AP reduced the vasodilatory effects of empagliflozin. Pretreatment with DPO-1 (K_v1.5 channel inhibitor), guangxitoxin (K_v2.1 channel inhibitor), or linopirdine (K_v7 channel inhibitor) had little effect on empagliflozin-induced vasodilation. Application of nifedipine (L-type Ca^{2+} channel inhibitor) or thapsigargin (sarco-endoplasmic reticulum Ca^{2+}-ATPase pump inhibitor) did not impact empagliflozin-induced vasodilation.
Conclusions: Empagliflozin induced vasodilation in thoracic aortic rings of rabbits. This was mediated by activation of PKG and thereby K_v channels. However, the vasodilatory effects of empagliflozin were not related to endothelial cells, other K^+ channels, the cAMP/PKA pathway, Ca^{2+} channels, or SERCA pumps.

Keywords: Empagliflozin, Voltage-gated K^+ channels, PKG, Rabbit aorta

P20-04-04

Physiological function and molecular composition of ATP-sensitive K^+ channel in human gastric smooth muscle

Hyo-Yung Yun[3], Sang Eok Lee[1], Young Chul Kim[2], Dae Hoon Kim[3], Seung Myeung Son[4], Song-Yi Choi[5], Ra Young You[2], Chan Hyung Kim[6], Woong Choi[6], Hun Sik Kim[6], Sang Jin Lee[2]

[1]Department of Surgery, College of Medicine, Konyang University, Daejeon, [2]Department of Physiology, College of Medicine, CBNU, Cheongju, [3]Department of Surgery, College of Medicine, CBNU, Cheongju, [4]Department of Pathology, CBNU, Cheongju, [5]Department of Pathology, School of Medicine, Chungnam National University, Daejeon, [6]Department of Pharmacology, College of Medicine, CBNU, Cheongju, Korea

Purpose: Gastric motility is controlled by slow waves. In general, the activation of the ATP-sensitive K^+ (KATP) channels in the smooth muscle opposes the membrane excitability and produces relaxation. Since metabolic inhibitions and/or diabetes mellitus are accompanied by dysfunctions of the gastric smooth muscle, possible roles of KATP channels in the human gastric motility were examined.
Methods: We used human gastric corpus and antrum smooth muscle preparations and the mechanical activities were recorded with a conventional contractile measuring system. We also identified the subunits of the KATP channels by Western blot.
Results: Pinacidil (10 μM), a KATP opener, suppressed contractions to 30 % (basal tone to -0.2 g) of the control. The inhibitory effect of pinacidil on contraction was reversed to 59 % of the control by glibenclamide (20 μM), a KATP blocker. The relaxation by pinacidil was not affected by a pretreatment with L-arginine methyl ester, tetraethylammonium, or 4-aminopyridine. Pinacidil also inhibited the acetylcholine (ACh)-induced tonic and phasic contractions in a glibenclamide-sensitive manner (42 % and 6 % of the control, respectively). Other KATP openers such as diazoxide, cromakalim and nicorandil also inhibited the spontaneous and ACh-induced contractions. One of gastric neuropeptides, calcitonin gene-related peptide (CGRP)-induced relaxation was produced by the activation of the KATP channel in the human gastric smooth muscle. Finally, Kir 6.2 and sulfonylurea receptor 2B (SUR2B) were detected by Western blots in the gastric corpus and antrum.
Conclusions: We firstly found human gastric smooth muscle express KATP channel with Kir 6.2 and SUR2B subunits.

Keywords: Human stomach, ATP-sensitive K^+ (KATP) channel, CGRP, Gastric antrum and corpus

P20-04-05

A muscular hypotonia-associated STIM1 mutant at R429 induces abnormalities in intracellular Ca^{2+} movement and extracellular Ca^{2+} entry in skeletal muscle

Jun Hee Choi[1,2], Mei Huang[1,2], Changdo Hyun[1,2], Mi Ri Oh[1,2], Keon Jin Lee[1,2], Chung-Hyun Cho[3], Eun Hui Lee[1,2]

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Department of Biomedicine & Health Sciences, Graduate School, The Catholic University of Korea, Seoul, [3]Department of Biomedical Sciences and Pharmacology, College of Medicine, Seoul National University, Seoul, Korea

Purpose: Stromal interaction molecule 1 (STIM1) mediates extracellular Ca^{2+} entry into the cytosol through a store-operated Ca^{2+} entry (SOCE) mechanism, which is involved in the physiological functions of various tissues, including skeletal muscle. STIM1 is also associated with skeletal muscle diseases, but its pathological mechanisms have not been well addressed.
Methods: The present study focused on examining the pathological mechanism(s) of a mutant STIM1 (R429C) that causes human muscular hypotonia. R429C was expressed in mouse primary skeletal myotubes, and the properties of the skeletal myotubes were examined using single-cell Ca^{2+} imaging of myotubes and transmission electron microscopy (TEM) along with biochemical approaches.
Results: R429C did not interfere with the terminal differentiation of myoblasts to myotubes. Unlike wild-type STIM1, there was no further increase of SOCE by R429C. R429C bound to endogenous STIM1 and slowed down the initial rate of SOCE that were mediated by endogenous STIM1. Moreover, R429C increased intracellular Ca^{2+} movement in response to membrane depolarization by eliminating the attenuation on dihydropyridine receptor-ryanodine receptor (DHPR-RyR1) coupling by endogenous STIM1. The cytosolic Ca^{2+} level was also increased due to the reduction in SR Ca^{2+} level. In addition, R429C-expressing myotubes showed abnormalities in mitochondrial shape, a significant decrease in ATP levels, and the higher expression levels of mitochondrial fission-mediating proteins.
Conclusions: Therefore, serial defects in SOCE, intracellular Ca^{2+} movement, and cytosolic Ca^{2+} level along with mitochondrial abnormalities in shape and ATP level could be a pathological mechanism of R429C for human skeletal muscular hypotonia. This study also suggests a novel clue that STIM1 in skeletal muscle could be related to mitochondria via regulating intra and extracellular Ca^{2+} movements.

Keywords: STIM1, SOCE, RyR1, DHPR, Mitochondria

P20-04-06

DPP-4 inhibitor sitagliptin induces vasorelaxation via the activation of PKA and voltage-gated K^+ channels in aortic smooth muscle

Mi Seon Seo, Won Sun Park

Department of Physiology, Kangwon National University School of Medicine, Korea

Purpose: We investigated the vasodilatory effects of sitagliptin (a dipeptidyl peptidase 4 inhibitor) and the underlying mechanisms using rabbit aorta.
Methods: Male New Zealand White rabbits were used in the experiment and its arterial tone was measured by using myogragh system.
Results: Sitagliptin induced vasorelaxation in a concentration-dependent manner but the inhibition of voltage-dependent K^+ (K_v) channels by pretreatment with 4-aminopyridine (4-AP) effectively reduced this effect. By contrast, the inhibition of inward rectifier K^+ (Kir) channels by pretreatment with barium (Ba^{2+}), large-conductance calcium (Ca^{2+})-activated K^+ (BKCa) channels with paxilline, and adenosine triphosphate (ATP)-sensitive K^+ (KATP) channels with glibenclamide did not change this effect. Although the application of SQ 22536, which is an adenylyl cyclase inhibitor, also did not change this effect, treatment with KT 5720, a protein kinase A (PKA) inhibitor, effectively reduced the vasorelaxant effects of sitagliptin. ODQ, which is a guanylyl cyclase inhibitor, and KT 5823, a protein kinase G (PKG) inhibitor, did not impact the effect. Furthermore, neither the inhibition of Ca^{2+} channels by pretreatment with nifedipine nor the inhibition of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps by pretreatment with thapsigargin changed the effect. Similarly, the effects of sitagliptin were not altered by eliminating the endothelium, by pretreatment with a nitric oxide (NO) synthase inhibitor (L-NAME), or by inhibition of small- and intermediate-conductance Ca^{2+}-activated K^+ channels (SKCa and IKCa) using apamin and TRAM-34.
Conclusions: Sitagliptin induces vasorelaxation by inhibiting both membrane potential (Em)-dependent and Em-independent vasoconstriction and activating PKA and K_v channels independent of PKG signaling pathways, other K^+ channels, SERCA pumps, and the endothelium.

Keywords: Sitagliptin, PKA, Voltage-gated K^+ channels, Rabbit aorta

P20-04-07

Identification of a potential exercise-induced myokine in C. elegans

Jessica Antonio, Jae Seung Chang, Kyoung-hye Yoon
Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Korea

Purpose: Exercise not only increases muscle strength and endurance, but also leads to beneficial effects in distant parts of the body, improving metabolic functions, immunity, and even cognitive functions. Studies in humans and mice have shown that such cross talk between different tissues are in part mediated through muscle-derived protein factors secreted during physical activity, collectively known as myokines. Using exercise paradigms recently established for *C. elegans*, we asked whether such myokines are secreted from exercising muscles of *C. elegans*.
Methods: Day 1 young adult *C. elegans* were subjected to a 90-minute swimming exercise in an unseeded nematode growth medium (NGM) plate covered with M9 buffer. A non-exercised control setup was prepared by transferring day 1 young adults to an unseeded NGM plate and allowed to crawl for 90 minutes. Worms were collected after 90 minutes for RNA extraction. cDNA was synthesized and let-756 expression was measured using RT-qPCR.
Results: In our preliminary studies using RT-qPCR, we found that there is a small but highly reproducible increase in let-756 expression in exercised worms, compared to non-exercised controls. LET-756 is an FGF ortholog expressed in a few tissues in worms including the muscle.
Conclusions: We are currently characterizing let-756 expression with different exercise regimens. We plan to investigate whether it plays a role in the observed effects of exercise in *C. elegans*.

Keywords: Exercise, *C. elegans*, Myokine

P20-05-01

The role of smooth muscle cell mineralocorticoid receptor in heart failure

Seung Kyum Kim[1,2], Iris Jaffe[2]
[1]Seoul National University of Science and Technology Sports Science, [2]Tufts Medical Center Molecular Cardiology Research Institute

Purpose: Mineralocorticoid receptor (MR) antagonists decrease heart failure (HF) mortality. Preclinical studies reveal that the benefits of MR antagonists on cardiac remodeling and dysfunction are not fully explained by MR inhibition in cardiomyocytes, fibroblasts or endothelial cells. The role of MR in smooth muscle cells (SMC) in HF is unknown.
Methods: HF in mice was induced by pressure-overload using transverse aortic constriction (TAC) with 27-gauge or sham surgery in male mice with SMC-specific MR deletion (SMC-MR-KO) and their MR-intact littermates. After four weeks, the effects of SMC-MR-KO on TAC-induced HF phenotypes and mechanisms were investigated using echocardiography, intra-cardiac PV loop analysis, exercise testing, histology and gene expression.
Results: TAC induced HF phenotypes in mouse. SMC-MR-KO attenuated TAC-induced HF with improvements in ejection fraction, cardiac stiffness, chamber dimensions, intra-cardiac pressure, pulmonary edema and exercise capacity. Mechanistically, SMC-MR-KO protected from adverse cardiac remodeling as evidenced by decreased cardiomyocyte hypertrophy and fetal gene expression, interstitial and perivascular fibrosis, and inflammatory and fibrotic gene expression. TAC induced a decline in cardiac capillary density and coronary flow reserve in MR-intact mice, while these parameters were improved in SMC-MR-KO mice.
Conclusions: These results provide a novel paradigm by which MR inhibition may be beneficial in HF by blocking MR in SMC, supported by improving cardiac blood supply in the setting of pressure overload-induced hypertrophy and thereby mitigating the adverse cardiac remodeling that contributes to HF progression and symptoms.

Keywords: Heart failure, Mouse model, Smooth muscle cell, Mineralocorticoid receptor, Coronary circulation

P20-05-02

Discordant interventricular differences in the action potentials, Ca^{2+} transients, and myocyte contractions explained by the lower levels of troponin expression and Ca^{2+} buffering capacity in the right ventricle of rats

Young Keul Jeon[1], Jae Won Kwon[1], Ji Hyun Jang[1], Jae Boum Youm[2], Yin Hua Zhang[1,3,4], Sung Joon Kim[1,3,4]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, [3]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: The left and right ventricles have distinctive functional characteristics as well as the anatomical differences. However, precise understanding of interventricular differences in the excitation-contraction (E-C) coupling mechanisms and the Ca^{2+} homeostasis is still lacking.
Methods: Here, we compared the results from the right and left cardiomyocytes (RVCMs and LVCMs) of rats by using whole-cell patch clamp and IonOptix measuring cytosolic Ca^{2+} ($[Ca^{2+}]_c$) and contractility.
Results: RVCMs showed significantly shorter action potential duration (APD), with higher density of transient outward K^+ current (Ito) while similar L-type Ca^{2+} current. The contractions triggered by field stimulation were smaller and the relaxation speed was slower in RVCMs than LVCMs. However, the triggered $[Ca^{2+}]_c$ changes (Ca^{2+}-transient) were not different while the decaying rate of Ca^{2+}-transient was slower in RVCMs. Interestingly, the immunoblot analysis revealed lower expression of cardiac troponin complex (cTnC, cTnI, cTnT) proteins in RVCMs. The lower Ca^{2+} binding cTnC implied smaller Ca^{2+} buffering capacity (κS), which might be responsible for, at least partly, the similar Ca^{2+}-transient despite the shorter APD in RVCMs. *In situ* analysis using known concentration of fura-2 revealed lower κS in the RVCMs. Introduction of the higher Ito and the lower cTn into the mathematical model of rat LVCM resulted smaller contraction despite the similar Ca^{2+} transient, along with the concomitant slower relaxation and Ca^{2+} decay, similar with the experimental data from the RVCMs.
Conclusions: Taken together, we firstly show the lower expression of cTn proteins in the RVCMs, which gives a clue to explain the inter-ventricular difference in the E-C coupling kinetics.

Keywords: Right ventricle, Cardiomyocyte, Excitation-contraction coupling, Action potential, Myofilmanent

P20-05-03

How to say NO to the excitation-contraction coupling; differential expression of nNOS in the right ventricular myocytes of rats

Jae Won Kwon[1,2], Young Keul Jeon[1,2], Sung Joon Kim[1,2,3]
[1]Department of Physiology, [2]Department of Biomedical Sciences, [3]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: There are constitutive NO synthase isoforms, neuronal NOS (nNOS) and endothelial NOS (eNOS) in myocardium, regulating the calcium dynamics and contractility. Physiological and and anatomical differences between the right (RV) and left ventricle (LV) are evident. However, there is no study of the regulatory roles of NOS in RV myocytes (RVCM).
Methods: Here we compared the roles of nNOS and eNOS in the RVCMs and LV myocytes (LVCMs) isolated by using Langendorff perfusion system from S-D rats. The sarcomere shortening (ΔSL) and changes of calcium transient ($\Delta[Ca^{2+}]_i$) paced by 2 Hz field stimulation were simultaneously measured us-

ing the Ionoptix system. An nNOS-specific inhibitor, S-methyl-L-thiocitrulline (SMTC), and the non-specific NOS inhibitor, L-NG-Nitroarginine methyl ester (L-NAME) were used to dissect the roles of NOS isoforms.
Results: RVCMs showed smaller $\Delta[Ca^{2+}]_i$ than LVCMs. The treatment with SMTC and L-NAME significantly decreased the $\Delta[Ca^{2+}]_i$ of RVCMs, while only slightly decreased in LVCMs. Perplexingly, the ΔSL of RVCMs was increased by SMTC, while not altered in LVCMs. The mismatched changes in ΔSL and $\Delta[Ca^{2+}]_i$ between RVCMs and LVCMs suggested differential changes in the calcium sensitivity of contraction. In fact, the Ca^{2+} sensitivity was increased by SMTC in RVCMs while not in LVCMs. In addition, the diastolic sarcomere length (SLD) of RVCMs were decreased by SMTC and L-NAME while not in LVCMs. As for the decreased myofilament calcium sensitivity through sGC-cGMP-PKG pathway, altered phosphorylation of troponin I (TnI) was suggested. In fact, the SMTC treatment decreased the TnI phosphorylation in RVCMs, while not LVCMs. Finally, differential distribution of splice variants of nNOS were supposed; the nNOSβ phosphorylation was higher in the myofilament from RVCMs than LVCMs.
Conclusions: Taken together, we firstly provide evidence that the inhibition of nNOSβ in RVCMs increases myofilament calcium sensitivity through decreased TnI phosphorylation.

Keywords: Right ventricle, Contraction, Intracellular Ca^{2+}, Nitric oxide synthase, Ca^{2+} sensitivity

P20-05-04

Downregulation of soluble guanylate cyclase and protein kinase G in the pulmonary artery leads to the sensitization to thromboxane A2 in the monocrotaline-induced pulmonary hypertensive rats

Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: Thromboxane A2 (TXA2) mediates various physiological responses including pulmonary artery (PA) contraction. A role of TXA2 receptor (TP)-mediated signaling was suggested in the pathophysiology of pulmonary arterial hypertension (PAH). The sensitivity of arteries to contractile agonists could be modulated by relaxing signals such as nitric oxide (NO), soluble gunyate cyclase (sGC) and cGMP-dependent kinase pathway. Here we investigated the changes of TP agonist (U46619)-induced contraction of PA in the monocrotaline-induced PAH rats (MCT-PAH).
Methods: The PA segments isolated from MCT-PAH were tested for contractile responsiveness of TXA2 using Mulvany-type myograph. Differential protein expressions of NO-dependent signaling pathway were examined with western blot in whole pulmonary arterial tissues.
Results: MCT-PAH model elicit lower body weight growth, increased medial layer of arteries, and increased right ventricle mass ratio. The PA from MCT-PAH showed smaller amplitude of U46619-induced contraction despite the increased contraction induced by 80 mM KCl-induced depolarization. Interestingly, however, the threshold concentration of U46619 was lower in MCT-PAH than control. Immunoblot analysis revealed lower expression of eNOS, sGC and PKG in the PA from MCT-PAH than control. In the MCT-PAH, the higher sensitivity to U46619 was reversed by 8-Br-cGMP (25 μM), a membrane permeable analogue of cGMP, while not by an NO donor, sodium nitroprusside (SNP 30 μM). Vice versa, in the control PA, inhibition of sGC by its inhibitor (ODQ, 10 μM) lowered the threshold of U46619-induced contraction. In the presence of ODQ, SNP treatment had no effect whereas the addition of 8-Br-cGMP lowered the sensitivity to U46619. The inhibition of rho A-dependent kinase (ROCK) by Y27632 (10 μM) commonly attenuated the sensitivity to U46619 in both control and MCT-PAH. The immunoblot analysis revealed an increase of ROCK-II while not significantly changed ROCK-I in MCT-PAH.
Conclusions: The study suggests that, despite the decreased maximum contraction by U46619, the attenuation of NO-sCG-cGMP signaling increases the sensitivity to TXA2 in the PAH animal. The reduced threshold of contraction by endogenous TXA2 might have pathophysiological implication in the inwardly hypertrophic PA of PAH patients.

Keywords: Pulmonary artery smooth msucle, Thromboxane A2, sGC-cGMP-PKG signaling

P20-05-05

Diphosphorylation of myosin regulatory light chain delays relaxation in the pulmonary arteries from monocrotaline-induced pulmonary hypertensive rats

Suhan Cho[1], Hyun Namgoong[1], Hae Jin Kim[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

Purpose: Myosin regulatory light chain (MLC2) is a regulatory protein of myosin holoenzyme which account for actin-myosin (AM) complex formation. MLC2 regulates vascular smooth muscle contraction and relaxation via phosphorylation at threonine 18 (T18), serine 19 (S19) or both. Other than S19 phosphorylation, T18 phosphorylation requires specific condition such as elevated rho A-dependent kinase (ROCK) or decreased myosin light chain phosphatase (MLCP) activity. S19 phosphorylation can easily be reversible by MLCP however, T18 phosphorylation or T18S19 diphosphorylation decrease the rate of MLC2 dephosphorylation. This study was designed to elaborate the delayed relaxation in PAH-MCT model was related with diphosphorylation of pulmonary arteries (PAs).
Methods: The PA segments were isolated from PAH-MCT model and tested for relaxation after high K^+-stimulation. Delayed relaxation was examined with comparison of half relaxation time of wash-out phase. All experiments were conducted in physiological salt solution at 37°C with presence of oxygen. Differential protein expressions were examined with western blot in whole pulmonary arterial tissues.
Results: PAs from PAH-MCT model elicit delayed relaxation comparing with its age-matched control. Diphosphorylation and monophosphorylation was highly expressed in PAH-MCT model. Furthermore, expression of MLCP was decreased while increased expression of ROCK was observed. Delayed relaxation in PAH-MCT model was reversed by ROCK inhibition by Y27632 (10 μM). Invrease of the MLCP activity via PKG activation with 8-Br-cGMP (25 μM) partially restored the delayed relaxation. To elaborate MLCP and ROCK role in delayed relaxation, ODQ was used to mimic the pathological condition in normal PA. Intrinsic inhibition of sGC-cGMP-PKG signaling showed delayed relaxation in normal PAs similar to PAH-MCT and this was not abolished by SNP treatment. Delayed relaxation in normal PAs was modulated by Y27632 and 8-Br-cGMP similar manner to when these were treated in PAH-MCT model.
Conclusions: This study suggests that diphosphorylation of MLC2 is accounted to delayed relaxation in PAH animal via loss of MLCP and elevated ROCK expression. The elongation of restore time after stimulation might have pathophysiological implication in the elevated pressure of PAH patients.

Keywords: Pulmonary artery vascular smooth muscle, Diphosphorylation of MLC2, Delayed relaxation

P20-05-06

Endogenous catalase prevents obesity by attenuating hypertrophy of white adipocytes

Su-Kyung Shin, Hyun-Woo Cho, Seung-Eun Song, Seung-Soon Im, Jae-Hoon Bae, Dae-Kyu Song
Department of Physiology & Obesity-mediated Disease Research Center, Keimyung University School of Medicine, Daegu, Korea

Purpose: Oxidative stress, mitochondrial dysfunction, and obesity are in a vicious relationship to each other. However, the evidence that oxidative stress itself is responsible for the development of obesity is not yet clear.

In particular, hydrogen peroxide (H_2O_2) among reactive oxygen species is known to cause mitochondrial dysfunction and metabolic complications in various cell types by inducing oxidative stress, but has not been studied as a cause of obesity. Therefore, we used catalase knockout (CKO) mice to show the effects of excessive H_2O_2 produced endogenously on pathogenesis of mitochondrial dysfunction and obesity.
Methods: Male C57BL/6J wild type (WT) and CKO mice used in the experiments were acclimated for a week and housed under controlled 12-h dark-light cycle and a constant temperature (25℃). Four-week old male C57BL/6J mice (n = 10) and CKO mice (n = 10) were bred for up 30 weeks old with the chow diet (CD). Seven-week old male C57BL/6J mice (n = 16) and CKO mice (n = 16) were randomly assigned to four groups of 8 mice per group as CD-fed WT and CKO, high-fat diet (HFD)-fed WT and CKO. Seven-week old male CKO mice (n = 24) were divided randomly into three groups of 8 mice per group as HFD-fed CKO, HFD-fed CKO with GKT137831 treatment and HFD-fed CKO with metformin treatment. GKT137831 (50 mg/kg B.W./day) or metformin (100 mg/kg B.W./day) was dissolved in saline solution and orally administered once a day for six weeks.
Results: CKO mice had a larger weight gain compared to WT mice as they got older, and the obesity rate was rapidly deteriorated by HFD. H_2O_2 concentration and NOX4 expression are increased in adipose tissue of obese mice compared to lean mice. And AMPKα is a key modulator of mitochondria biogenesis and a cell energy sensor. HFD-fed CKO mice had reduced about 60% AMPKα phosphorylation at Thr172 in epididymal fat than HFD-fed WT mice. Using GKT137831 and metformin, we found that NOX4 and AMPKα are involved in hypertrophy of adipocyte due to oxidative stress caused by H_2O_2.
Conclusions: These findings suggest that therapeutic strategy to restrain H_2O_2/NOX4/AMPKα signaling pathway could be to prevent hypertrophy of adipocytes-induced obesity.

Keywords: Catalase, Hypertrophy, NOX4, AMPK, Obesity

P20-05-07

Deletion of TRPC6 aggravates lipid accumulation and insulin resistance in mice

Phan Anh Nguyen[1,2], Kyu-Hee Hwang[1,2], Ji-Hee Kim[1,2], Bao TN Dang[1,2], Kwon Jin[1,2], Kyu-Sang Park[1,2], Seung-Kuy Cha[1,2]

[1]Department of Physiology, [2]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea

Purpose: Deregulation of Ca^{2+} signaling has been implicated in metabolic diseases such as obesity, diabetes and non-alcoholic liver diseases. Transient receptor potential channel canonical type 6 (TRPC6) is widely expressed and its altered activity has been associated with various diabetic complications, but its role in lipid metabolism is unknown.
Results: We explored this by deletion of TRPC6 in mice and found that TRPC6 knockout resulted in lipid accumulation. Mice lacking TRPC6 showed increased body weight and higher adiposity compared to wild-type. Regarding metabolic phenotypes, TRPC6 knockout mice have increased food intake and decreased heat production, abnormal respiratory exchange ratio, and reduced physical activity. Deletion of TRPC6 aggravated glucose intolerance and insulin sensitivity in mice and showed blunted insulin signaling pathway in liver, indicating that insulin signaling and glucose homeostasis is disturbed in TRPC6 knockout mice.
Conclusions: These data suggest a new mechanism for Ca^{2+} signaling on fat metabolism and provide a clue for treatment of the metabolic diseases.

Keywords: TRPC6, Lipid accumulation, Insulin resistance

P20-05-08

Role of CXCR4 in differentiation of embryonic submandibular gland

Junchul Kim, Sang Woo Lee, Kyungpyo Park

Department of Physiology, School of Dentistry, Seoul National University and Dental Research Institute, Seoul, Korea

Purpose: The CXC Chemokine receptor type 4 (CXCR4), a member of 7-transmembrane receptor family, is known to facilitate migration of immune cells as well as stem cell homeostasis. The most distinctive feature, however, is that this receptor, alone among the chemokine receptor family members, is ubiquitously expressed in all types of cells including epithelial and mesenchymal cells. Its ligand, Stromal cell derived factor 1 (SDF1), is highly chemotactic for both lymphocytes and mesenchymal stem cells, and such roles indicate that its spatiotemporal expressions are crucial in determining whether the host system moves toward a homeostatic organogenesis or inflammation-led degradation. Many studies have been conducted on the contributions of CXCR4 to organogenesis and, specifically, to neurogenesis and angiogenesis. However, the function of CXCR4 in the early branching stages of embryo submandibular gland is yet to be explored. Here we investigated the relations between CXCR4 and glandular branching morphogenesis.
Methods: Mouse embryos were harvested on embryonic days 13 and 14 (E13-14) for isolation of submandibular glands (eSMGs). AMD3100, a small-molecule CXCR4 inhibitor, was applied to polycarbonate membrane-cultured eSMGs. In other experiments, the epithelial rudiments were separated from mesenchymes, cultured in Matrigel, and treated with AMD3100. Bright field images of eSMGs at 0, 24 and 48 h were obtained with Nikon Ti microscope. 3' mRNA sequencing was performed on E13 and E14 eSMGs for system-wide analysis of changes in gene expressions. Sequential time lapse images of AMD3100-treated E14 eSMGs were acquired with Evos FL Auto 2 cell imaging system. Marker genes for acinar and ductal progenitor cells were analyzed with immunofluorescence staining and realtime PCR.
Results: Different levels of morphological defects were observed in CXCR4-inhibited E13 and E14 eSMGs. E14 eSMGs showed a retarded growth compared to control group whereas the branching and expansion were nearly abolished in E13 eSMGs. Similar results were found in AMD3100-treated epithelial rudiments without mesenchyme. Both control and AMD3100-treated groups, however, showed no significant difference in their expressions of cleaved caspase-3. Live imaging of the eSMGs demonstrated a significant decline in branching morphogenesis from 9 h. Analysis of mRNA sequencing data revealed considerable increase in the expressions of acinar and ductal progenitor as well as of differentiation marker genes.
Conclusions: CXCR4 regulates the spatiotemporal differentiation of acinar and ductal cells in glandular branching morphogenesis.

Keywords: CXCR4, Differentiation, Embryo, Submandibular gland

P20-05-09

Atrial dilation and dysfunction are events accompanied with ventricular hypertrophy at early stage of aortic constriction

Nipa Eslenur, Qui Le, Sun-Hee Woo

Chungnam National University College of Pharmacy, Korea

Purpose: Transverse aortic constriction (TAC) method has been used to make an animal model having hypertrophy followed by heart failure to investigate pathogenesis of heart failure due to high blood pressure. Although the changes in ventricular morphology and function have been well-established in small animal models, atrial changes caused by TAC remain unknown.
Methods: We made TAC rats and assessed changes in atrial structure and function in different TAC durations together with those of ventricles using

an ultrasound cardiac imaging ("echocardiography") at 20 MHz.
Results: An M-mode analysis in long axis revealed significantly increased left ventricular posterior wall thickness (mm) at 4-6 weeks post-TAC (diastole: TAC, 2.0±0.14, n=4 vs. sham, 1.4±0.07, n=3, P<0.05; systole: TAC, 3.3±0.16 vs. sham, 2.3±0.19, P<0.05), representing ventricular hypertrophy. At this stage, ejection fraction (EF) (sham, 71.7±0.86%; TAC, 87.0±0.42%, P<0.05) and fractional shortening (FS) of ventricles were increased (sham, 41.3±0.73%; TAC, 57.0±0.71%, P<0.05).
A B-mode analysis in long axis in atria showed that left atrium (LA) was dilated at 4-6 weeks post-TAC, exhibited by two-fold increase in LA inner diameter (end diastole, mm: sham, 2.4±0.24, n=3; TAC, 4.6±0.58, n=6, P<0.05). Contractility of LA was reduced in proportion to its enlargement at ~5 weeks post-TAC (FS, %: sham, 24.1±2.1; TAC, 15.5±2.9, P<0.05). Prolonged TAC more than 20 weeks induced reverse of ventricular ejection (EF, %: sham, 70.0±1.78, n=5; TAC, 59.1±3.51, n=6, P<0.05) and FS (%: sham, 38.8±1.54; TAC, 34.0±2.58, P<0.05), in that they were reduced. At this stage, LA dilation became more severe (end diastole, mm: sham, 3.1±0.09, n=4; TAC, 5.1±0.5, n=6, P<0.05) with lower contractility compared with sham (FS, %: sham, 25.5±2.6; TAC, 13.3±3.5, P<0.05).
Conclusions: Our data suggest that atrium dilates and fails at the time when enhanced ventricular ejection occurs with its wall thickening during early stage of pressure overload.

Keywords: Atrial dilation, Transverse aortic constriction, Hypertropy, Heart failure, Echocardiography

P20-06-01

Impaired fatty acid-dependent mitochondrial oxygen consumption was modulated by reduced nNOS activity in atrial myocardium from hypertensive rat

Yu Na Wu, Yin Hua Zhang
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

Purpose: Fatty acid (FA)-dependent mitochondrial activities of atrial myocardium in hypertension (HTN) and its regulation by nitric oxide (NO) remain unidentified. Here, we have studied palmitic acid (PA) regulation of cardiac mitochondrial oxygen consumption rate (OCR) in left atrial (LA) myocardium of sham and angiotensin II-induced HTN rats and their regulations by endothelial NO synthase (eNOS) and neuronal NO synthase (nNOS). The effects were compared with those of left ventricular (LV) myocytes.
Methods: 1. Preparation of left atria and left ventricular myocytes. 2. Measurement of oxygen consumption rate from cardiac LA and LV. 3. Immunoblotting. 4. Measurement of NO production.
Results: Our results showed that OCR was greater in HTN-LA compared with that in sham-LA. PA increased OCR in sham-LA, sham-LV, and HTN-LV but reduced it in HTN-LA. Inhibition of nNOS (S-methyl-L-thiocitrulline, SMTC) or eNOS/nNOS (Nω-nitro-L-arginine methyl ester hydrochloride, L-NAME) reduced PA increment of OCR in sham-LA but exerted no effect on OCR in HTN-LA. SMTC reduced OCR in HTN-LV and L-NAME reduced OCR in sham-LV. nNOS was the predominant source of NO in LA and LV. nNOS-derived NO was increased in HTN-LA and HTN-LV. PA reduced eNOSSer1177, nNOSSer1417, and NO level in HTN-LA but exerted no effect in sham-LA. In contrast, PA increased NO in HTN-LV and enhanced nNOSSer1417 but reduced NO level in sham-LV without affecting eNOSSer1177, eNOSThr495, or nNOSSer1417. 2-Bromopalmitate (2BP), which blocks the S-palmitoylation of target proteins, prevented PA-dependent decrease of nNOSSer1417 and OCR in HTN-LA. In HTN-LV, 2BP prevented PA-induced OCR without affecting nNOSSer1417.
Conclusions: Our results reveal that FA-induced mitochondrial activity in atrial myocardium is impaired in HTN which is mediated by reduced nNOS activity and NO bioavailability. Metabolic dysregulation may underlie diastolic dysfunction of atrial myocardium in HTN.

Keywords: Left atrium, Hypertension, nNOS, Oxygen consumption

P20-06-02

Lactate accelerates fatty acid oxidation as an anti-obesity metabolite

Jin-Ho Koh[1], Sol-Yi Park[1], Soo-Ryun Jung[1], Jong-Yeon Kim[1], Yong-Woon Kim[1], Hoon-Ki Sung[2], So-Young Park[1], Kyung-Oh Doh[1]
[1]Department of Physiology, Yeungnam University College of Medicine, [2]Department of Laboratory Medicine and Pathobiology, University of Toronto

Purpose: Lactate is a key factor to regulate the shuttling of the energetic substrate between glycolysis and oxidative phosphorylation. Thus, we speculated that lactate regulates the fat metabolism axis of muscle-liver-adipose tissue, and may reduce fat mass. The aim of this study was to evaluate the specific mechanisms by which lactate mainly regulates fat oxidation in muscle and which influences fat metabolism in liver and adipose tissue in normal chow diet (NCD) and high-fat diet (HFD).
Methods: Lactate was administered in the mice via intraperitoneal, and muscle, liver, and adipose tissue were isolated. And lactate also treated in C2C12 to evaluate specific mechanisms.
Results: The study reveals an increase in oxygen consumption and energy expenditure due to fat metabolism on lactate administration. Further, lactate increases angiogenesis via VEGFa and facilitates fatty acid uptake to the muscle. In obese mice, lactate diminishes glycolysis and accelerates fatty acid oxidation in muscles via uncoupling protein 3 (UCP3)-induced mitochondrial uncoupling enhancement. In obese mice liver, lactate increases fatty acid oxidation to generate β-hydroxybutyrate via fibroblast growth factor 21. Lactate enhances fatty acid oxidation in the muscle liver axis which induces lipolysis via hormone-sensitive lipase activation in the adipose tissue. Finally, we demonstrate that lactate alters the mitochondrial NAD+/NADH redox state through monocarboxylate transporter-1, and changes in the NAD+/NADH ratio lead to UCP3 and VEGF mRNA expression.
Conclusions: These findings suggest that lactate induced by administration or exercise training accelerates fatty acid oxidation via alteration of mitochondria in the muscle, and lactate influences fat metabolism in liver and adipose tissue, eventually decreasing fat mass. Thus, lactate can be used as an exercise-mimic for the treatment of metabolic diseases.

Keywords: Lactate, Fat metabolism, Mitochondrial uncoupling, Skeletal muscle, Exercise

P20-06-03

Estrogen-regulated miR-10a/b as gender- and diabetes-associated biomarkers in Korean diabetes mellitus patients

Min Seob Kim[1], Hyun Seok Choi[1], Moxin Wu[1], JiYeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[2], Han-Seung Ryu[2], Suck Chei Choi[2], Tae Yang Yu[3], Se Eun Ha[4], Seungil Ro[4], Moon Young Lee[1]
[1]Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Department of Gastroenterology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, [3]Department of Endocrinology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, Korea, [4]Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, USA

Purpose: Diabetes prevalence is rapidly rising in Asian countries, including South Korea. The incidence of type 2 diabetes is related to age and gender-specific hormones as it is higher in men than in women and increased with aging. Interestingly, there are more male diabetes patients before 45, and the pattern is reversed after age 45, with more female patients showing diabetic phenotypes. Previously, we found that miRNAs, miR-10a, and miR-10b, were significantly depleted in KIT+ pancreatic b cells from animal model diabetic samples. In further studies, injection of a synthesized miR-10a-5p mimic or miR-10b-5p mimic into diabetic mice markedly rescued the diabetic phenotypes by lowering blood glucose and improving insu-

lin production and sensitivity. We also found that the mir-10a or mir-10b knockout (KD) effect in mice is sex-dependent. mir-10b KO in male mice, but not female mice, led to the development of diabetes. mir-10b KO females eventually developed diabetes when both ovaries were removed where estrogen was lost.
Furthermore, expression of miR-10a/b was increased by estrogen and significantly reduced in ovariectomy mice. Based on animal studies, we hypothesized that females are protected from diabetes by estrogen-regulated miR-10a/b. In this study, we examined if miR-10a/b are differentially expressed in Korean male and female diabetic patients and healthy controls and if the miRNAs are also differentially expressed in the female patients and healthy controls before and after menopause.
Methods: We analyzed blood plasma samples of 100 Korean male and female diabetic patients and 40 healthy participants, which were obtained from the Biobank in the Wonkwang University Hospital in Korea. Fasting A1C, glucose, insulin, and C-peptide were measured. For miR-10a/b measurement in plasma, a TaqMan probe-based qPCR assay was performed. Expression levels of each miRNA were normalized by an endogenous control snoRNA.
Results: Substantial increases in the plasma concentration of A1C, glucose, C-peptide, and insulin from both male and female diabetes patients compared to healthy controls were confirmed. The expression of miR-10a/b was significantly decreased in both males and females with diabetes. Expression levels of both miR-10a/b were higher in healthy females than males, and the miRNAs levels were even higher in younger females before menopause (45 years old).
Conclusions: These results suggest that estrogen-induced miR-10a/b may protect females from diabetes before menopause, but postmenopausal females may lose the protection by reduction of miR-10a/b. Therefore, estrogen-regulated miR-10a/b may serve as gender and diabetic biomarkers.

Keywords: Type 2 diabetes, miR-10a/b, Menopause, Estrogen

P20-06-04

The suppressive effect of carbon monoxide on ANP secretion via Akt pathway

Weijian Li, Suhn Hee Kim

Department of Physiology, Research Institute for Endocrine Sciences Jeonbuk National University Medical School, Jeonju, Korea

Purpose: Similarly with hydrogen sulfide (H_2S) and nitric oxide (NO), carbon monoxide (CO) which is one of the cytoprotective byproducts of heme oxygenase (HO) and shows pleietrepie bebeficial function on various animal models has been regarded as a gaseous bioactive substance in numerous body systems. However, it is known as "silent killer" on account of high affinity to hemoglobin at high concentrations. It has been indicated that CO treatment exerts anti-inflammatory, anti-apoptotic, anti-hypertensive, cellular proliferation programs and vasodilatory effects in many experimental models at a low concentration. Recently, it has been reported that CORMs have cardioprotective actions against Ischemia-Reperfusion Injury *in vitro* and *in vivo*, reversal effect on pulmonary hypertensive rats, vasorelaxant effect on two-kidney, one-clip hypertensive rats, and spontaneously hypertensive rats. Nevertheless, whether CORMs have effects on ANP secretion remains unclear. The purpose of the present study is to explore the effect of CORMs on ANP secretion and to detect its signaling pathway.
Methods: The atria were perfused for 80 min to stabilize secretion of ANP. The atrial perfusate was collected at 2-min intervals at 4 ℃ for 10 min while paced at 1.2 Hz. To induce atrial stretch, the height of the outflow catheter was increased from 5.0 to 7.5 cmH_2O by a connecting 2.5-cm-long catheter after a 10-min collection period and the atrial perfusate was collected for 50 min.
Results: CORM-2 (50μM) but not CORM-3 decreased high stretch induced-ANP secretion significantly in normoxic condition. In addition, hemin, a potent inducer of HO-1, decreased high stretch induced-ANP secretion which is similar with CORM-2 did. Moreover, the decrease of CORM-2 induced-ANP secretion did not be reversed by the pretreatment of PD98059 (MAPK inhibitor) and LY294002 (PI3K inhibitor) but SH-5, a Akt inhibitor.
Conclusions: These results suggest that CORM-2 decreases ANP secretion in high stretch condition via Akt pathway.

Keywords: CORM-2, Akt pathway, ANP, MAPK pathway and PI3K pathway

P20-06-05

Ca^{2+} inhibition on proteasomal degradation of mitochondrial proteins in mouse brown adipocytes

Dat Da Ly[1,2], Nuoc Non Tran[3], Hanh Minh Thi Nguyen[1,2], Nhung Thi Nguyen[1,2], Minho Shong[4], Byung-Hoon Lee[3], Kyu-Sang Park[1,2]

[1]Mitohormesis Research Center, [1]Department of Physiology, Yonsei University Wonju College of Medicine, [3]Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology, [4]Research Center for Endocrine and Metabolic Diseases, Chungnam National University School of Medicine, Korea

Purpose: Investigate the molecular mechanism of acute sympathetic stimulation on thermogenesis and mitochondrial activities in mouse brown adipocytes, which may imply a therapeutic meaning for overweight/obesity patients and weight management.
Methods: Western blot, End-point PCR, qPCR were performed to check mitochondrial transcriptional level under acute norepinephrine (NE) activation.
Nanolive imaging was used to demonstrate lipid accumulation and mitochondria distribution in differentiated brown adipocytes.OCR/ECAR measurement by Seahorse, ROS, ATP measurement, Live-cell imaging were conducted to illustrate mitochondrial activities and Ca^{2+} signaling change under NE/dibutyryl-cyclic AMP/Forskolin treatment with or without the presence of Ca^{2+} chelator.
Pulse-chase assay was done to show the turnover rate of mitochondrial proteins under translation (cycloheximide) or proteasome inhibitors (MG132, lactacystin), LLVY-AMC assay was carried out to examine proteasome activity under NE treatment with or without Ca^{2+} chelator.
Results: Brown fat mass declines with ageing, while thermogenic induction in brown adipose tissue (BAT) improves insulin sensitivity and adiposity. We investigated the mechanism of acute sympathetic regulation on BAT thermogenesis. Norepinephrine (NE) activated mitochondrial respiration with upregulations of uncoupling protein 1 (UCP1) and mitochondrial calcium uniporter (MCU) within 1 hour, whilst the transcriptional level of mitochondrial proteins remained unchanged. BAT has an active mitochondrial protein turnover rate. In the presence of MG132 and lactacystin, proteasome inhibitors, UCP1 and MCU maintained high protein levels without further increases by NE. Forskolin, an activator of adenylate cyclase, or dibutyryl-cyclic AMP (db-cAMP), a membrane permeable cAMP derivative, mimicked the NE-induced upregulations of mitochondrial proteins and respiration, all of which were blocked by inhibition of protein kinase A. However, neither NE- nor db-cAMP-induced activation was affected by the inhibition of mitochondrial fatty acid uptake. NE acutely increased cytosolic Ca^{2+} and inhibited proteasome activities. Intriguingly, Ca^{2+} chelator (BAPTA-AM) abolished NE-induced mitochondrial activities and protein abundance as well as NE-reduced proteasomal activities.
Conclusions: Besides the well-known canonical pathway of norepinephrine induction via cAMP-PKA axis, we suggest a novel molecular mechanism of sympathetic stimulation on acute thermogenesis of brown and beige adipocytes, which is the acute activation of mitochondrial respiration and biogenesis mediated by intracellular Ca^{2+}-dependent proteasome inhibition, implying a potential therapeutic application in obesity and various metabolic diseases.

Keywords: Brown adipose tissue (BAT), Browning, Thermogenesis, Mitochondria, Uncoupling protein 1 (UCP1), Sympathetic stimulation, Norepinephrine (NE), Mitochondrial calcium uniporter (MCU)

P20-06-06

Ca^{2+}-activated mitochondrial biogenesis and functions improve stem cell fate in Rg3-treated human mesenchymal stem cells

Taeui Hong[1,2], Dat Da Ly[1,2], Su Jung Park[1,3], Young Woo Eom[4], Moon Young Kim[1,3,4], Soon Koo Baik[1,3,4], Kyu-Sang Park[1,2]

[1]Mitohormesis Research Center, [2]Department of Physiology, [3]Department of Internal Medicine, [4]Regeneration Medicine Research Center, Yonsei University Wonju College of Medicine, Korea

Purpose: Although mitochondrial functions are essential for cell survival, their critical roles in stem cell fate, including proliferation, differentiation, and senescence, remain elusive. Ginsenoside Rg3 exhibits various biological activities and reportedly increases mitochondrial biogenesis and respiration.
Methods: To confirm cell proliferation, MTT assay was performed and population doubling time was calculated. Gene expression was evaluated by immunoblotting and real-time PCR. The degree of aging was confirmed by Senescence-associated β-galactosidase staining. To investigate differentiation potency, induced cell differentiation (osteogenesis and adipogenesis). After differentiation, lipid or calcuim amount were confirmed through Oil-red O staining and alizarin-red S staining. Oxygen consumption rate and ExtraCellular acidification rate were evaluated by Seahorse XF cell mito stress test or glycolysis stress test. Reactive oxygen species and Ca^{2+} were measured using ROS or Ca^{2+} sensing dye.
Results: Herein, we observed that Rg3 increased proliferation and suppressed senescence of human bone marrow-derived mesenchymal stem cells. Osteogenic, but not adipogenic, differentiation was facilitated by Rg3 treatment. Rg3 suppressed reactive oxygen species production and upregulated mitochondrial biogenesis and antioxidant enzymes, including superoxide dismutase. Consistently, Rg3 strongly augmented basal and ATP synthesis-linked respiration with high spare respiratory capacity. Rg3 treatment elevated cytosolic Ca^{2+} concentration contributing to mitochondrial activation. Reduction of intracellular or extracellular Ca^{2+} levels strongly inhibited Rg3-induced activation of mitochondrial respiration and biogenesis.
Conclusions: Taken together, Rg3 enhances capabilities of mitochondrial and antioxidant functions mainly through a Ca^{2+}-dependent pathway, which improves the proliferation and differentiation potentials and prevents the senescence of human mesenchymal stem cells.

Keywords: Mesenchymal stem cells, Ginsenoside Rg3, Cellular senescence, Oxidative stress, Mitochondria

P20-06-07

The effects of neurophysiological social engagement system DMT on psychological and physical·physiological function of the juvenile delinquent

Eon-Ah Choo[1], Hyun-Woo Nam[2], Jeong-Beom Lee[3]

[1]Department of Physiology, College of Medicine, Soonchunhyang University,
[2]Department of Youth Education and Counseling, Soonchunhyang University,
[3]Department of Physiology, College of Medicine, Soonchunhyang University, Korea

Purpose: This study verifies the effects of the neurophysiological social engagement system DMT ('SES-DMT') on psychological and physical·physiological functions of the juvenile delinquent and applies neurophysiological SES-DMT activities to orthodontic education to identify psychological and physical·physiological aspects of male and female adolescents and the effect of environmental adaptation.
Methods: The pre- and post-program change was compared to 8 experimental groups and 8 control groups in 16 female adolescents. The neurophysiological SES-DMT program was conducted twice a week, 12 times a week, and the control group did not participate in any other healing and counseling programs in addition to the regular curriculum in the juvenile school. Psychological changes were analyzed by Beck's Depression Inventory (BDI), behavioral and psychological changes, and physical and physiological changes were analyzed by WBCs and their subtypes as well as serotonin and cortisol.
Results: Analyses of the collected data through the paired t-test and repeated measure design ANOVA verify the following: First, the BDI of the experimental group indicated the subjects being very severely depressed prior to the program, but significantly decreased after applying neurophysiological SES-DMT. The average BDI after the program decreased significantly compared to the control group, which was found to maintain a very severe depressive state ($P<0.05$). Second, the level of serotonin in the experimental group increased to a significant level after applying neurophysiological DMT ($P<0.05$) while that in the control group statistically significantly decreased ($P<0.01$). Serotonin levels after 3 months in the experimental group were significantly higher ($P<0.05$). Third, cortisol in the experimental group decreased to a significant level after neurophysiological SES-DMT intervention ($P<0.05$) while that in the control group statistically significantly increased ($P<0.05$). Cortisol levels after 3 months in the experimental group were significantly lower ($P<0.05$).
Conclusions: In conclusion, the neurophysiological SES-DMT program has a statistically meaningful impact both on psychological factors in female adolescents, such as depression, physical and emotional stabilization, emotional control, and social relationships, and physiological factors, such as WBCs and their subtypes, serotonin, and cortisol.

Keywords: Neurophysiological social engagement system DMT, Juvenile delinquent, Depression, Serotonin, Cortisol

P20-06-08

Senotherapeutic agent attenuates obesity and insulin resistance in high-fat diet-fed mice

Han-Byul Jung, Hye-Na Cha, Min-Ah Choi, Soyoung Park, Eok-Cheon Kim, Jae-Ryong Kim, So-Young Park

Department of Physiology, Yeungnam University of Medicine, Korea

Purpose: Senotheraputics is emerging strategy for the treatment of chronic diseases, such as diabetes, cardiovascular diseases, and cancers. Therefore, we searched and found an effective senotherapueitc agent (X) to improve obesity-related insulin resistance.
Methods: Mice were fed with chow diet or high-fat diet (HFD) for 6 weeks and then mice in HFD group were randomly divided into two groups: phosphate buffered saline (PBS)-treated mice or X-treated mice. Then, the mice were further fed with HFD for 6-8 weeks.
Results: In 3T3L1 adipocytes, X reduced SA-beta-gal staining and triglyceride contents, which was accompanied with reduced p21 protein expression. Increases in LDH activity in X-treated cells suggests senolytic effect of X on adipocytes. The administration of X prevents further increases in body weight and fat mass following HFD, whereas X increases skeletal muscle mass. However, there was no difference in cumulative food intake. The new agent improved insulin resistance, which was contributed by increased glucose uptake in the skeletal muscle and adipose tissue. X reduced the number of large adipocytes, crown like structures and inflammation in adipose tissue, which was followed by reduced SA-beta-gal staining and p53 protein levels. The protein levels of lipolytic enzymes and PPAR-gamma were increased in the white adipose tissue of X-treated mice. Acute cold exposure increased browning-related gene expression. In brown adipose tissue, UCP-1 protein levels were increased in X-treated mice. X increased oxygen consumption and energy expenditure.
Conclusions: These results suggest that a new senotherapeutic agent suppresses diet-induced obesity and improved insulin resistance.

Keywords: Anti-obesity, Anti-diabetic, Senotherapeutic, Browning, Lipolysis

P20-06-09

Effect of melatonin on Dapagliflozin-induced diabetic ketoacidosis in type 2 diabetic mice

Jae-Hyung Park[1], Hae-Min Shim[1], Hochan Cho[2]

[1]Department of Physiology, [2]Division of Endocrinology, Department of Internal Medicine, Keimyung University School of Medicine, Korea

Purpose: Sodium-glucose cotransporter-2 inhibitors (SGLT2i) are effective hypoglycemic agents that can induce glycosuria. However, there are increasing concerns that they might induce diabetic ketoacidosis. This study investigated the effect of melatonin on SGTL2i-induced ketoacidosis in insulin-deficient type 2 diabetic (T2D) mice.
Methods: To obtain an insulin-deficient T2D mouse model, high fat-fed mice were injected with low-dose streptozotocin and nicotinamide. 6-week-old C57BL/6 mice were fed a high-fat diet (HFD) for 4 weeks. High fat-fed mice were injected with streptozotocin and nicotinamide and were then fed a HFD continuously for 2 weeks. Mice were treated with an intraperitoneal injection of 1 mg/kg dapagliflozin after 4-hour food deprivation. Melatonin (0, 1, 10, 20, or 50 mg/kg) was administered intraperitoneally shortly before the treatment with dapagliflozin. Six hours after the injection of dapagliflozin, plasma, urine, and tissue samples were collected.
Results: The SGLT2i dapagliflozin reduced blood glucose level and plasma insulin concentrations in T2D mice, but induced increases in the concentrations of plasma β-hydroxybutyrate, acetoacetate, and free fatty acid and a decrease in the concentration of plasma bicarbonate, resulting in ketoacidosis. Melatonin inhibited dapagliflozin-induced ketoacidosis without inducing any change in blood glucose level or plasma insulin concentration. In white adipose tissue, melatonin inhibited lipolysis and downregulated phosphorylation of PKA, HSL, and perilipin-1. In liver tissue, melatonin suppressed cellular cyclic AMP levels and downregulated phosphorylation of PKA, AMPK, and acetyl-CoA carboxylase (ACC). In addition, melatonin increased hepatic ACC activity, but decreased hepatic CPT1a activity and acetyl-CoA content. These effects of melatonin on lipolysis and hepatic ketogenesis were blocked by pretreatment with melatonin receptor antagonist or PKA activator.
Conclusions: Collectively, these results suggest that melatonin can ameliorate SGLT2i-induced ketoacidosis by inhibiting lipolysis and hepatic ketogenesis though cyclic AMP/PKA signaling pathways in T2D mice. Thus, melatonin treatment may offer protection against SGLT2i-induced ketoacidosis.

Keywords: Dapagliflozin, Diabetic ketoacidosis, Melatonin, SGLT2 inhibitor, Type 2 diabetes

P20-06-10

Melatonin prevents transforming growth factor-β1-stimulated transdifferentiation of renal interstitial fibroblasts to myofibroblasts by suppressing reactive oxygen species-dependent mechanisms

Jae-Hyung Park[1], Jung-Yeon Kim[2], Jaechan Leem[2]

[1]Department of Physiology, Keimyung University School of Medicine, [2]Department of Immunology, Catholic University of Daegu School of Medicine, Korea

Purpose: Accumulating evidence suggests that the pineal hormone melatonin displays protective effects against renal fibrosis, but the mechanisms remain poorly understood. Here, we investigate the effect of the pineal hormone on transdifferentiation of renal fibroblasts to myofibroblasts invoked by transforming growth factor-β1 (TGF-β1).
Methods: The rat kidney interstitial fibroblast cell line NRK-49F cells were used. To explore the effect of melatonin on TGF-β1-stimulated activation of fibroblasts, the cells were incubated with TGF-β1 (5 ng/mL) for 24 h after pretreatment with melatonin (0.1 mM or 1 mM) for 30 min in the presence or absence of luzindole (20 μM or 100 μM).
Results: Increased proliferation and activation of renal interstitial fibroblasts after TGF-β1 treatment were attenuated by melatonin pretreatment. Mechanistically, melatonin suppressed Smad2/3 phosphorylation and nuclear co-localization of their phosphorylated forms and Smad4 after TGF-β1 stimulation. In addition, increased phosphorylations of Akt, extracellular signal-regulated kinase 1/2, and p38 after TGF-β1 treatment were also suppressed by the hormone. These effects of melatonin were not affected by pharmacological and genetic inhibition of its membrane receptors. Furthermore, melatonin significantly reversed an increase of intracellular reactive oxygen species (ROS) and malondialdehyde levels, and a decrease of the reduced glutathione/oxidized glutathione ratio after TGF-β1 treatment. Finally, TGF-β1-induced proliferation and activation were also suppressed by N-acetylcysteine.
Conclusions: Altogether, these findings suggest that the pineal hormone melatonin prevents TGF-β1-induced transdifferentiation of renal interstitial fibroblasts to myofibroblasts via inhibition of Smad and non-Smad signaling cadcades by inhibiting ROS-mediated mechanisms in its receptor-independent manner.

Keywords: Transforming growth factor-β1, Fibroblast-myofibroblast transdifferentiation, Reactive oxygen species, Renal interstitial fibroblasts, Melatonin

P20-06-11

Melatonin inhibits transforming growth factor-β1-induced epithelial-mesenchymal transition in AML12 hepatocytes

Jae-Hyung Park[1], Jung-Yeon Kim[2], Jaechan Leem[2]

[1]Department of Physiology, Keimyung University School of Medicine, [2]Department of Immunology, Catholic University of Daegu School of Medicine, Korea

Purpose: Recent studies showed that melatonin, a well-known pineal hormone that modulates the circadianrhythm, exerts beneficial effects again-stliver fibrosis. However, mechanisms for its protective action against the fibrotic processes remain incompletely understood. Here, we aimed to explore the effects of the hormone on transforming growth factor-β1 (TGF-β1)-stimulated epithelial-mesenchymal transition (EMT) in AML12 hepatocytes.
Methods: AML12 cells, a mouse hepatocyte cell line, were used. To examine the effects of melatonin on EMT stimulated by TGF-β1, cells were preincubated with melatonin (0.1 mM or 1 mM) or 0.1% dimethyl sulfoxide (DMSO; vehicle) for 30 min and then treated with TGF-β1 (2 ng/mL) for 24 or 48 h.
Results: Pretreatment with melatonin dose-dependently reversed downregulation of an epithelial marker and upregulation of mesenchymal markers after TGF-β1 stimulation. Additionally, melatonin dose-dependently suppressed an increased phosphorylation of Smad2/3 after TGF-β1 treatment. Besides the canonical Smad signaling pathway, an increase in phosphorylation of extracellular signal-regulated kinase 1/2 and p38 was also dose-dependently attenuated by melatonin. The suppressive effect of the hormone on EMT stimulated by TGF-β1 was not affected by luzindole, an antagonist of melatonin membrane receptors, suggesting that its membrane receptors are not required for the inhibitory action of melatonin. Moreover, melatonin suppressed elevation of intracellular reactive oxygen species (ROS) levels in TGF-β1-treated cells. Finally, TGF-β1-stimulated EMT was also inhibited by the antioxidant N-acetylcysteine.
Conclusions: Collectively, these results suggest that melatonin prevents TGF-β1-stimulated EMT through suppression of Smad andmitogen-activated protein kinase signaling cascades by deactivating ROS-dependent mechanisms in a membrane receptor-independent manner.

Keywords: Melatonin, Transforming growth factor-β1, Liver fibrosis, Epithelial-mesenchymal transition, Reactive oxygen species

P20-06-12

The effects of thermotherapy on the C-reactive protein, FGF-21, adiponectin, irisin and orexin in obese subjects

Hye-Jin Lee[1], Mi-Young Lee[2], Jeong-Beom Lee[3]

[1]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, [2]Global Graduate School of Healthcare, Soonchunhyang University, Asan, [3]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan, Korea

Purpose: Many women gaining weight as they transition as they approach menopause. Weight gain during menopause is predominantly due to a reduction in physical activity. For women who are obesity menopause women, appropriate therapy about controlling weight and increasing lipid metabolism is required to prevent metabolic syndrome.
Methods: The main aim of this study was to analyze the how thermotherapy (half bath in hot water, 42±0.5°C, 3-4 times/week, 30 min/time, 15 times for 4 weeks) affects the orexin A (OxA), adiponectin and c-reactive protein (CRP) expression in menopausal overweight-obese women (n=15, age, 55.12±3.27 yrs; height, 157.92±4.87 cm; weight, 62.19±7.52 kg).
Results: OxA, FGF-21, adiponectin and CRP were significantly increased after thermotherapy.
Conclusions: We found that the increased lipid metabolism with thermotherapy was associated with the OxA, FGF-21 and adiponectin. Also, the role of OxA on lifestyle and eating behavior in menopausal overweight-obese women can be further explored to identify obesity and lifestyle-related diseases.

Keywords: Thermal sweating, Heat acclimatization, Sweat glands density, Sweat gland output, Tropical, Temperate

P20-06-13

Mitochondrial activation by acute exposure of thyroid hormone in brown adipocytes

Minh-Hanh Thi Nguyen[1,2], Dat Da Ly[1,2], Nhung Thi Nguyen[1,2], Ha Thu Nguyen[1,2], Soo-Jin Kim[1,2], Seung-Kuy Cha[1,2], Kyu-Sang Park[1,2]

[1]Mitohormesis Research Center, [2]Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

Purpose: Thyroid hormone including 3,5,3′-triiodothyroinine (T3) has been well-known for its wide spectrum of genomic actions on cellular metabolism and bioenergetic regulation in various tissues. However, the nongenomic actions of T3, which are initiated rapidly within several minutes or a few hours, have been reported, but not been understood completely yet.
Results: Immortalized mature brown adipocytes were treated with T3 for 30 minutes. After 30 minutes of T3 treatment on brown adipocytes, oxygen consumption rate (OCR) including basal, maximal, proton-leak and ATP-linked OCR was increased significantly accompanied with increased protein abundance of uncoupling protein 1 (UCP1). T3 depolarized resting mitochondrial membrane potential (ΔΨm), but augmented oligomycin-induced hyperpolarization of ΔΨm in brown adipocytes after T3 incubation for 30 minutes. The mammalian target of rapamycin (mTOR) was activated by T3, leading to the inhibition of autophagy. Rapamycin, as a mTOR inhibitor, blocked UCP1 upregulation and autophagy inhibition induced by T3, suggesting a relation between the autophagy inhibition and the higher UCP1 level. T3 increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in Fura-2-loaded brown adipocytes. The pretreatment of BAPTA-AM, an intracellular Ca^{2+} chelator, abrogated T3-induced increases in mTOR activation, UCP1 upregulation, and OCR increase. Furthermore, edelfosine, a phospholipase C (PLC) inhibitor, prevented T3's actions on $[Ca^{2+}]_i$, ΔΨm, OCR and mTOR activity.
Conclusions: We suggest that short-term exposure of T3 induces mitochondrial activation and UCP1 upregulation via PLC-mediated $[Ca^{2+}]_i$ increases in brown adipocytes.

Keywords: Brown adipose tissue, Thyroid hormone, Mitochondria, Uncoupling protein 1 (UCP1), Ca^{2+} signaling

P20-06-14

The transcriptome analysis of the isolated brain microvessles in endothelial-specific mitochondrial OXPHOS defect mouse

Min Joung Lee[1,2,4], Yunseon Jang[1,2,4], Jiebo Zhu[1,2,4], Da Hyun Go[1,2,4], Changjun Seo[1,2,4], Eunji Namgung[1,2,4], Xianshu Ju[1,4], Yu Lim Lee[1,4], Jianchen Cui[1,4], Hyoeun Kang[1,2], Woosuk Chung[1,4,5], Hyeongseok Kim[1,2], Gi Ryang Kweon[1,2], Jun Young Heo[1,2,4]

[1]Department of Medical Science, [2]Department of Biochemistry, [3]Department of Anesthesiology and Pain Medicine, Chungnam National University School of Medicine, [4]Infection Control Convergence Research Center, College of Medicine, Chungnam National University, [5]Department of Anesthesiology and Pain Medicine, Chungnam National University Hospital, Korea

Purpose: Cerebral endothelial cells (ECs) which composed of blood-brain barrier (BBB) have more mitochondria that ECs of peripheral capillaries. The dysfunction of mitochondrial oxidative phosphorylation causes the defects in the maintenance of BBB integrity. However, the genes and pathways related in mitochondria of ECs are not investigated well. Here, we report the profiling of the transcriptome in TEKCRIF1 mice which show BBB disruption and abnormal behavior to understand the molecular mechanism that regulate BBB maintenance by mitochondrial function.
Methods: We isolated the cerebral microvessels containing endothelial cells, pericytes and astrocyte endfeet from WT and TEKCRIF1 mice. We analyzed the difference of gene expression between two groups.
Results: We found that Crif1 deletion in ECs causes the change of the gene expression related to metabolism and cell cycle, inflammation in ECs, pericytes and astrocytes between WT and TEKCRIF1 mice.
Conclusions: The identification of gene functions and related pathways in TEKCRIF1 mice suggests therapeutic targets to help maintain BBB integrity in cerebrovascular disease and neurodegenerative disease.

Keywords: Endothelial cell, Mitochondria, Trascriptome, Blood-brain barrier

P20-06-15

Effects of thermotherapy on plasma irisin levels and FGF21 and metabolic of glucose regulating factors in overweight and obesity

RyeoWon Kwon[1], HyunWoo Nam[2], JinSun Park[1], EonAh Choo[1], HyeJin Lee[1], Jeong-Beom Lee[1]

[1]Department of Physiology, College of Medicine, Soonchunhyang University, [2]Department of Youth Education and Counseling, Soonchunhyang University, Korea

Purpose: In this study we verified the effect of thermotherapy on the expression of irisin and fibroblast growth factor 21 (FGF21). In addition, we investigated the associations between irisin and glucose homeostasis in overweight and obesity.
Methods: The subjects who did not experience thermotherapy had overweight and obesity patients. The participants underwent thermotherapy of half bath in hot water. Venous blood samples were obtained before and after thermotherapy.
Results: Thermotherapy was effective for the expression of irisin and FGF21. After thermotherapy blood glucose and HbA1c significantly decreased. In addition, weight, waist size (%), and body fat were also decreased and free fatty acid was increased.
Conclusions: In conclusion, thermotherapy was effective in elevating plasma irisin and FGF21, and in lowering blood glucose and HbA1c in overweight and obese human. Therefore, thermotherapy may be expected to help reduction of body fat, prevent obesity and maintain normal glucose metabolism.

Keywords: Thermotherapy, Irisin, FGF21, Glucose, Obesity

P20-07-01

Blood pressure and feeding behavior change in tas2r108 knock-out mouse

Ji-Young Heo[1], Ki-Myung Chung[1,2], Young-Kyung Cho[1,2], Kyung-Nyun Kim[1,2]

[1]Department of Physiology & Neuroscience, [2]Research Institute for Oral Science, Gangneung-Wonju National University, Korea

Purpose: Taste plays an important role as the selection of nutrient and energy source essential for survival or the avoidance of probable harmful substances or spoiled food. Among the five basic tastes, bitter taste is important for suppressing the intake of possible toxic substances such as life-threatening toxic substances. Bitter taste is detected in the vertebrates by type 2 taste receptors (t2rs). Tas2r108 of 35 murine t2rs was found to be most highly expressed in various exocrine tissues as well as the tongue. The physiological function of tas2r108 would be elucidated by pursuing change in blood pressure (BP) during aging in tas2r108 knock-out (KO) mice.
Methods: Tas2r108 KO mice were produced with CRISPR/Cas9 technology. BPs were measured with noninvasive blood pressure using volume pressure recording (VPR) sensor technology. Wild type (WT) C57BL/6 mice or tas2r108 KO mice from 8-week-old were used.
Results: From 8 weeks to 26 weeks age, the difference in BPs between WT and KO mice was not found. The body weight of both groups were gradually increased, however, were not different from each other.
Conclusions: The results suggest that at least by young adult periods tas2r108 KO would not elicit change in metabolism or feeding behavior. However, more continuous research is needed to confirm the exact physiological role of bitter taste.

Keywords: Tas2r108, Bitter taste, Blood pressure, Metabolism, Feeding behavior

P20-07-02

Study the relationship of isocytrate dehydrogenase 2 and mitophagy in human umbilical vein endothelial cells

Su-Jeong Choi[1,2], Harsha Nagar[1], Shuyu Piao[1], Seonhee Kim[1,2], Ikjun Lee[1,2], Jeen-Woo Park[3], Byeong Hwa Jeon[1,2], Hee-Jung Song[4], Cuk-Seong Kim[1,2]

[1]Department of Physiology, College of Medicine, Chungnam National University, [2]BK21Plus CNU Integrative Biomedical Education Initiative, College of Medicine, Chungnam National University, [3]Department of Thoracic and Cardiovascular Surgery, School of Life Sciences, College of Natural Science, Kyungbook National University, [4]Department of Neurology, School of Medicine, Chungnam National University Hospital, Korea

Purpose: Mitochondrial dysfunction is a risk factor for vascular disease by overexpressing ROS caused by some stimuli in mitochondria which causes oxidative stress and enhances vascular inflammatory response. Isocitrate dehydrogenase 2 (IDH2) is $NADP^+$ dependent mitochondrial enzyme and an antioxidant enzyme that produces NADPH in the antioxidant system. Mitophagy and mitochondrial Unfolding Protein Response (mtUPR) are internal defense mechanism in mitochondria. In this study, we investigated whether IDH2 knock down causes mitochondrial dysfunction then Mitophagy and mtUPR in vitro in HUVECs and in vivo in IDH2 knock out (KO) mice.
Methods: We examined expression of mitophagy and mtUPR-related gene in IDH2-deficient human umbilical vein endothelial cells (HUVECs) and its role in mitophagy and mtUPR in the endothelial cell and IDH2 KO animal tissues. Mitophagy and mtUPR were measured using the Western blotting and qPCR.
Results: We showed that knockdown of IDH2 expression induced depolarization of mitochondrial membrane potential (MMP). Mitochondrial dynamics is mitochondrial fusion and fission. Knockdown of IDH2 increased Drp1 (fission protein) and mfn1 (fusion protein) compared with Tom20 (control). IDH2 deficiency increased mitophagy-related protein PINK-1 and Parkin expression and mRNA level (PINK-1, Parkin, BNIP3, NIX and FUNDC-1). Moreover, knockdown of IDH2 induced mtUPR mRNA level (USP30 and Clpp) *in vitro*. In addition, IDH2 deficiency increases mtUPR mRNA level and decreases PINK-1 and Parkin protein expression in vivo.
Conclusions: Our data show that IDH2 deficiency induces mitochondrial dysfunction and then mitophagy and mtUPR expression in endothelial cells. These findings provide a novel strategy for the development of therapeutic agents for restoring mitochondrial and endothelial function.

Keywords: IDH2, Mitochondria, Mitophagy, mtUPR, Endothelial cells

P20-07-03

The establishment of an in vitro three-dimensional human conjunctival tissue model

Ji Woo Im, Chae Young Lee, Hae-Rahn Bae

Department of Physiology, College of Medicine, Dong-A University, Korea

Purpose: The needs for alternatives to animal models in drug testing are increasing due to not only ethical issues but also low relevance to human physiology. Three-dimensional (3D) culture models based on human derived cells can closely mimic in vivo tissues, and thus have become essential research tools in cell biology as well as drug testing. As the conjunctiva serves as a route for administration of ophthalmic drugs, there is an increasing demand for more physiologically mimetic in vitro conjunctival models to assess drug delivery and safety of new ocular medicines. However, 3D human conjunctiva models using human conjunctival cells and nanofiber scaffolds have not yet been developed. We have established an in vitro human conjunctival model using 3D co-cultures of human primary conjunctival epithelial cells and fibroblasts using polyvinylalcohol(PVA) and polycaprolactone(PCL) micro/nanofibrous scaffolds, respectively.
Methods: The primary cultures of human conjunctival epithelial cells and fibroblasts were established from surplus tissue from surgical resection in cataract patients.
Results: Conjunctival cells cultured on PVA scaffolds reached confluence earlier than those cultured on both surfaces of PCL scaffolds in which conjunctival fibroblasts had been grown for 7 days. However, conjunctival epithelial cells cultured on PVA scaffolds alone were detached from the scaffolds after 7 days of culture while those cells co-cultured on fibroblast grown PCL scaffolds were not detached until 14 days, but exhibited more abundant thick stress fibers and less localization of ZO-1 at the interfaces of neighboring cells. Conjunctival epithelial cells cultured on PVA scaffolds displayed more distinct localization patterns of ZO-1 at the junctional region of the cell periphery as well as less stress fiber formation.
Conclusions: Taken together, co-cultures of both human conjunctival epithelial cells on PVA scaffolds and conjunctival fibroblasts in PCL scaffolds might provide a useful 3D human conjunctiva tissue model for studying ocular drug toxicity and delivery.

Keywords: Human conjunctival epithelial cells, Human conjunctival fibroblasts, 3D cell culture, Polyvinylalcohol (PVA) scaffold, Polycaprolactone (PCL) scaffold

P20-07-04

Differential expressions of aquaporin subtypes in female reproductive tract of mice

Ji Woo Im, Hae-Rahn Bae

Department of Physiology, College of Medicine, Dong-A University, Korea

Purpose: Although many aquaporin (AQP) transcripts have been demonstrated to express in the female reproductive tract, the defined localizations and functions of AQP subtype proteins remain unclear. In this study, we investigated the expression of AQP1, AQP3, AQP5, AQP6 and AQP9 proteins in female reproductive tract of mouse and characterized their precise localizations at the cellular and subcellular levels.

Methods: Immunofluorescence staining was performed on the frozen sections of female CD-1 mice aged 30-week-old and observed using laser scanning confocal microscope or fluorescence microscopy.
Results: Immunofluorescence analyses for AQP1, AQP3, AQP6 and AQP9 showed that these proteins were abundantly expressed in female reproductive tract and that intense immunoreactivities were observed in mucosa epithelial cells with a subtype-specific pattern. The most abundant aquaporin in both vagina and uterine cervix was AQP3. Each of AQP1, AQP3, AQP6 and AQP9 exhibited its distinct distribution in stratified squamous or columnar epithelial cells. AQP9 expression was predominant in oviduct and ovary. AQP1, AQP3, AQP6 and AQP9 proteins were mostly seen in apical membrane of ciliated epithelial cells of the oviduct as well as in both granulosa and theca cells of ovarian follicles. Most of AQP subtypes were also expressed in surface epithelial cells and glandular cells of endometrium in the uterus, but their expression levels were relatively lower than those observed in the vagina, uterine cervix, oviduct and ovary. This is the first study to investigate the expression and localization of 5 AQP subtype proteins simultaneously in female reproductive tract of mouse.
Conclusions: Our results suggest that AQP subtypes work together to transport water and glycerol efficiently across the mucosa epithelia for lubrication, proliferation, energy metabolism and pH regulation in female reproductive tract.

Keywords: AQP1, AQP3, AQP5, AQP6, AQP9, Female reproductive tract

P20-07-05

Significance of hyaluronate during organogenesis of salivary glands and its application in tissue engineering

Sang-woo Lee, Kyungpyo Park
Department of Oral Physiology, School of Dentistry, Seoul National University, Korea

Purpose: Dry mouth, or xerostomia, caused by salivary gland dysfunction significantly impacts oral/systemic health and quality of life. Although in vitro-generated artificial salivary glands have been considered as the fundamental solution, its structural complexity is difficult to reproduce using current biomaterials. Therefore, understanding and recapitulating the roles of biomacromolecules in salivary gland organogenesis is needed to solve these problems. Hyaluronic acid (HA) is a macromolecule abundant during salivary gland organogenesis, but its role remains unknown. Here, we verify the effects of HA on salivary gland organogenesis and artificial organ germ formation in solubilized and substrate-immobilized forms.
Methods: e13.5 mouse embryonic submandibular glands were isolated and ex-vivo cultured with various molecules (ie. hyaluronate) or drugs (ie. HAS2 inhibitor, hyaluronidase, or CD44 blocking antibody). One-pot coating method was used to immobilize hyaluronic acids on culture surfaces. Briefly, polycarbonate membrane is dipped into mixture of 2mg/ml dopamine, 1000 kDa hyaluronic acid dissolved in pH=8.5 Tris HCl buffer for 24 hrs.
Results: In embryonic submandibular glands (eSMG), we found dense HA layers encapsulating proliferative c-Kit+ progenitor cells that were expressing CD44, an HA receptor. The blockage of HA synthesis, or degradation of HA, impaired eSMG growth by ablating the c-Kit+ progenitor cell population. We also found that high-molecular-weight (HMW) HA has a significant role in eSMG growth. Based on these findings, we discovered that HA is also crucial for in vitro formation of salivary gland organ germs, one of the most promising candidates for salivary gland tissue regeneration.
Conclusions: We significantly enhanced salivary gland organ germ formation by supplementing HMW HA in solution; this effect was further increased when the HMW HA was immobilized on the substrate by polydopamine/HA co-immobilization. Our study suggests that the current use of HA in salivary gland tissue engineering can be further optimized.

Keywords: Salivary gland tissue engineering, Polydopamine, Hyaluronate, Branching morphogenesis

P20-08-01

Apoptosis inhibitor of macrophage (AIM) contributes to IL-10-induced anti-inflammatory response in LPS-induced acute peritonitis through inhibition of inflammasome activation

Kyungwon Yang[1,2], Jihee Lee Kang[1,2]
[1]Department of Physiology, [2]Inflammation-Cancer Microenvironment Research Center, Ewha Womans University, College of Medicine, Korea

Purpose: Apoptosis inhibitor of macrophage (AIM, also known as CD5L) modulates the signaling in inflammatory responses, including infection, cancer, or other immune diseases. Recent studies suggest that like interleukin-10 (IL-10), AIM is involved in alternatively activated (M2) macrophage polarization. We investigated whether and how AIM is involved in IL-10-induced inhibition of inflammasome activation and resolution of inflammation using a model of lipopolysaccharide (LPS)-induced peritonitis in AIM-/- and WT mice.
Methods: AIM-/- mouse embryos were purchased from Dr. Toru Miyazaki (Kumamoto University). Recombinant IL-10 was injected into peritoneum of mice. After 24hr, peritonitis was developed with LPS. The plasma and peritoneal fluid levels of IL-1b, IL-18, and TNF-α were measured by ELISA. Peritoneal lavage fluid cells were counted using an electronic Coulter Counter. Caspase-1 activity were measured using Caspase-Glo-1 Inflammasome Assay according to the manufacturer's instructions.
Results: Administration of IL-10 reduced production of proinflammatory cytokines, such as IL-1β, IL-18, and TNF-α, at 6 h after LPS injection in peritoneal lavage fluid (PLF) and serum from WT mice. These inhibitory effects of IL-10 on IL-1β and IL-18 were reversed in PLF and serum from AIM-/- mice. In addition, administration of IL-10 significantly reduced neutrophil recruitment and macrophage recruitment into peritoneal cavity after LPS injection. These inhibitory effects of IL-10 on recruitment of inflammatory cells were not shown in AIM-/- mice. Furthermore, enhanced amount of mature caspase-1 and IL-1β expression in the culture supernatants as well as caspase-1 activity in lysates of peritoneal macrophage isolated from WT mice at 6 h after LPS injection were reduced by administration of IL-10. However, these decreases were not shown in AIM-/- mice.
Conclusions: These in vivo results support the hypothesis that AIM mediates the negative regulation by IL-10 through the inhibition of NLRP3 inflammasome activation-mediating caspase-1-related IL-1β and IL-18 production.

Keywords: AIM, IL-10, Inflammasome, IL-1β, Caspase-1, Peritonitis

P20-08-02

Reducing effects of anthocyanin-rich red Chinese cabbage extract on vascular inflammation in atherosclerosis-induced mouse model

Eun Ok Lee, Hee Kyoung Joo, Yu Ran Lee, Sung Min Kim, Hao Jin, Yeon Hee Choi, Byeong Hwa Jeon
Department of Physiology, Chungnam National University School of Medicine, Korea

Purpose: Chronic vascular diseases, such as atherosclerosis, result from complex, multistep immune reactions, which eventually lead to the formation of atheromatous plaques, vascular endothelial dysfunction, and disruption of blood flow. Anthocyanins, the most prevalent flavonoids in red/purple fruits and vegetables, are known to improve immune responses and reduce chronic disease risks. So in the this study we investigated the effects of ArCC (Anthocyanin-rich Red Chinese Cabbage).
Methods: In this study, the anti-inflammatory activities of anthocyanin-rich extract from red Chinese cabbage (ArCC) (18% cyanidin) were demonstrated based on inhibitory effects on vascular inflammation in vitro and daily oral administration of ArCC in atherosclerosis-induced mouse model were also evaluated.
Results: We observed that ArCC treatment regulates TNF-α-induced in-

flammatory markers in cultured endothelial cells and inhibits vascular inflammation in hyperlipidemic ApoE-/- mice, without causing weight loss or other side effects. ArCC-mediated suppression of vascular inflammation in hyperlipidemic ApoE-/- mice paralleled the inhibition of circulating inflammatory cytokines and plaque formation on the surface of arterial endothelium and suppressed vascular adhesion molecule expression.
Conclusions: This study suggests that regular consumption of anthocyanin-rich red Chinese cabbage is useful to lower the risk of chronic vascular inflammatory diseases, and highlights the need for improved cultivation of functional vegetables with anti-inflammatory activities.

Keywords: Red Chinese Cabbage, Anthocyanin, Vascular inflammation, Anti-atherosclerotic

P20-08-03

SCAP deficiency in macrophage promotes M1 polarization and obesity in adipose tissue

Jae-Ho Lee, Dae-Kyu Song, Jae-Hoon Bae, Seung-Soon Im
Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

Purpose: Sterol regulatory element binding proteins (SREBPs) cleavage-activating protein (SCAP) plays an important role in regulating cholesterol balance. SCAP, a cholesterol sensor on proteolytic cleavage activates SREBPs in endoplasmic reticulum membrane to produce mature SREBPs. Macrophages play a crucial role in development, metabolism and maintenance of homeostasis, as well as mediating inflammation. Regulation of the macrophage polarization has been reported to be effective therapeutic approaches for inflammatory diseases. Depending on the different microenvironment stimulations, macrophages may be polarized into pro-inflammatory M1 phenotype and anti-inflammatory M2 phenotype. The present study aims to investigate the roles of SCAP in macrophage polarization and inflammation.
Methods: In this study SCAP macrophage-specific knockout (SCAP mKO) mice were generated using the Lys2-Cre model. To address the hypothesis that SCAP regulates macrophage phenotypic polarization, we isolated bone marrow-derived macrophage (BMDMs) from $SCAP^{fl/fl}$ or SCAP mKO mice. To explore the potential function of SCAP in macrophage during high fat/high sucrose (HFHS) diets, we compared weight gain in $SCAP^{fl/fl}$ vs SCAP mKO mice which are placed on a HFHS or a matched control diet.
Results: In the absence of SCAP, macrophages polarized with LPS expressed significantly higher levels of M1-marker genes, such as iNOS, IL-12β, CD80, IL-6, and IL-1β. Unlike, IL-4 treated BMDMs showed reduced expression of M2-marker genes Arg1, CD206, IL-10, CLEC10A, and Fizz1. In flow cytometry analysis, SCAP mKO BMDMs treated with LPS showed a significant increase in $F4/80^{+}CD80^{+}$ cells, whereas a significant decrease was reported in $F4/80^{+}CD206^{+}$ cells with IL-4 treatment. Indeed, lack of SCAP uplift the biosynthesis of the pro-inflammatory cytokine IL-1β upon LPS treatment. Here, we demonstrate that HFHS diet-fed SCAP mKO mice increased fat accumulation due to classical activated macrophages polarization in adipose tissue through regulation of suppression of intracellular cholesterol efflux.
Conclusions: Our finding demonstrates that SCAP deficiency in macrophages accelerates obesity in response to HFHS diets. The function of SCAP KO macrophages is identified as a key regulator of cholesterol efflux and cholesterol synthesis as well as inflammatory macrophage polarization. Finally, the results provide a mechanism that regulates macrophage polarization and function by SCAP, suggesting that open therapeutic avenues toward accelerating obesity and adipose differentiation.

Keywords: Macrophage, SCAP, M1 polarization, Inflammation, BMDM

P20-08-04

Protective effect of kaempferol in RINm5F β-cells under exposure to inflammatory cytokines

Seo-Yoon Chang[1], Yong-Jun Ko[1], Dong-Bin Kim[2], Myung-Jun Kim[1]
[1]Departments of Physiology, [2]Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Korea

Purpose: Pro-inflammatory cytokines and nitric oxide (NO) have been implicated in both dysfunction and destruction of pancreatic β-cells during the development of diabetes. Kaempferol, a natural flavonoid, has the beneficial effects of preserving pancreatic β-cell mass and function. In this study, we investigated the effects and the underlying mechanisms of kaempferol on RINm5F β-cells.
Methods: RINm5F β-cells were treated with IL-1β in the presence or absence of kaempferol (5-50 μM). The production of NO was detected with the Griess reagent system. iNOS expression was determined with qRT-PCR and Western blot. The transcriptional activity of NF-κB was evaluated by luciferase reporter assay and EMSA. iNOS mRNA and protein stability assay were determined by actinomycin D (Act.D) and cycloheximide (CHX) chase analysis, respectively. Also, to assess the effect of kaempferol on pancreatic β-cells' function, cell viability, apoptosis, and insulin secretion assays were conducted.
Results: In pro-inflammatory cytokine interleukin-1β (IL-1β)-stimulated RINm5F β-cells, kaempferol inhibited the production of NO dose-dependently, and reduced the levels of iNOS protein and its mRNA expression. Kaempferol also inhibited NF-κB activation for iNOS induction, IκB phosphorylation and NF-κB p65 nuclear translocation. Kaempferol decreased the expression of iNOS mRNA through both inhibitions of NF-κB DNA binding activity and mRNA stabilization. In addition, kaempferol reduced the stability of iNOS protein in cycloheximide-treated cells and inhibited enzyme activity of iNOS. Kaempferol pretreatment partially preserved cell viability in IL-1β-treated RINm5F β-cells and improved IL-1β-induced reduction of insulin release.
Conclusions: These finding suggest that kaempferol inhibits NO production in RINm5F β-cells by reducing the expression of IL-1β-induced iNOS protein, and its reduction could occur through inhibition of NF-κB pathway, transcriptional, post-transcriptional, and post-translational mechanisms. Taken together, kaempferol protects RINm5F β-cells by suppressing of iNOS expression and improving of β-cell functions.

Keywords: Kaempferol, Interleukin-1β, NO, iNOS, RINm5F β-cells

P20-08-05

STAT6 induces PPARγ expression and activation to resolve acute sterile inflammation

Ye-Ji Lee, Bo-Min Kim, Jihee Lee
Department of Physiology, Inflammation-Cancer Microenvironment Research Center, College of Medicine, Ewha Womans University, Seoul, Korea

Purpose: The signal transducer and activator of transcription 6 (STAT6) transcription factor leads to activation of peroxisome proliferator-activated receptor γ (PPARγ) pathway in macrophages. Little is known about the impact of proximal signal transduction leading to PPARγ activation for the resolution of acute inflammation. Here, we studied to define the role of STAT6 signaling in PPARγ expression and activation.
Methods: BALB/C mice(6 to 8 weeks old weighing 19 to 21g) were purchased from Orient Bio. BALB/C(STAT6-/-) and wild-type control mice. STAT6(-/-) and wild-type mice were age-matched (7 to 13 weeks old) and sex-matched for all experiments. STAT6(-/-) and wild-type mice were also injected intraperitoneally with 1mg of zymosan in 500 μl of PBS. All animals were euthanized at 6,24,72 hours after injection with zymosan.
Results: we demonstrated that deficiency of STAT6 aggravated and delayed inflammatory responses. In particular, mRNA and protein levels of PPARγ and its target molecules were lower over the course of inflammation in peritoneal macrophages and spleen from STAT6-/- mice than those in WT mice.

In particular, efferocytosis by peritoneal macrophages was reduced during peritonitis in STAT6-/- mice.
Conclusions: These results suggest that enhanced STAT6 signaling results in PPARγ-mediated macrophage programming, contributing to increased efferocytosis and resolution of inflammation.

Keywords: STAT6, PPARγ, Efferocytosis, Macrophages, Resolution of inflammation

P20-08-06

The THIK-1 C-terminus is responsible for LPS-induced reduction of THIK-1 expression levels in macrophages and sensory neurons

Marie Merci Nyiramana[1,2], Eun Jin Kim[1], Adrian S. Siregar[1,2], Dong Kun Lee[1,2], Seong-Geun Hong[1,2], Jaehee Han[1], Dawon Kang[1,2]

[1]Department of Physiology, College of Medicine and Institute of Health Sciences, Gyeongsang National University, Jinju, [2]Department of Convergence Medical Science, Gyeongsang National University, Jinju, Korea

Purpose: Macrophage-sensory neuron crosstalk causes peripheral pain sensations, which are regulated by ion channels. Tandem-pore domain potassium (K_{2P}) channels contribute to the background K^+ currents in sensory neurons. Tandem-pore domain halothane inhibited K^+ channel-1 (THIK-1) K_{2P} channel is expressed in sensory neurons and microglial cells, and its activity is essential for the regulation of inflammation. This study was performed to identify the role of THIK-1 in sensory neurons and macrophages under inflammatory condition.
Methods: Changes in THIK-1 expression level were measured in RAW264.7 cells and rat dorsal root ganglion neurons activated by LPS treatment using RT-PCR and Western blotting assay. In addition, LPS-induced changes in THIK-1 expression was measured in cells transfected with C-terminus truncated mutant (THIK-1ΔC). Pro-inflammatory cytokine level was determined by ELISA assay.
Results: LPS and inflammatory mediators decreased THIK-1 mRNA and protein expression levels in mouse macrophages and sensory neurons. Among inflammatory mediators, IL-1β and NO significantly reduced THIK-1 expression levels in macrophages and sensory neurons in a time- and dose-dependent manner. However, in cells transfected with C-terminus truncated mutant (THIK-1ΔC), THIK-1 expression levels were not reduced in response to LPS treatment. Production of NO and secretion of IL-1β were reduced in THIK-1ΔC-transfected cells. THIK-1 overexpressed and knockdowned cells showed increased and decreased production of NO and IL-1, respectively. THIK-1 activators increased IL-1β secretion, whereas THIK-1 blockers decreased it in LPS-stimulated macrophage and sensory neurons. The THIK-1 C-terminal region possesses kinase motif sites. Protein kinase modulators regulated THIK-1 expression levels.
Conclusions: These results show that LPS-induced reduction of THIK-1 expression is manifested by inflammatory mediators through the C-terminus of THIK-1 channel. We suggest that THIK-1 could be a target of inflammatory mediators in sensory neurons and macrophages.

Keywords: Inflammation, Macrophage cells, Sensory neurons, K_{2P} channel, THIK-1

P20-08-07

Secretory Ref-1 inhibits the inflammatory responses in lipopolysaccharide-induced septic mice

Hee Kyoung Joo, Yu Ran Lee, Eun-Ok Lee, Sungmin Kim, Hao Jin, Yeon Hee Choi, Byeong Hwa Jeon

Department of Physiology, School of Medicine, Chungnam National University, Korea

Purpose: Redox factor-1 is a multifunctional protein identified as a DNA base excision repair enzyme and redox modulator for several transcriptional factors. The aim of this study is to evaluate the role of secreted Ref-1 on lipopolysaccharide-induced vascular inflammation in cultured cells and in vivo.
Methods: We generated a secretory Ref-1 adenoviral vector system, AdPPT-LS-Ref-1, by conjugation of preprotrypsin leading sequence with full-length Ref-1 sequences. We used two vascular cells (HUVEC endothelial cells, RAW 264.7 macrophages) and a septic mouse model to study the anti-inflammatory effects of secreted APE1/Ref-1.
Results: Our results demonstrated that secretory APE1/Ref-1 treatment inhibited the expression of inflammation markers in vascular cells, decreased the production of LPS-induced cytokines and chemokine, protected LPS-induced tissue damage from LPS-induced septic mouse.
Conclusions: Our data highlight secretory Ref-1 as potential candidate for the development of new strategies for the treatment of systemic inflammation.

Keywords: Secretory Ref-1, Inflammation, Septic mice, Cytokines, Lipopolysaccharide

P20-08-08

Chios Mastic Gum suppresses the LPS-induced upregulation of inflammatory cytokine via regulating MAPK pathway in the HDPCs

Ye-Won Seol, Sam Young Park, Kyung Joo Seong, Sun-Woong Bae, Song-Yeon Park, So-Ra Kim, Won-Jae Kim, Ji-Yeon Jung

Department of Physiology, School of Dentistry, Dental Science Research Institute, Hard-tissue Biointerface Research Center, Chonnam National University, Korea

Purpose: The aim of this study is to investigate the effect of CGM on lipopolysaccharide (LPS) stimulated inflammatory cytokines and MAPK pathway in HDPCs.
Methods: The effect of LPS and CGM on HDPCs viability was measured using a 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT) assay. The expression of inflammatory cytokines was evaluated by reverse-transcription polymerase chain reaction (RT-PCR) and Western blot analysis. Also, phosphorylation of MAPK family (p-38, JNK, ERK) were evaluated by Western blot analysis. Statistical analysis was performed with analysis of variance (ANOVA).
Results: The viability of cells exposed to different concentrations of CGM and E. coli LPS was not significantly different compared with that of control cells ($P > 0.05$). LPS significantly increased interleukin-6 (IL-6) mRNA expression ($P < 0.05$), whereas treatment with CGM significantly attenuated LPS-stimulated production of IL-6 ($P < 0.05$). Treatment with CGM significantly decreased LPS-induced phosphorylation of p38, JNK and ERK ($P < 0.05$).
Conclusions: CGM has a suppressing effect on LPS-induced inflammatory cytokine and activation of MAPK pathway in HDPCs. Therefore, CGM might be a useful candidate as anti-inflammatory agent for vital pulp therapy.

Keywords: Chios Mastic Gum, Human dental pulp cells, Inflammation, MAPK

P20-08-09

The role of APE1/Ref-1 in atherosclerotic mice model

Sungmin Kim[1,2,3], Hao Jin[1,2,3], Hee Kyoung Joo[2], Yu Ran Lee[2], Eun Ok Lee[2], Yeon Hee Choi[1,2,3], Byeong Hwa Jeon[1,2,3]

[1]Department of Physiology, [2]Research Institute for Medical Sciences, [3]Department of BK21Plus CNU Integrative Biomedical Education Initiative, College of Medicine, Chungnam National University, Daejeon, Korea

Purpose: Atherosclerosis is a disease caused by the accumulation of fat and cholesterol in the walls of blood vessels, and is characterized by the expression of pro-inflammatory molecules and plaque formation. We previously reported that secretory APE1/Ref-1 inhibited vascular inflammation caused by oxidative stress. However, the relationship between APE1/Ref-1 and pathology of the arteriosclerosis was not revealed. So we investigated

the role of APE1/Ref-1 in atherosclerosis using ApoE -/- mouse fed with a western-type diet.
Methods: We assessed the relationship between APE1/Ref-1 and atherosclerosis through two approaches: (1) Correlation of the secretory APE1/Ref-1 levels with hematological parameter and (2) co-localization of APE1/Ref-1 expression and inflammatory proteins.
Results: We found that APE1/Ref-1 protein level in blood was increased with vascular inflammation of these mice. The cut-off value for APE1/Ref-1 for predicting atherosclerotic inflammation at 4.903 ng/ml showed sensitivity of 100% and specificity of 91%. Neutrophil/lymphocyte ratio (NLR), endothelial cell/macrophage activation and atherosclerotic plaque formation were increased in the ApoE knock out mice fed with a western-type diet. APE1/Ref-1 expression was increased in aortic tissues of these mice, and was co-localized with cells positive for platelet endothelial cell adhesion molecule (PECAM1; also known as CD31) and galectin-3, showing endothelial cell/macrophage expression of APE1/Ref-1.
Conclusions: In the mouse model of atherosclerosis, we suggested that the increased inflammatory response and increased serological APE1/Ref-1 levels is correlated, and the increased APE1/Ref-1 levels could be used as a marker for predicting atherosclerosis.

Keywords: APE1/Ref-1, Atherosclerosis, ApoE knockout mouse, Atherosclerotic mice, Atherosclerotic inflammation

P20-08-10

APE1/Ref-1 associated with chronic inflammation and fibrosis in the renal tubular and interstitial tissues in mouse CKD model

Soo Yeon An[1,2], Sungmin Kim[1,3], Hee Kyong Joo[1,3], Hao Jin[1,3], Yu Ran Lee[1,3], Eon Ok Lee[1,3], Yeon Hee Choi[1,3], Byeong Hwa Jeon[1,3]

[1]Department of Physiology, College of Medicine, Chungnam National University, [2]Department of Internal Medicine, Chungnam National University Hospital, [3]Research Institute for Medical Sciences, College of Medicine, Chungnam National University, Korea

Purpose: Apurinic/apyrimidinic endonuclease 1/Reduction-oxidation factor-1(APE1/Ref-1) is a multifunctional protein regulating the cell response to oxidative stress and operating as a DNA-repair enzyme. Previous studies indicated that APE1/Ref-1 has important roles in diseases involved with acute and chronic inflammation and tissue repair. However, its function and therapeutic potential in chronic kidney injury are unknown. The present study investigated the expression level of APE1/Ref-1 in inflammatory and fibrotic changes induced by ischemic reperfusion in mouse kidneys.
Methods: Ischemia reperfusion injury was induced surgically to develop chronic renal injury and tubulointerstitial fibrosis in mice. In male BALB/c nude mice aged eight weeks, kidney vessels were clamped for 30 minutes, followed by reperfusion. Sham group was not subjected to renal ischemia reperfusion, and their kidney vessels were only dissected off the surrounding peri-renal fat. Serum creatinine, Urea nitrogen, Neutrophil gelatinase-associated lipocalin (NGAL) and urinary NGAL were measured to evaluate the renal function in response to the injury. Kidneys were resected on day 21 post-operation. Inflammatory changes including tubular damage and interstitial fibrosis were quantified as H&E, Masson's Trichrome, and immunohistochemistry stains.
Results: The kidney function decreased in response to ischemic reperfusion stress induced by bilateral clamping of renal vessels. APE1/Ref-1 expression levels increased significantly in the injured kidneys on day 21 post-operation compared to sham kidneys. It suggests that APE1/Ref-1 might play a central role in the pathogenesis of kidney fibrosis after ischemic reperfusion injury. Furthermore, the injured kidneys showed extensive localization of APE1/Ref-1 in the cytoplasm of tubular and interstitial cells, whereas sham kidneys exhibited localization in the cell nucleus. Thus, APE1/Ref-1 was up-regulated in the process of chronic inflammation and fibrosis.
Conclusions: This report demonstrates that APE1/Ref-1 expression is associated with the development of chronic inflammation and tubulointerstitial fibrosis of the kidney. APE1/Ref-1 might be one of the key factors in the progression of inflammation and fibrosis in CKD. Different molecular pathways are involved with DNA-repair functions, redox function, or modulation of the immune system of APE1/Ref-1, but it needs further investigation. APE1/Ref-1 could be a diagnostic marker for chronic inflammation and fibrosis in CKD.

Keywords: APE1/Ref-1, Chronic kidney disease, Ischemia reperfusion injury, Chronic inflammation, Fibrosis

P20-09-01

CR6-interacting factor 1 Deficiency Promotes Premature Senescence via SIRT3 Inhibition in endothelial cells

Seonhee Kim, Shuyu Piao, Ikjun Lee, Harsha Nagar, Su-Jeong Choi, Cuk-Seong Kim

Department of Physiology, Chungnam National University, Korea

Purpose: Vascular endothelial cell senescence is an important cause of cardiac-related diseases. Mitochondrial reactive oxygen species (mtROS) has been implicated in cellular senescence and multiple cardiovascular disorders. CR6 interacting factor (CRIF1) deficiency increased mtROS via impairing mitochondrial oxidative phosphorylation; however, the mechanisms by which mtROS regulate vascular endothelial cell senescence has not been thoroughly explored. The aim of this study is to investigate whether CRIF1 deficiency could accelerate endothelial cell senescence and attempt to elucidate the underlying mechanism. We observed that CRIF1 deficiency increased the activity of senescence-associated β-galactosidase (SA-β-gal) and the protein expression of p53 phosphorylation, p21 and p16. CRIF1 deficiency exerted the senescence effect by reducing the expression of Sirtuin 3 (SIRT3) through the degradation of transcription factor PGC1α and NRF2 expression by ubiquitination. CRIF1 downregulation also destroyed the function of mitochondrial antioxidant enzymes (manganese superoxide dismutase (MnSOD), Foxo3a, nicotinamide-adenine dinucleotide phosphate, glutathione) by decreased SIRT3 expression. Interestingly, overexpression of SIRT3 in CRIF1 deficient endothelial cells not only reduced mtROS levels by elevating antioxidant enzyme MnSOD, but lessened the expression of cell senescence markers. In conclusion, these results suggest that CRIF1 deficiency induces vascular endothelial cell senescence through suppressing SIRT3 generation, which was destroyed by inhibiting transcription coactivators PGC1α and NRF2 expression.
Methods: western blot, SA-β-gal, realtime-PCR, immunostaining, conditional knockout mice
Results: In this study, we found that several key senescence markers, including SA-β-gal activity, p53 phosphorylation, p21, and p16 were significantly increased not only in vascular endothelial cells of CRIF1 KO mice, but also in CRIF1-deficient HUVECs. These results suggest that endothelial mitochondrial dysfunction is strongly associated with endothelial cellular senescence.
Conclusions: Taken together, the data presented here demonstrate that SIRT3 plays a role in regulation of mitochondrial dysfunction-induced endothelial cellular senescence and the effect may mediate through the alteration of the mitochondrial antioxidant machinery. This pathway may therefore represent a promising therapeutic target for the treatment of vascular senescence in cardiovascular disease.

Keywords: Vascular endothelial cell, Mitochondria, Oxidative stress, Antioxidant system

P20-09-02

Enhanced expression of GABRD predicts poor prognosis in patients with colon adenocarcinoma

Moxin Wu[1,4], Keun Young Kim[2], Won Cheol Park[2], Han-Seung Ryu[3], Suck Chei Choi[3], Min Seob Kim[4], Ji Yeon Myung[4], Hyun Seok Choi[4], Eui Joong Kim[4], Moon Young Lee[4]

[1]Department of Medical Laboratory, Affiliated Hospital of Jiujiang University, Jiujiang, China, [2]Department of General Surgery, Wonkwang University, School of Medicine, Wonkwang Digestive Disease Research Institute, Iksan, [3]Department of Gastroenterology, Wonkwang University, School of Medicine, Wonkwang Digestive Disease Research Institute, Iksan, [4]Department of Physiology, School of Medicine, Wonkwang University, Wonkwang Digestive Disease Research Institute & Institute of Wonkwang Medical Science, Iksan, Korea

Purpose: Neurotransmitters are reported to be involved in tumor initiation and progression. This study aimed to elucidate the prognostic value of γ-aminobutyric acid type A receptor δ subunit (GABRD) in colon adenocarcinoma (COAD) using the data from The Cancer Genome Atlas (TCGA) database.
Methods: The GABRD mRNA expression levels in the COAD and normal tissues were compared using the Wilcoxon rank-sum test. The correlation between clinicopathologic characteristics and GABRD expression was analyzed by Wilcoxon rank-sum test or Kruskal-Wallis test and logistic regression. The prognostic value of GABRD mRNA expression in patients with COAD was determined using the Kaplan-Meier curve and Cox regression analysis. Finally, the molecular mechanisms of GABRD in COAD were predicted by gene set enrichment analysis (GSEA).
Results: The COAD tissues exhibited higher GABRD mRNA expression levels than the normal tissues. The logistic regression analysis revealed that GABRD mRNA expression was correlated with TNM stage, N stage, M stage, and microsatellite instability (MSI) status. The Kaplan-Meier survival curve and log-rank test revealed that patients with COAD exhibiting high GABRD mRNA expression were associated with poor overall survival (OS). The multivariate analysis indicated that increased GABRD mRNA expression was an independent prognostic factor and was correlated with a poor OS. The GSEA revealed that GABRD was involved in signaling pathways, including cell adhesion molecules, gap junction, melanogenesis, and mTOR signaling pathway, as well as the signaling pathways associated with basal cell carcinoma or bladder cancer development.
Conclusions: In summary, enhanced GABRD mRNA expression may be a potential independent prognostic biomarker for COAD. This study demonstrated that the COAD tissues exhibited GABRD mRNA overexpression and that GABRD mRNA expression may be a potential prognostic marker for patients with COAD. Further studies are needed to elucidate the molecular mechanism underlying the role of GABRD in the tumor microenvironment in facilitating cancer invasion and metastasis.

Keywords: γ-aminobutyric acid type A receptor δ subunit (GABRD), Colon adenocarcinoma (COAD), Multivariate analysis, Prognostic biomarker, Gene set enrichment analysis (GSEA)

P20-09-03

Propyl gallate induces human pulmonary fibroblast cell death through the regulation of Bax and caspase-3

Mina Ryu, Woo Hyun Park

[1]Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Korea

Purpose: Propyl gallate (PG) has an anti-growth effect on various cell types, including lung cancer cells. However, little is known about the cytotoxicological effects of PG on normal primary lung cells.
Methods: The present study evaluated the cellular effects and levels of cell death with PG treatment in human pulmonary fibroblast (HPF) cells and investigated the effects of various caspase inhibitors, apoptosis-related siRNAs, and mitogen-activated protein kinase (MAPK) inhibitors on PG-induced HPF cell death.
Results: DNA flow cytometry showed that PG (100-1600 μM) significantly induced G1 phase arrest of the cell cycle. Only the highest concentration of PG (1600 μM) slightly increased the number of sub-G1 cells. Treatment with PG (400-1600 μM) induced cell death, which was accompanied by mitochondrial membrane potential (ΔΨm) loss, as assessed by FACS cytometer. Inhibitors of pan-caspase, caspase-3, caspase-8, and caspase-9 did not significantly alter the number of annexin V-positive and ΔΨm loss cells in PG-treated HPF cells. Administration of Bax or caspase-3 siRNA attenuated HPF cell death by PG, whereas p53, Bcl-2, or caspase-3 siRNAs did not affect the cell death. In addition, none of the MAPK [MEK, c-Jun N-terminal kinase (JNK), and p38] inhibitors tested in the present study had effects on PG-induced cell death or ΔΨm loss in HPF cells.
Conclusions: In conclusion, PG induces G1 phase arrest of the cell cycle and cell death in HPF cells via apoptosis and/or necrosis. PG induced-HPF cell death occurred through the regulation of Bax and caspase-3.

Keywords: Propyl gallate (PG), Human pulmonary fibroblast cell, Cell death, Bax, Caspase-3

P20-09-04

Propyl gallate induces cell death in human pulmonary fibroblast through increasing reactive oxygen species levels and depleting glutathione

Mina Ryu, Woo Hyun Park

Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Korea

Purpose: Propyl gallate (PG) has an anti-growth effect on numerous cell types. The present study investigated the effects of PG on the levels of reactive oxygen species (ROS) and glutathione (GSH) in primary human pulmonary fibroblast (HPF) cells.
Methods: Moreover, the effects of N-acetyl cysteine (NAC, an antioxidant), L-buthionine sulfoximine (BSO, a GSH synthesis inhibitor), and small interfering RNA (siRNAs) against various antioxidant genes on ROS and GSH levels and cell death were examined in PG-treated HPF cells.
Results: PG (100-800 μM) increased the levels of total ROS and O_2^- at early time points of 30-180 min and 24 h, whereas PG (800-1,600 μM) increased GSH-depleted cell number at 24 h and reduced GSH levels at 30-180 min. PG downregulated the activity of superoxide dismutase (SOD) and upregulated the activity of catalase in HPF cells. Treatment with 800 μM PG increased the number of apoptotic cells and cells that lost mitochondrial membrane potential (ΔΨm). NAC treatment attenuated HPF cell death and ΔΨm loss induced by PG, accompanied by a decrease in GSH depletion, whereas BSO exacerbated the cell death and ΔΨm loss without altering ROS and GSH depletion levels. Furthermore, siRNA against SOD1, SOD2, or catalase attenuated cell death in PG-treated HPF cells, whereas siRNA against GSH peroxidase enhanced cell death.
Conclusions: In conclusion, PG induced HPF cell death by increasing ROS levels and depleting GSH. NAC decreased HPF cell death induced by PG, whereas BSO enhanced cell death.

Keywords: Propyl gallate, Reactive oxygen species, SOD, Glutathione, Mitochondrial membrane potential (MMP)

P20-09-05

Auranofin induces cell death via increasing intracellular oxidative stress and GSH depletion in human lung cancer cells

Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

Purpose: Auranofin is known to inhibit thioredoxin reductase (TrxR) and exhibits the promising anti-cancer activity in several cancer types. However, at present there is no clear explanation for the mechanism underlying the inhibitory effects of Auranofin on lung cancer cell growth.
Methods: In this study, we evaluated the effects of Auranofin on various lung cancer cells in relation to cell death, reactive oxygen species (ROS) and glutathione (GSH) levels.
Results: Treatment with Auranofin inhibited cell growth and induced cell death at 24 h in Calu-6, A549, SK-LU-1, NCI-H460, and NCI-H1299. In addition, Auranofin led to increased ROS levels including mitochondrial O_2^- and induced GSH depletion levels in these cells. Treatment with N-acetyl cysteine (NAC, a well-known antioxidant) attenuated apoptotic cell death, mitochondrial membrane potential (ΔΨm) loss, ROS levels, and GSH depletion levels in Auranofin-treated Calu-6 and A549 cells. By contrast, L-buthionine sulfoximine (BSO, an inhibitor of GSH synthesis) increased the number of dead cells via the induction of oxidative stress and the depletion of GSH. Similarly, analysis of protein expression levels in these cells showed that Auranofin down-regulated poly (ADP ribose) polymerase (PARP) and caspase-3, which were prevented by NAC, whereas the combination treatment of Auranofin and BSO enhanced the degradation of PARP and caspase-3 induced by Auranofin alone. Furthermore, we also demonstrated that Auranofin strongly inhibited TrxR enzyme activity in both Calu-6 and A549 cells.
Conclusions: Taken together, our present data suggest that Auranofin-induced cell death is tightly related to intracellular oxidative stress.

Keywords: Auranofin, Lung cancer, Anti-cancer effect, Reactive oxygen species, Glutathione, Thioredoxin reductase

P20-09-06

Enhanced cell death effects of MAP Kinase inhibitors in propyl gallate-treated lung cancer cells are related to increased ROS levels and GSH depletion

Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

Purpose: Propyl gallate (PG), a synthetic antioxidant, has an anti-growth effect in various cell types, including lung cancer cells.
Methods: The present study investigated the effects of mitogen-activated protein kinase (MAPK; MEK, JNK, and p38) inhibitors on PG-treated Calu-6 and A549 lung cancer cells in relation to cell death as well as reactive oxygen species (ROS) and glutathione (GSH) levels.
Results: PG induced cell death in both Calu-6 and A549 cell types at 24 h post treatment, and this was accompanied by loss of mitochondrial membrane potential (ΔΨm). All of the tested MAPK inhibitors increased the numbers of sub-G1 or annexin V-stained cells in both PG-treated cell lines. In particular, the MEK inhibitor strongly enhanced cell death and complete ΔΨm loss in Calu-6 cells, and the p38 inhibitor had the same effects in A549 cells. PG increased ROS levels and caused GSH depletion in both lung cancer cell lines at 24 h post treatment. All of the MAPK inhibitors increased $O_2^{\bullet-}$ levels and caused GSH depletion in PG-treated Calu-6 cells, and the JNK and p38 inhibitors increased general ROS levels and caused GSH depletion in PG-treated A549 cells.
Conclusions: In conclusion, all of the tested MAPK inhibitors increased cell death in PG-treated Calu-6 and A549 lung cancer cells. Enhanced cell death and GSH depletion in Calu-6 cells caused by the MEK inhibitor were related to increased $O_2^{\bullet-}$ levels, and the effects of the p38 inhibitor in A549 cells were correlated with increased general ROS levels.

Keywords: Propyl gallate, Lung cancer, Mitogen-activated protein kinase, Reactive oxygen species, Glutathione

P20-09-07

Effects of N-acetyl cysteine and buthionine sulfoximine in propyl gallate-treated lung cancer cells: cell death, reactive oxygen species, and glutathione

Sun Hyang Park, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Jeonju, Korea

Purpose: Propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester; PG) displays an anti-growth effect in lung cancer cells.
Methods: The present study investigated cell growth and death effects of N-acetyl cysteine (NAC) and L-buthionine sulfoximine (BSO) in PG-treated Calu-6 and A549 lung cancer cell lines with regard to reactive oxygen species (ROS) and glutathione (GSH) levels.
Results: PG induced growth inhibition and cell death in Calu-6 and A549 cells at 24 h, accompanied by mitochondrial membrane potential (ΔΨm) loss. NAC attenuated this inhibition in Calu-6, but not A549 cells and significantly increased the numbers of sub-G1 or annexin V-positive cells in PG-treated A549, but not Calu-6 cells. BSO enhanced cell death and ΔΨm loss resulting from PG treatment in both lung cancer cell lines. Moreover, PG increased ROS levels and caused GSH depletion in both cancer cell lines. NAC decreased ROS levels in PG-treated Calu-6 cells but increased $O_2^{\bullet-}$ levels in both PG-treated lung cancer cells. BSO increased ROS levels in PG-treated A549 cells and increased $O_2^{\bullet-}$ levels in PG-treated Calu-6 cells. In addition, NAC strongly enhanced GSH depletion in both PG-treated lung cancer cells. BSO also increased GSH depletion in PG-treated Calu-6, but not A549 cells.
Conclusions: In conclusion, NAC enhanced PG-induced A549 cell death via increasing ROS levels and GSH depletion. BSO increased PG-induced Calu-6 cell death via increasing $O_2^{\bullet-}$ levels and GSH depletion.

Keywords: Propyl gallate, Lung cancer, N-acetyl cysteine, L-buthionine sulfoximine, Reactive oxygen species, Glutathione

P20-09-08

Tetrahydrobiopterin regulated cardiac energy metabolism in diabetic hearts

Hyoung Kyu Kim, Nam Mi Park, Dae Yun Seo, Pham Trong Kha, Sun-Woo Kim, Jae Boum Youm, Nari Kim, Jong Chul Won, Jin Han
Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Korea

Purpose: Diabetic cardiomyopathy (DCM) is a major cause of mortality/morbidity in diabetes mellitus patients. Although tetrahydrobiopterin (BH4) shows therapeutic potential as an endogenous cardiovascular target, its effect on myocardial cells and mitochondria in DCM and the underlying mechanisms remain unknown. Here, we determined the involvement of BH4 deficiency in DCM and the therapeutic potential of BH4 supplementation in a rodent DCM model.
Methods: We observed a decreased BH4:total biopterin ratio in heart and mitochondria accompanied by cardiac remodeling, lower cardiac contractility, and mitochondrial dysfunction. Prolonged BH4 supplementation improved cardiac function, corrected morphological abnormalities in cardiac muscle, and increased mitochondrial activity.
Results: Proteomics analysis revealed oxidative phosphorylation (OXPHOS) as the BH4-targeted biological pathway in diabetic hearts as well as BH4-mediated rescue of downregulated peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) signaling as a key modulator of

OXPHOS and mitochondrial biogenesis. Mechanistically, BH4 bound to calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2) and activated downstream AMP-activated protein kinase/cAMP response element binding protein/PGC-1α signaling to rescue mitochondrial and cardiac dysfunction in DCM.
Conclusions: These results suggest BH4 as a novel endogenous activator of CaMKK2.

Keywords: CaMKK2, Diabetic cardiomyopathy, Mitochondria, PGC-1α, Tetrahydrobiopterin

P20-09-09

Macrophages induce migration in A549 cells

Hee Ju Song, Taehee Kim, Sang Do Lee
Department of Physiolosy, Medical Science, Chungnam National University, Korea

Purpose: Macrophage is known to be involved in cancer progression and migration. In addition, macrophages secrete many cytokines, among which HB-EGF and IL-1β are known to be involved in cancer progression and migration. Cell migration is induced due to actin polymerization or depolymerization, the most representative signals of the upstream signal are the cdc42 and rac1 pathways. It is known that cdc42 and rac1 interact to induce phosphorylation of PAK1 after conversion to GTP, and it has been found that cdc42 alone activates N-WASP after conversion to GTP to activate actin polymerization. In this study, we investigate the effects and mechanisms of macrophage and cytokine on the migration of A549 cells.
Methods: We performed WHA (wound healing assay) by treating A549 cells with macrophage culture medium, HB-EGF, and IL-1β. It was confirmed that HB-EGF and IL-1β were secreted from macrophage by ELISA. PAK1 and N-WSAP inhibitors were treated in A549 cells, and the effect of migration inhibition was confirmed with WHA.
Results: We show that a culture medium of macrophages increased the migration of A549 cells, lung cancer cells. In addition, it was confirmed that macrophages secrete HB-EGF and IL-1β. Increased secretion of HB-EGF and IL-1b further increased the migration of A549 cells. To confirm the migration pathway of A549 cells, WHA was performed using inhibitors of PAK1 and N-WASP. All groups showed a tendency to decrease migration when the inhibitor was treated.
Conclusions: The culture medium of macrophage increases the migration of A549 cells. The levels of HB-EGF and IL-1β, which are shown to be secreted from macrophage also increase the migration. Increased migration showed a tendency to be reduced by inhibitors of PAK1 and N-WASP, and consequently suggests the possibility of passing through the PAK1 or N-WASP pathway.

Keywords: Lung cancer, Macrophage, Cytokine, Migration

P20-09-10

The effects of autophagy regulators on Auranofin-treated lung cancer cells in relation to cell growth and reactive oxygen species

XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

Purpose: Auranofin, an inhibitor of thioredoxin reductase (TrxR), inhibits the growth of a variety of cancer cells.
Methods: Here, we investigated the effects of Rapamycin (Rapa; an inducer of autophagy) or Hydroxychloroquine (HQ; an inhibitor of autophagy) on Auranofin-treated Calu-6 and A549 lung cancer cells in relation to cell growth and ROS levels.
Results: Auranofin inhibited the growth of Calu-6 and A549 cells with IC_{50} values of approximately 5 μM and 3 μM at 24 h, respectively. Auranofin induced apoptosis and necrosis in both cell lines, which were accompanied by the loss of mitochondrial membrane potential. ROS levels including O2•- were increased in both Auranofin-treated lung cancer cells. Auranofin lead to autophagy, as evidenced by the increased of LC3B form and the decreased of P62 in these cells. Treatment with 20 μM HQ or 100 nM Rapa affected cell death and ROS levels in Auranofin-treated Calu-6 and A549 cells. However, these changes did not influence cell growth and death in these cells. Rapamycin prevented cell growth inhibition and death in Auranofin-treated Calu-6 and A549 cells, which were accompanied by decreasing ROS levels. However, HQ significantly increased cell death and ROS levels in Auranofin-treated Calu-6 and A549 cells.
Conclusions: In conclusion the changes of autophagy by Auranofin, Rapamycin or HQ were partially related to cell growth and death in Calu-6 and A549 lung cancer cells.

Keywords: Auranofin, Lung cancer, Rapamycin, Hydroxychloroquine, Autophagy, Reactive oxygen species

P20-09-11

Auranofin inhibits the proliferation of lung cancer cells via necrosis and caspase-dependent apoptosis

XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

Purpose: Auranofin, an inhibitor of thioredoxin reductase (TrxR), inhibits the growth of a variety of cancer cells.
Methods: In the present study, various lung cancer cells were used to investigate the molecular basis of anti-cancer effects of auranofin, including cell death via apoptosis or necrosis and cell cycle arrest.
Results: Generally, auranofin inhibited the growth of the tested lung cancer cell lines in a dose-dependent manner with an IC_{50} of 3-4 μM at 24 h. This agent significantly decreased the activity of TrxR in Calu-6 and A549 lung cancer cells. In addition, auranofin (3-5 μM) triggered necrosis in lung cancer cells measured by the release of lactate dehydrogenase (LDH) into culture media. Auranofin increased the percentages of sub-G1 cells in Calu-6 and A549 cells. DNA flow cytometry showed that auranofin induced G2/M phase arrest of Calu-6 cells. This agent also efficiently induced apoptosis, accompanied by loss of mitochondrial membrane potential (ΔΨm), increases in cleavage forms of caspase-3 and poly (ADP-ribose) polymerase (PARP), and a high ratio of BAX to Bcl-2 proteins. Furthermore, various caspase inhibitors reduced apoptosis and ΔΨm loss in auranofin-treated Calu-6 cells. In particular, the pan-caspase inhibitor, benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD), decreased cleavage forms of caspase-3, -8, and -9 in these cells.
Conclusions: In conclusion, auranofin inhibited the proliferation of lung cancer cells, especially Calu-6 cells, via cell cycle arrest and cell death due to necrosis or caspase-dependent apoptosis.

Keywords: Auranofin, Lung cancer, Thioredoxin reductase, Apoptosis, Necrosis, Caspase

P20-09-12

Propyl gallate reduces the growth of lung cancer cells through caspase-dependent apoptosis and G1 phase arrest of the cell cycle

XiaYing Cui, Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

Purpose: Propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester, PG) is a synthetic phenolic antioxidant which exerts many effects on tissue and cell functions.
Methods: In the present study, Calu-6 and A549 lung cancer cells were used to examine the molecular mechanism of PG's anti-growth effects in relation

to apoptosis and cell cycle arrest.
Results: PG inhibited the growths of both lung cancer cell types in a dose-dependent manner with an IC_{50} of 800 μM at 24 h based on MTT assays. DNA flow cytometry showed that PG induced G1 phase arrest of the cell cycle in Calu-6 and A549 cells. In addition, PG induces apoptosis in both lung cancer cell types, as evidenced by sub-G1 cells and annexin V-staining cells. Western blot results demonstrated that PG decreased Bcl-2 level which accompanied an increase in the cleavage form of poly (ADP-ribose) polymerase (PARP). PG also triggered loss of mitochondrial membrane potential (ΔΨm) and decreased ΔΨm levels in both lung cancer cell types, as assessed by FACS cytometer. Furthermore, PG upregulated the activities of caspase-3 and caspase-8 in Calu-6 cells.
Conclusions: In conclusion, PG treatment inhibited the growth of lung cancer cells, especially Calu-6 cells via caspase-dependent apoptosis as well as G1 phase arrest of the cell cycle.

Keywords: Propyl gallate, Lung cancer cells, Apoptosis, Cell cycle

P20-09-13

The anti-apoptotic effects of caspase inhibitors in propyl gallate-treated lung cancer cells are somewhat related to changes in reactive oxygen species and glutathione levels

XiaYing Cui, Woo Hyun Park

Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

Purpose: Propyl gallate [3,4,5-trihydroxybenzoic acid propyl ester; PG] exhibits an anti-growth effect in various cells.
Methods: In this study, the anti-apoptotic effects of caspase inhibitors were evaluated in PG-treated Calu-6 and A549 lung cancer cells in relation to reactive oxygen species (ROS) and glutathione (GSH) levels.
Results: PG inhibited the proliferation and induced the cell death of both Calu-6 and A549 cells at 24 h. Inhibitors of pan-caspase, caspase-3, caspase-8, and caspase-9 reduced the number of dead and sub-G1 cells in both PG-treated cells at 24 h. PG increased ROS levels, including O_2^-, in both lung cancer cell lines at 24 h. Generally, caspase inhibitors appeared to decrease ROS levels in PG-treated lung cancer cells at 24 h and somewhat reduced O_2^- levels. PG augmented the number of GSH-depleted Calu-6 and A549 cells at 24 h. Caspase inhibitors did not affect the level of GSH depletion in PG-treated A549 cells but differently and partially altered the depletion level in PG-treated Calu-6 cells.
Conclusions: In conclusion, PG exhibits an anti-proliferative effect in Calu-6 and A549 lung cancer cells and induced their cell death. PG-induced lung cancer death was accompanied by increases in ROS levels and GSH depletion. Therefore, the anti-apoptotic effects of caspase inhibitors were, at least in part, related to changes in ROS and GSH levels.

Keywords: Propyl gallate, Caspase inhibitors, Lung cancer cells, Reactive oxygen species, Glutathione

P20-09-14

Propyl gallate inhibits the proliferation of Calu-6 and A549 lung cancer cells via affecting ROS and GSH levels

XiaYing Cui, Woo Hyun Park

Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Jeonbuk National University, Deokjin, Jeonju, Korea

Purpose: Propyl gallate (3,4,5-trihydroxybenzoic acid propyl ester, PG) has an anti-growth effect.
Methods: In this study, Calu-6 and A549 lung cancer cells were used to examine the anti-proliferative effect of PG in relation to reactive oxygen species (ROS) and glutathione (GSH) levels.
Results: PG (100-1600 μM) dose-dependently inhibited the proliferation of Calu-6 and A549 cells at 24 h, and 800-1600 μM PG treatment strongly induced cell death in both cell lines. PG (800-1600 μM) increased cellular metabolism in Calu-6 but not A549 cells at 4 h. PG either increased or decreased ROS levels, including O_2•- and •OH, depending on the incubation doses and times of 1 or 24 h. Even these effects differed between Calu-6 and A549 cell types. PG reduced the activity of superoxide dismutase (SOD) in Calu-6 cells, and it augmented the activity of catalase in A549 cells. PG dose-dependently increased GSH depleted cells at 24 h. PG decreased GSH levels in both lung cancer cells at 1 h whereas it increased the levels in Calu-6 cells at 24 h. Furthermore, diethyldithiocarbamate (DDC; an inhibitor of Cu/Zn-SOD) and 3-amino-1,2,4-Triazole (AT; an inhibitor of catalase) differently either increased or decreased cellular metabolism, ROS and GSH levels in 800 μM PG-treated and PG-untreated Calu-6 and A549 cells at 1 h.
Conclusions: In conclusion, PG showed an anti-proliferative effect in Calu-6 and A549 lung cancer cells in a dose-dependent manner, which was related to changes in ROS levels and the depletion of GSH.

Keywords: Propyl gallate, Lung cancer cells, ROS, GSH levels

P20-09-15

CRBN overexpression reverses drug resistance of multiple myeloma cells by regulating mitochondrial function

Jubert Marquez, Nam-mi Park, Hyoung Kyu Kim, Jin Han

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

Purpose: Cereblon (CRBN) is a thalidomide binding protein which plays an essential role in anti-myeloma effects associated with the teratogenicity of immunomodulatory drugs (IMiDs). However, CRBN-related mitochondrial biomarkers in multiple myeloma have not been reported. This study aimed to identify the correlation between CRBN and mitochondrial function modification in multiple myeloma.
Methods: KMS20, KMS26, and KMS28BM multiple myeloma cell lines were used to evaluate for the effects of overexpressing and knockdown of CRBN in vitro during thalidomide treatment. Mitochondrial function and protein expression analyses were carried out to determine for its effect. A xenograft model was developed using BALB/C mice injected with KMS20 cells. Ad-CRBN or Ad-control were i.t. injected on day -1 to 2 of thalidomide treatment in the xenograft model. Mice were treated with 50 mg/kg thalidomide by i.p. injection from day 11 to 24 after tumor cell inoculation. Survival was monitored.
Results: Multiple myeloma patients displayed higher CRBN levels than normal patients, and its expression depends on drug resistance and susceptibility. Intriguingly, CRBN negatively regulates mitochondrial function. Mitochondrial proteins (PGC-1α, NRF1, TFAM, ERRα), mitochondrial membrane potential (ΔΨm), mitochondrial ATP levels and mitochondrial mass were higher in drug-resistant multiple myeloma cells and lower in drug-sensitive multiple myeloma cells. After silencing CRBN, expression of mitochondrial proteins, ΔΨm, mitochondrial ATP level, and mitochondrial mass were significantly increased in drug-sensitive multiple myeloma cells. Conversely, adenovirus-infected CRBN leads to decreased mitochondrial function. Using a xenograft model, tumor growth was decreased and survivals were extended significantly in KMS20 tumor-bearing mice by thalidomide treatment. Furthermore, CRBN overexpression in KMS20 tumor by adenovirus successfully increased susceptibility to thalidomide.
Conclusions: These findings suggest that CRBN is a novel therapeutic target for overcoming drug resistance in multiple myeloma via the mediation of mitochondrial dysfunction. Furthermore, combined CRBN and mitochondria marker evaluation is more effective in patients suited for thalidomide therapy.

Keywords: CRBN, Multiple myeloma, Thalidomide, Mitochondria

P20-09-16

NecroX-5 mitigates hypoxia/reoxygenation injury by preserving PGC1α expression levels and protecting phosphorylation capacity during mitochondrial oxidation

Vu Thi Thu[1,2], Hyoung Kyu Kim[1], Maria Victoria Faith Garcia[1], Le Thanh Long[1], Bayalagmaa Nyamaa[1], In-Sung Song[1], To Thanh Thuy[2], Nguyen Quang Huy[2], Jubert Marquez[1], Soon Ha Kim[3], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], Jin Han[1]

[1]Department of Physiology, National Research Laboratory for Mitochondrial Signaling, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea, [2]Faculty of Biology, VNU University of Science, Hanoi, Vietnam, [3]Product Strategy and Development, LG Life Sciences Ltd, Seoul, Korea

Purpose: The role of NecroX-5 in protecting mitochondrial oxidative phosphorylation capacity during hypoxia-reoxygenation (HR) is verified
Methods: Isolated rat hearts were subjected to 10 μM Necrox-5 treatment during hypoxia/reoxygenation treatment using an ex vivo Langendorff system. Proteomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS) and non-labeling peptide count protein quantification. Real-time PCR, western blot, citrate synthases and mitochondrial complex activity assays were then performed to assess heart function.
Results: Treatment with NecroX-5 during hypoxia significantly preserved electron transport chain proteins involved in oxidative phosphorylation and metabolic functions. NecroX-5 also improved mitochondrial complex I, II, and V function. Additionally, markedly higher peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1α) expression levels were observed in NecroX-5-treated rat hearts.
Conclusions: These novel results provide convincing evidence for the role of NecroX-5 in protecting mitochondrial oxidative phosphorylation capacity and in preserving PGC1α during cardiac HR injuries.

Keywords: NecroX, Hypoxia, Mitochondria, Oxidative phosphorylation, PGC1α

P20-09-17

CR6-interacting factor 1 deficiency inhibits activity of endothelial nitric oxide synthase activity by impeding biosynthesis of tetrahydrobiopterin

Ikjun Lee, Seonhee Kim, Harsha Nagar, Su-jeong Choi, Byeonghwa Jeon, Shuyu Piao, Cuk-Seong Kim

Department of Phyisology & Medical Science, Chungnam National University, Korea

Purpose: Tetrahydrobiopterin is an important cofactor in regulating the balance between NO and superoxide production. In CRIF1 deficiency cells and mouse, nitric oxide synthesis activity was significantly decreased but whether the decreased eNOS and NO production in CRIF1-deficient cells is associated with relative BH4 deficiency-induced eNOS uncoupling remains unknown.
Methods: Fluorometric HPLC analysis was performed to quantify biopterin. A 120 SB-C18 column (Poroshell) was used to perform HPLC with a mobile phase of 5% MeOH (5:95 MeOH:water, v/v) at a rate of 1 ml/min. Fluorescence was measured using a 1290 series fluorescence detector (Agilent) at 350 nm excitation and 450 nm emission. The content of BH4 was determined by subtracting alkaline oxidation from acid oxidation.
Results: CRIF1 deficiency significantly decreased BH4 content in HUVECs and mouse endothelial cells through inbhition of BH4 biosynthesis and recycling pathway.
Conclusions: CRIF1-deficeincy-induced oxidative stress reduces the BH4-biosynthesis pathway and increased cell cycle regulation markers inhibits BH4-recycling pathway. These results lead to decreased NO production via eNOS uncoupling in the pathogenesis of cardiovascular diseases.

Keywords: Bh4, Crif1, Nitric oxide, eNOS, ROS

P20-09-18

Study on the regulation of mitochondrial dynamics and vascular regeneration in BM-derived mesenchymal stem cells for the treatment of ischemic disease

Jin su Kim, Seung Taek Ji, Jin Sup Jung, Sang-Mo Kwon

Pusan National University School of Medicine, Korea

Purpose: This study demonstrated the function of FAM210A that a novel factor induced by hypoxia in bone marrow-derived mesenchymal stem cells(BM-MSCs) for the treatment of myocardial infarction.
Methods: We first made a genetically modified mouse that knocked out FAM210A to confirm the function of FAM210A and isolated the BM-MSC from this mouse and performed functional evaluation.
We also demonstrated that over-expression of the FAM210A cell line using lentivirus.
In order to confirm the therapeutic function of myocardial infarction, the isolated MSC was transplanted into MI model nude mice.
Results: In results, We demonstrated that FAM210A expression was induced and increased migration and tube formation capacity in the hypoxia condition(1% O_2, 5% CO_2, 94% N_2). And it was demonstrated that the function was reduced in FAM210A-/- BM-MSC.
We also demonstrated that over-expression of the FAM210A cell line using lentivirus increased migration and tube formation capacity in human BM-MSCs.
We measured echocardiography on day 28 and demonstrated that the LV ejection fraction and LV fractional shortening of the heart decreased and fibrosis was more advanced compared to the control group. In addition, we demonstrated that the amount of endothelial cell-specific markers (CD31, E-SMA) decreased in heart tissue on day 28.
Conclusions: In conclusion, we demonstrated that FAM210A performs angiogenic function in BM-MSC.

Keywords: BM-MSC, Angiogenesis, Myocardial infarction, FAM210A

P20-09-19

Inhibitory effect of sirtuin 6 on cancer proliferation and metastasis in hepatocellular carcinoma

Congshan Li, Soomi Kim

Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

Purpose: Sirtuin (SIRT) 6 as a member of the sirtuin family which may be the targets in the treatment of tumors. In this study, we investigated the role of SIRT6 in hepatocellular carcinoma cell line
Results: Overexpression of SIRT6 significantly suppressed the viability of HCC cells whereas silencing of SIRT6 stimulated the viability of HCC cells. Overexpression of SIRT6 increased expression of cleaved-PARP and cleaved-caspase9 and decreased the PARP, caspase9, and caspase3. Knockdown of SIRT6 increased the number and size of colonies. In addition, overexpression of SIRT6 significantly inhibited the invasion and metastasis of HCC cells whereas silencing of SIRT6 increased the invasion and metastasis abilities of HCC cells in a time dependent manner. Moreover, overexpression of SIRT6 inhibited vimentin, UPA, and MMP9 protein levels while silencing of SIRT6 in HCC cells increased the protein levels of vimentin, UPA, and MMP9. P-β-catenin levels was increased by overexpression of SIRT6 and was decreased by silencing of SIRT6. In vitro, knockdown of SIRT6 significantly promoted the tumor growth.
Conclusions: SIRT6 suppresses the proliferation, invasion and metastasis of HCC cells and may play as a tumor suppressor in HCC cells. SIRT6 is an important tumor regulatory factor in liver carcinogenesis.

Keywords: SIRT6, Hepatocellular carcinoma cells, Metastasis, Cell proliferation, β-catenin

P20-09-20

3,3'-diindolylmethane induces synergistic anticancer effect with 5-fluorouracil in gastric carcinoma cancer

Congshan Li, Soomi Kim

Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

Purpose: Our study aims to explore whether 3,3'-diindolylmethane (DIM) potentiates chemotherapeutic agents 5-fluorouracil (5-Fu) of gastric cancer cells and investigate the possible mechanisms of this process.
Results: The data of MTT assay and colony formation assay revealed that combination treatment could suppress gastric cancer cells viability, proliferation and migration. In addition, DIM combined with 5-Fu significantly induce apoptosis in vitro through enhancing the degree of cleavage caspase-9 and cleaved poly ADP-ribose polymerase (PARP). Co-administration of DIM with 5-Fu resulted in a significantly increased the percentage of sub G1 phase and total apoptotic cells. Western blot also showed that MMP-9 and uPA were decreased while E-cad was increased after two drugs combination. DIM and 5-FU alone or in combination treatment could reduce the expression of p-Akt, c-Myc and cyclin D1 after 48 hours. In addition, in the group treated with DIM and 5-Fu combined, the size of the cancer was significantly reduced compared to the group treated with only 5-FU in vivo experiment.
Conclusions: DIM could strengthen the chemotherapy effect of 5-FU on gastric cancer cells through Akt signaling pathway.

Keywords: 3'-diindolylmethane, 5-fluorouracil, Gastric carcinoma, Akt

P20-09-21

Ursolic acid-enhanced antitumor effect of DIM via activation of Hippo signaling pathway in esophageal cancer cells

Ruoyu Meng, Soomi Kim

Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

Purpose: The purpose of our present study is to research how ursolic acid (UA) enhanced diindolylmethane (DIM) anti-cancer efficacy in esophageal squamouse cancer cells (ESCC).
Results: In TE-8 and TE-12 cell lines, treatment with UA and DIM declined the cell viability and cell proliferation more considerably compared to single reagent treatment. Treatment with UA and DIM markedly induced cell apoptosis were proven by increased levels of cleaved PARP with cleaved caspase-9 protein and subG1 phase. UA with DIM treatment also inhibited the metastasis. FACS analysis showed that combination treatment stimulated G1 phase arrest and decreased CDK4, CDK6 and cyclin D1 were observed in TE-8 and TE-12 cell lines. Akt activation was also suppressed by combination treatment. In addition, combination treatment regulated the Hippo pathway by enhancing the dephosphorylation of MST1 and MST2, resulting in induced phosphorylation of Mob1 but reduced Yes-associated protein (YAP) and increased the protein level of Rassf1 compare to single agent treatment. These results indicated that combination treatment effectively potentiates the anti-tumor effect via inhibition of proliferation and induced apoptosis by activation of hippo signaling pathway in ESCC cells.
Conclusions: Taken together, UA and DIM improve the therapeutic efficacy in esophageal cancer cell compare with treatment with UA or DIM alone.

Keywords: Ursolic acid, 3,3-diindolylmethane, Esophageal squamous cell carcinoma, Apoptosis, Hippo signaling pathway

P20-09-22

Chemosensitizing and cytotoxic effects of ursolic acid and doxorubicin on colorectal cancer cells

Dan Hu, Soomi Kim

Department of Physiology, Institute for Medical Sciences, Jeonbuk National University Medical School, Jeonju, Korea

Purpose: Colorectal cancer is the third most common cancer worldwide, and its incidence in South Korea has dramatically increased as a result of changes in the economy and lifestyle. Although ursolic acid (UA) and doxorubicin (DXR) have been suggested to reduce the risk of adenocarcinomas in humans, their combined effects on human colorectal cancer cells have never been fully elucidated. Here, we report for the first time that the biological effects of UA and DXR in human colorectal cancer cells.
Results: Combination treatment with UA and DXR significantly inhibited the viability of HCT116 and HT-29 cells and colony formation in soft agar. Treatment with UA and DXR substantially increased apoptosis as indicated by increased cleaved poly ADP-ribose polymerase (PARP) and cleaved caspase-9 levels. In addition, combination treatment with UA and DXR also strongly inhibited the migration of colorectal cancer cells as assessed by wound healing assays. Treatment with the combination also decreased the protein levels of uPA and MMP9, yet significantly increased E-cadherin protein expression when compared to treatment with a single agent. UA sensitized colorectal cancer cells through downregulation of FOXM1 and potentiated the effects of DXR. Furthermore, UA strongly inhibited phosphorylation of Akt and potentiated DXR-induced inhibition of Akt function in colorectal cancer cells, which led to a concomitant reduction of the anti-apoptotic protein cyclinD1, c-myc altogether resulting in the activation of intrinsic apoptosis.
Conclusions: Therefore, our results indicate that UA effectively potentiates the efficacy of chemotherapeutic agents such as DXR by downregulation of the Akt/ FOXM1 signaling cascade in colorectal cancer cells. Our findings suggest that UA enhances the therapeutic efficacy of DXR in colorectal cancer and is a potential clinical anticancer agent for the prevention and/or treatment of colorectal cancer.

Keywords: Ursolic acid, Doxorubicin, Colorectal cancer cells

P20-09-23

Knockdown of catalase promotes adipocyte differentiation and expression of the NADPH oxidase NOX4

Hyun-Woo Cho[1], Su-Kyung Shin[1], Seung-En Song[1], Sang Pyo Kim[2], Seung-Soon Im[1], Jae-Hoon Bae[1], Dae-Kyu Song[1]

[1]Department of Physiology & Obesity-mediated Disease Research Center,
[2]Department of Pathology, Keimyung University School of Medicine, Daegu, Korea

Purpose: Catalase that functions as an antioxidant by catalyzing the decomposing of hydrogen peroxide (H_2O_2) to oxygen and water is distributed in entire all tissues. H_2O_2 is one of reactive oxygen species (ROS). It is a beneficial second messenger in some signal transduction but excessive ROS can cause various pathological change and toxicity within cells. We founded that obesity was induced in catalase knockout mice (CKO) even fed with normal chow diet. Therefore, our central hypothesis is that lipid accumulation during adipocyte differentiation is due to knockdown of catalase
Methods: Male C57BL/6J mice (wild-type, WT), a same strain with catalase knockout mice (CKO), were purchased from Jung Ang Experimental Animals. 3T3-L1 cells at day 4 of differentiation were transfected with 100 nM control (siCON) or catalase-small interfering RNA (siCAT) using Lipofectamine for 72 h, according to the manufacturer's protocol. Cells were treated with 1 μM metformin (an AMPK activator) or 20 μM GKT137831 (a NOX4 inhibitor) for that 72 h. For early-stage adipogenesis assays, 3T3-L1 cells were transfected with 50 nM siCON or siCAT for 24 h at 2 days before differentiation. ROS were detected using a H_2O_2 Assay Kit, according to the manufacturer's protocol.

Results: We investigated the effect of catalase deficiency on adipocyte differentiation in mice embryonic fibroblast (MEF) and 3T3-L1. In catalase deficient 3T3-L1 and MEF, concentration of H_2O_2 was higher than in each control group. mRNA expression of preadipocyte factor 1 (Pref-1) and GATA-binding protein 2 (GATA2), well known as preadipocyte markers, was decreased in CKO MEF and siCAT during the early stage of adipocyte differentiation. Also, protein expression of adipogenic transcription factors, CCAAT/enhancer-binding proteins beta (C/EBPβ) and proliferator-activated receptor γ (PPARγ), was increased in siCAT. These results suggest that CKO MEF and catalase knockdown 3T3-L1 facilitated adipocyte hyperplasia with increased production of H_2O_2. Additionally, oil-red-o staining was performed to analyze lipid droplet accumulation. As a result, the lipid droplet accumulation was increased in siCAT during the late stage of adipocyte differentiation. 3T3-L1 in siCAT showed up-regulated NADPH oxidase 4 (NOX4) expression, but decreased phospho-AMP-activated protein kinase (p-AMPK). H_2O_2 production and lipid droplet accumulation in the siCAT cells were suppressed by metformin or GKT137831. Also, with H_2O_2 increased by siCAT transfection, protein expression of NOX4 was increased at 12 h and 72 h. However, protein expression of AMPKα phosphorylation was not changed at 12 h but decreased at 72 h.
Conclusions: These results indicate that the role of catalase in adipocytes is important to decrease cellular H_2O_2 concentration. In addition, the increase in H_2O_2 induced by catalase knockdown promotes adipocyte differentiation toward mature adipocytes. Although further research is required, we found that the protein expression of NOX4 after siCAT transfection was increased at 12 h, suggesting that NOX4 might augment earlier than the decrease AMPKα phosphorylation.

Keywords: Catalase, Adipoctyte, Differentiation

Acknowledgement: This study was supported by grant from the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (No. 2018R1A2B2004429).

P20-09-24

Activation of ERK1/2-mTORC1-NOX4 mediates TGF-β1-induced epithelial-mesenchymal transition and fibrosis in retinal pigment epithelial cells

Soo-Jin Kim, Dat Da Ly, Ha Thu Nguyen, Hanh Minh T. Nguyen, Seung-Kuy Cha, Kyu-Sang Park

Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

Purpose: Transforming growth factor-β (TGF-β) plays a crucial role in the development of epithelial to mesenchymal transition (EMT) and fibrosis, particularly in an ocular disorder such as proliferative vitreoretinopathy (PVR). However, the key molecular mechanism underlying its pathogenesis remains unknown.
Methods: In the present study, using cultured ARPE-19 cells, we determined that TGF-β initiates a signaling pathway through extracellular signal-regulated kinase (ERK)-mammalian target of rapamycin complex 1 (mTORC1) that stimulates trans-differentiation and fibrosis of retinal pigment epithelium.
Results: Blocking this pathway by a TGF-βRI, ERK or mTORC1 inhibitor protected cells from EMT and fibrotic protein expression. TGF-β1 treatment increased reactive oxygen species (ROS) via NOX4 upregulation, which acts downstream of ERK and mTORC1, as the ROS scavenger N-acetylcysteine and a pan-NADPH oxidase (NOX) inhibitor DPI dissipated excess ROS generation. TGF-β1-induced oxidative stress resulted in EMT and fibrotic changes, as NAC and DPI prevented α-SMA, Col4a3 expression and cell migration.
Conclusions: All these inhibitors blocked the downstream pathway activation in addition to clearly preventing the activation of its upstream molecules, indicating the presence of a feedback loop system that may boost the upstream events. Furthermore, the FDA-approved drug trametinib (10 nM) blunted TGF-β1-induced mTORC1 activation and downstream pathogenic alterations through ERK1/2 inhibition, which opens a therapeutic avenue for the treatment of PVR in the future.

Keywords: Retinal pigment epithelium (RPE), Transforming growth factor-β (TGF-β), Epithelial-mesenchymal transition (EMT), Extracellular signal-regulated kinase (ERK), Mammalian target of rapamycin C1 (mTORC1)

P20-09-25

Acetylated APE1/Ref-1 is secreted through ATP-binding cassette transporter A1

Yu Ran Lee[1,2], Hee Kyoung Joo[1,2], Eun-Ok Lee[1,2], Sungmin Kim[1,2], Hao Jin[1,2], Byeong Hwa Jeon[1,2]

[1]Department of Physiology, [2]Research Institute for Medical Sciences, College of Medicine, Chungnam National University, Korea

Purpose: APE1/Ref-1 protein secretion showed remarkable regulatory effects on inflammatory cytokine-stimulated cells both in vitro and in vivo. Moreover, in different tumor cell types, the level of APE1/Ref-1 was abnormally high, suggesting its possible use as a cancer biomarker. The different subcellular distribution of APE1/Ref-1 and the evidence of its extracellular secretion together suggest that its distribution and translocation are controlled not only by stimulatory agents like chemicals or hormones but also by plasma membrane transporters. Although APE1/Ref-1 has been demonstrated to accumulate along the plasma membrane in the presence of a deacetylase inhibitor, trichostatin A (TSA), the mechanism of its translocation across the plasma membrane remains unknown
Methods: Human embryonic kidney 293T (HEK293T) cells were cultured in DMEM with 10% fetal bovine serum and 1% antibiotics. The cells were treated with the inhibitor for 2 h, followed by TSA treatment for 1 h, as indicated. Cell viability was analyzed using the RealTime-GloTM MT luminescent kit. The final supernatant was used for detection of secretory APE1/Ref-1. The amount of APE1/Ref-1 in each sample was determined by ELISA analysis. The plasma membrane of HEK293T cells, which had been transfected with ABCA1 siRNA, was prepared by sucrose density gradient centrifugation. The membrane pellet was analyzed by immunoblotting using anti-ABCA1 and anti-N-cadherin antibodies. Interaction of ABCA1 and APE1/Ref-1 was visualized using a Duolink II fluorescence kit. Co-immunoprecipitation using monoclonal anti-ABCA1 antibody and polyclonal anti-APE1/Ref-1 was also performed to analyze the binding between ABCA1 and APE1/Ref-1 in cell lysate.
Results: While APE1/Ref-1 targeting was not affected by inhibition of the endoplasmic reticulum/Golgi-dependent secretion, its secretion was reduced by inhibitors of ATP-binding cassette (ABC) transporters, and siRNA-mediated down-regulation of ABC transporter A1. TheassociationbetweenAPE1/Ref-1 and ABCA1 transporter was confirmed by proximalligation assay and immunoprecipitation experiments. An APE1/Ref-1 construct with mutated acetylation sites (K6/K7R) showed reduced co-localization with ABC transporter A1. Exposure of trichostatin A (TSA) induced the acetylation of APE1/Ref-1, which translocated into membrane fraction.
Conclusions: Acetylation of APE1/Ref-1 is considered to be necessary for its extracellular targeting via non-classical secretory pathway using the ABCA1 transporter.

Keywords: APE1/Ref-1, Acetylation, Secretion, ABC transporter A1, Secretion pathway

P20-09-26

Hematopoietic- and neurologic-expressed 1 inhibited autophagy in colorectal cancer

Ruoyu Meng, Soomi Kim

Department of Physiology, Institute for Medical Sciences, Jeonbuk National University Medical School, Korea

Purpose: The purpose of our study is investigated the underlying molecular mechanisms by which HN1 regulates proliferation, metastasis, apoptosis and autophagy in colorectal cancer cell.
Results: Knockdown of HN1 significantly decreased the viability of colorectal cancer cells, inducing G1 cell cycle arrest and apoptosis. And knockdown of HN1 also inhibited the invasion and metastasis of colorectal cancer cells. Additionally, knocking down of HN1 induced autophagy whereas EGF treatment to CRC cells induced HN1 expression. Knockdown of HN1 on EGF treated cells counteracted the effects of EGF induction. Co-immunoprecipi-

tation demonstrated that HN1 regulates colorectal cancer cell proliferation, metastasis and autophagy through directly acting with Akt/mTOR signaling. Moreover, knockdown of HN1 inhibited tumor growth in xenograft model.
Conclusions: Therefore, our results suggest that in vivo and vitro HN1 regulates growth, metastasis, apoptosis and autophagy of colorectal cancer cells and targeting HN1 may constitute a therapeutic strategy for colorectal cancer.

Keywords: Colorectal cancer, HN1, Proliferation, Metastasis, Autophagy

P20-09-27

Recombinant human BMP-2 induced cell apoptosis in Human colorectal cancer cells

Ruoyu Meng, Soomi Kim

Department of Physiology, Jeonbuk National University Medical School, Korea

Purpose: In our present study, we investigate the role of rhBMP-2 in colorectal cancer (CRC) cells with its associated signaling pathway using HT-29 and HCT116 cells.
Results: RhBMP-2 significantly suppressed proliferation of CRC cells in dose-dependent way by MTT assay. Cell cycle arrest in G1 phase was induced at 24h after rhBMP-2 treatment. RhBMP-2 also stimulated Smad4, p53 and p21 levels, and reduced cyclin D1, cyclin-dependent kinase (CDK) 4 and CDK6 activities. rhBMP-2 treatment resulted in reduced protein expression levels of poly (ADP-ribose) polymerase (PARP) and caspase-9 whereas those of cleaved PARP and cleaved caspase-9 were significantly increased in CRC cells. In addition, rhBMP-2 activated Hippo signaling pathway.
Conclusions: Therefore, our results indicate that rhBMP-2 suppresses colorectal cell proliferation which is mediated via activation of hippo signaling pathway. Therefore, targeting BMP-2 may constitute a potential therapeutic strategy for human colorectal cancer.

Keywords: Colorectal cancer, rhBMP-2, Hippo signaling pathway, Apoptosis, Cell cycle

P20-09-28

Hematopoietic- and eurologic-expressed sequence 1 reduced Autophagy via Akt/mTOR sinaling pathway in Hepatocellular Carcinoma cell

Ruoyu Meng, Soomi Kim

Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

Purpose: The purpose of our present study is to research underlying mechanism of HN1 in HCC. Although HN1 gene is high expressed in hepatocellular carcinoma cell but its underlying mechanism in remains unclear.
Results: Knockdown of HN1 enhanced cell apoptosis and induced G1 cell cycle arrest. In addition, knockdown of HN1 induced autophagy whereas overexpression of HN1 reduced autophagy. Knockdown of HN1 under starvation condition or Torin 1 treatment further induced autophagy but reversed chloroquine-induced autophagy reduction. Knockdown of HN1 down-regulated mTOR/AKT signaling pathway and Bcl-2/Bax signaling pathway. Knockdown of HN1 increased nuclear-translocation of transcription factor EB (TFEB), a key transcriptional regulator of lysosome biogenesis and autophagy, under starvation condition or Torin 1 treatment. All these results indicate knockdown of HN1 induced autophagy in HCC. Moreover, knockdown of HN1 induced ER stress and overexpression of HN1 counteract part of tunicamycin induced-ER stress.
Conclusions: Take together, our results suggest that HN1 regulates cell apoptosis, autophagy and ER-stress of hepatocellular carcinoma cells.

Keywords: Hepatocellular carcinoma, HN1, Apoptosis, Autophagy, TFEB

P20-09-29

Ursolic acid plus paclitaxel inhibited cell proliferation via Akt signaling pathway in esophageal cancer cells

Ruoyu Meng, Soomi Kim

Department of Physiology, Institute for Medical, Jeonbuk National University Medical School, Korea

Purpose: The purpose of our present study is demonstrate ursolic acid plus paclitaxel inhibited cell proliferation via Akt signaling pathway in esophageal cancer cells.
Results: Combination treatment showed more significant inhibition effect on cell proliferation and colony formation in TE-12 cells and TE-8 cells compared to treatment with UA or paclitaxel alone. Combination treatment substantially induced apoptosis as indicated by increased levels of cleaved poly ADP-ribose polymerase (PARP) and cleaved caspase-9 protein. UA plus paclitaxel treatment more effectively reduced the invasion rate and metastasis rate whereas the metastasis-related protein E-cadherin was increased in TE-12 and TE-8 cells. In addition, combination treatment increased the protein levels of p-Akt and decreased FOXM1 in ESCC cells. These results indicated that combination treatment of ursolic acid and paclitaxel more effectively potentiates the efficacy compare with single chemotherapeutic agent via inhibition of proliferation and metastasis by inactivation of FOXM1 in ESCC cells.
Conclusions: Taken together, combination treatment of ursolic acid and paclitaxel promote anti-cancer efficacy through Akt/FOXM1 signaling cascade and UA is a potential clinical anticancer agent for the prevention and/or treatment of esophageal cancer.

Keywords: Ursolic acid, Esophageal squamous cell carcinoma, Apoptosis, FOXM1

P20-09-30

Two-pore domain K^+ channels involve autophagy in bladder cancer cell lines

Yangmi Kim

Department of Physiology, College of Medicine, Chungbuk National University, Cheongju, Korea

Purpose: Potassium channels are widely distributed in various cells and are involved in maintaining the physiological processes and homeostasis of cells, including regulation of resting membrane potential, cellular signal transduction. However, little data is available on the role of potassium channels in autophagy in cancer cells. The two-pore domain K^+ channel (K2P channel) contributes to maintaining resting membrane potential and is regulated by pH, heat, hypoxia, polyunsaturated fatty acids, cell membrane stretch. In the present study, we investigated whether K2P channels regulate autophagy in bladder cancer cell lines.
Methods : The human bladder cancer cell lines were maintained in RPMI supplemented with 10% fetal bovine serum. To knockdown of K2P, approximately 60-80% confluent cells were treated with negative control siRNA and K2P siRNA for 72-96 hours using Lipofectamine RNAiMax reagent. Electrophysiological recording was performed in whole cell configurations and using a patch clamp amplifier Axopatch 200B. To measure resting membrane potential, the current was clamped (I=0) in a whole cell patch configuration. Western blot assay Samples containing 40 μg total protein were separated by 15% sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel, transferred onto immobilon-P membranes and incubated with polyclonal or monoclonal antibodies.
Results: The knockdown of K2P channel was depolarized from -15 mV to -8 mV, and the cancer cell line overexpressed K2P channel was hyperpolarized to -60 mV. Transient overexpression of K2P channel was enhanced the formation of autophagosomes. Knockdown using small interfering RNA (siRNA) targeting K2P channels confirmed that autophagy was suppressed in bladder cancer cell lines, this process was regulated by inhibition of p38-mitogen-activated protein kinase(MAPK)/c-Jun N-terminal kinase

(JNK) signaling proteins.
Conclusions: These results suggest that K2P channel act as regulators of autophagy in cancer cells and may be new therapeutic targets for bladder cancer

Keywords: Two-pore domain K channel, K2P, Cancer cell line, Autophagy, Autophagosome, MAPK

P20-10-01

Association between serum irisin concentration and bone stiffness in Korean adults

Jae Seung Chang[1], Jong-Whan Choi[2], In Deok Kong[1]
[1]Department of Physiology, [2]Department of Biochemistry, Yonsei University Wonju College of Medicine, Korea

Purpose: Muscle interacts with bone each other. Irisin, which is a myokine induced by exercise, can promote osteoblast differentiation in vitro and shows anabolic actions on the skeleton in rodents. Moreover, circulating irisin levels were associated with osteoporotic fractures in postmenopausal women. However, there is a paucity of data on the relationship between serum irisin levels and bone health in Asians. The aim of the study was to investigate the association of serum irisin concentrations with bone strength in Korean adults.
Methods: We evaluated the osteoporotic and sarcopenic risk factors and circulating irisin levels of 472 adults (307 women) aged 19-89 years. Bone status was assessed using a calcaneal quantitative ultrasound method. Appendicular lean mass (ALM) was measured by bioelectrical impedance analysis and muscle function was evaluated by handgrip strength (HS) test. Serum irisin level was measured with ELISA methods. Sarcopenia and pre-sarcopenia were determined by the presence of muscle atrophy (ALM/height2 < 7.0 kg/m^2 in men, and < 5.7 kg/m^2 in women) and/or weakness (HS < 26 kg in men and < 18 kg in women), respectively. Subjects were classified into four groups according to sex and quartiles of irisin levels.
Results: As expected, the prevalence of those with sarcopenia tended to increase in the lowest quartile of irisin, whereas bone stiffness index (BSI) was significantly higher in the highest quartile of circulating irisin compared to the lowest one (93.6 ± 16.9 vs. 81.7 ± 13.9 in men and 79.7 ± 18.1 vs. 71.6 ± 13.3, all for $p < 0.05$). Moreover, serum irisin levels had positive linear correlation with BSI in both sexes ($r = 0.1441$ in men and $r = 0.1438$ in women, all for $p < 0.05$).
Conclusions: Our results suggest that circulating irisin is associated with bone strength as well as sarcopenia in Korean subjects. Further investigations are needed to clarify the role of irisin as a mediator of bone-muscle unit.

Keywords: Irisin, Myokine, Bone strength, Sarcopenia, Biomarker

P20-10-02

Resistance exercise improves cardiac contractility by preserving mitochondrial function, which ameliorates diabetic cardiomyopathy

Tae Hee Ko, Seung Hun Jeong, Hyoung Kyu Kim, Min Sung Kim, Jubert C. Marquez, SungRyul Lee, Jae Boum Youm, Dae Yun Seo, Byoung Doo Rhee, Kyung Soo Ko, Nari Kim, Jin Han
Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

Purpose: Diabetic cardiomyopathy is one of the latent-phase complications of diabetes. Although there are a lot of ongoing researches and evidence that physical activity can affect the progression of the diabetic complication, it remains unclear whether resistance exercise (RE) can attenuate the diabetic cardiomyopathy and it's underlying mechanism. In this study, therefore, we tried to investigate the effect of RE on mitochondrial function, ultimately preservation of cardiac contractility and attenuation of the diabetic cardiomyopathy.
Methods: Fourteen Otsuka Long-Evans Tokushima Fatty (OLETF) rats were assigned to sedentary control (SC, n=7) and RE (n=7) groups at 28 weeks of age. Long-Evans Tokushima Otsuka (LETO) rats were used as the non-diabetic control. The RE rats were trained by 20 repetitions of climbing a ladder 5 days per week.
Results: The increase in energy metabolism in RE group has been shown, through the higher glucose consumption and lower lipid profile. In cardiac contractility, RE group had higher ejection fraction and fractional shortening. Energy metabolism, especially glucose and fat, has been altered between RE group and SC group. Higher expression of PGC-1α and TFAM in RE group indicates increased biogenesis of mitochondria. Also, proton leakage and ROS generation were reduced while membrane potential increased in RE group. Finally, RE group had higher SOD2 and lower UCP2, UCP3. These results suggest that the RE conserves mitochondrial function in hyperglycemic condition.
Conclusions: RE protects mitochondrial function in three ways. First, RE regulates glucose and fat metabolism. Next, RE accelerates mitochondrial biogenesis. Lastly, RE itself can lower ROS generation and proton leakage. The preservation of mitochondrial function might affect the cardiac contractility and eventually ameliorates diabetic cardiomyopathy.

Keywords: Resistance exercise, Diabetic cardiomyopathy, Mitochondrial function

P20-11-01

Co-occurrence of acute food restriction and microinjection of D1 dopamine receptor agonist in the nucleus accumbens core produces sensitized-locomotor activity in amphetamine pre-exposed rat

Seohyun Lee, Hyung Shin Yoon, Jeong-Hoon Kim
Department of Physiology, Yonsei University College of Medicine, Korea

Purpose: Ghrelin is an orexigenic peptide hormone derived from the stomach and its receptor is known to exist in several brain areas including the nucleus accumbens (NAcc), a region important for the incentive motivational and locomotor activating properties of psychostimulant drugs. We have previously showed that co-microinjection of ghrelin and dopamine (DA) D1 receptor agonist, SKF81297, into the NAcc core produced the expression of locomotor sensitization in amphetamine (AMPH) pre-exposed rat. In this presentation, we investigated whether actual food restriction in replace of microinjection of ghrelin may produce similar effects.
Methods: We first pre-exposed rats to either saline or AMPH (1 mg/kg, IP) every 2 or 3 days for a total of 4 times. One day before 2 weeks of drug-free withdrawal period ends, the half of each pre-exposure group received food pellet with 50 % restriction, while the other half continued to receive pellet with no restriction. Then, we examined the effect of either saline or D1 DA receptor agonist, SKF 81297 (0.5 μg/side), directly microinjected into the NAcc core on locomotor activity.
Results: Microinjection of SKF 81297 produced sensitized-locomotor activity only in AMPH pre-exposed group with acute food restriction, while these effects were not observed in all other groups.
Conclusions: These results indicate that food restriction, probably by ghrelin working directly to the NAcc as shown in our previous findings, positively regulates psychomotor stimulants-induced locomotor activity to the direction of its increase resulting in locomotor sensitization, and further suggest that D1 DA receptors are necessarily involved in this process.

Keywords: Food restriction, Ghrelin, SKF81297, Nucleus accumbens, Amphetamine

P20-11-02

Deep learning based parameter estimation for ion channel kinetics

Yun Wan Jeon[1], Young Seon Lee[1], Kyu Ri Kim[1], Jae Boum Youm[2], Chae Hun Leem[1]

[1]Department of Physiology, University of Ulsan College of Medicine, Seoul, [2]Department of Physiology, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, Korea

Purpose: Ion channels are the membrane proteins that transport the specific ions to generate and transmit electrical signals. In electrophysiology, the computational models of ion channels express the voltage-gated or pharmacological characteristics. Using computational models, ionic current simulations can predict basic biological principles and drug responses on cardiomyocytes. For this purpose, model parameter fitting is an essential process and time consuming. Appropriate initial guess estimation can facilitate to find the best parameters for the model. Deep learning might help to find the most appropriate initial guess of the model parameters.
Methods: We used a deep learning model that combined Convolutional Neural Network (CNN) and Long Short Term Memory (LSTM) to find parameters of hERG channel. CNN was used to extract important features inherent in data and LSTM was used to learn the temporal relationship in time-series data. For training the deep learning model, we used the hERG channel model and the dataset in previous report (Lei et al. 2019) to construct the hERG channel model and simulation environment. The deep learning model in which the input was IKr current and the outputs were 8 estimated parameters was performed using Python with Keras library.
Results: For the verification of the proposed method, we conducted demonstration that the deep learning model can help to estimate parameter. The fitting model from previous report (Lei et al. 2019) have taken about 4 hours and 40 minutes to fit the data of 211 hERG channel currents with 15,400 data points (1 minute and 20 secnod per each cell data). On the other hand, deep learning model completed the training in 4 minutes and 30 seconds and predicted parameters in 1 second. In addition, the ininial guess estimated by deep learning makes fitting process faster with an average of 1 minute and 5 seconds.
Conclusions: The results suggest that deep learning improve the parameter estimation process and could be leveraged in electrophysiology.

Keywords: Electrophysiology, Ion channel kinetics, Computational models, Deep learning, hERG

P20-12-01

Hidden translational initiation of 453delC-KCNH2 mutant in LQT2 patient generates hERG K^+ channels with reduced activity

Na kyeong Park, Seong Woo Choi, Sung Joon Kim

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

Purpose: Patient-specific cardiomyocytes from human induced pluripotent stem cells (hiPSC-CM) are employed to investigate the inherited cardiac diseases. A recent study reported single nucleotide C deletion mutation in the exon 3 of KCNH2 gene (c.453delC-KCNH2, p.151Pfs +15X in hERG) associated with LQT syndrome (Park JK et al., 2013). Since the 453delC-KCNH2 resulted in the frameshift of the coding sequences, a premature termination codon in N-terminal region was suggested. However, neither hERG protein assay nor functional investigation was conducted yet.
Methods: In this study, we investigated the electrophysiological properties of hiPSC-CMs derived from a LQT2 patient (453delC-KNCH2) and HEK293 cells transfected with 453delC-KCNH2. Electrophysiological analysis was performed using conventional whole-cell patch clamp method. Immunoblot assay was evaluated to detect formation of 453delC-KCNH2 protein.
Results: The 453delC-KCNH2 hiPSC-CM showed significantly prolonged action potential duration (APD) and reduced density of the rapidly activating delayed rectifier K^+ current (I_{Kr}). The density of I_{hERG} in HEK293 cells transfected with 453delC-KCNH2 was 10 % of wild type IhERG. However, the voltage dependency of activation, inactivation and deactivation kinetics of 453delC-KCNH2 were not significantly different from those of WT. Immunoblot analysis of WT consistently indicate double bands at 135kDa (core-glycosylated hERG) and 155kDa (fully-glycosylated hERG). In contrast, the 453delC-KCNH2 overexpressed cells showed a single dominant size at 220kDa that might reflect dimeric proteins of the short-form mutant or putative excessive glycosylation of monomeric proteins.
Conclusions: These results suggest that 453delC-KCNH2 could escape the premature termination via a hidden initiation site. Nevertheless, the markedly reduced I_{hERG} and the prolonged APD was still consistent with the LQT2 phenotype.

Keywords: Human induced pluripotent stem cells-cardiomyocyte, Long QT syndrome type 2, KCNH2 mutation

P20-12-02

The changes of colonic contractility in chronic sertraline treated mice

Min Seob Kim[1], Hyun Seok Choi[1], Moxin Wu[1], JiYeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[2], Seung Ho Jang[3], Moon Young Lee[1]

[1]Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Department of Gastroenterology, Digestive Disease Research Institute, School of Medicine, Wonkwang University, Iksan, [3]Department of Psychiatry, Wonkwang University School of Medicine, Iksan, Korea

Purpose: Sertraline is one of the selective serotonin reuptake inhibitors commonly used as antidepressant at present. However, several gastrointestinal tract side effects such as nausea, diarrhea, and constipation have been reported for sertraline. The purpose of this study was to investigate the mechanism of adverse effects on GI tract function by sertraline, we measured the changes of colonic contractility and the immunoreactivity of acetylcholine and soluble guanylyl cyclase receptors as well as inflammatory markers in chronically treated mice by sertraline.
Methods: C57BL6 mice were intraperitoneally administered at a concentration of 20mg/kg sertraline for 14 days. After sacrifice, the organ bath study was performed to measure the changes of colonic contractility for atropine, L-NAME, Ach, and SNP and immunofluorescence and western blot were performed for nNOS, sGC, CHRM2, and IL-6.
Results: The sertraline-administered group showed elevated basal contractile tone and no atropine effect for colonic contractility. Additionally, the colonic contractility in the sertraline group was increased less after Ach treatment, as well as was decreased less after SNP treatment compared to the control group. In the sertraline group, the expression of nNOS, sGC, and CHRM2 was decreased in muscular layer and the expression of IL-6 was increased in mucosal layer compared to the control group.
Conclusions: The patterns of colonic contractility observed in the sertraline group may be due to decreased expression of sGC and CHRM2. After treatment of exogenous Ach and NO, the less changes in colonic contractility compared to the control also implies that sGC and CHRM2 may be involved. Meanwhile, the increased IL-6 in mucosal layer of the sertraline group suggests that sertraline may cause colonic inflammation as well as the motility changes. Therefore, chronic administration of sertraline can cause several disorders by altering colonic motility and increasing inflammation.

Keywords: Sertraline, Colonic contractility, sGC, CHRM2, IL-6

P20-12-03

Effect of oral famotidine with mosapride compared with famotidine alone on the intragastric pH in the rat stress model

Hyeon Seok Choi[1], Min Seob Kim[1], Jiyeon Myung[1], Eui Joong Kim[1], Yong Sung Kim[1,2], Moon Young Lee[1]

Department of Physiology, Digestive Disease Research Institute, and Institute of Wonkwang Medical Science, School of Medicine, Wonkwang University, Iksan, [2]Good Breath Clinic, Gunpo, Korea

Purpose: Gastric acid-suppressive agents, histamine-2 receptor antagonists (H2RA), and proton pump inhibitor (PPI) are used to treat GERD and peptic ulcers. Studies in healthy humans have reported that adding mosapride to H2RA and PPI resulted in faster gastric acid suppression than a single administration. In this study, we investigated the effect of adding mosapride to famotidine on gastric pH and gastric emptying rate in the rat stress model.
Methods: Male Wistar rats were used. Experimental groups were divided into the control, famotidine only, mosapride only, famotidine with mosapride (combination). Mosapride was administered one hour before the meal, and famotidine was administered just before the meal. Rats were provided food for 30 minutes of mealtime. After isoflurane anesthesia, gastric pH was measured at the antrum, and then emptying rate of the stomach was measured. For the stress model, the restraint stress was provided for one hour immediately after the mosapride administration.
Results: Compared to the control group, famotidine only and combination group showed significantly lower gastric pH levels in the antrum in both non-stress and stress models. Combination group also showed significantly lower gastric pH levels in the antrum than famotidine only group in both the non-stress and stress model. Mosapride only and combination group showed significantly higher gastric emptying rate compared with famotidine only, except mosapride only in the stress model with a tendency ($p=0.056$).
Conclusions: Mosapride and famotidine combination treatment showed an earlier effect for reducing gastric acid secretion than famotidine only treatment even in the stressed state in rats.

Keywords: Famotidine, Mosapride, Gastric pH, Gastric emptying

P20-12-04

Human physio-types: from body shape to inner functions

Duong Duc Pham, Chae Hun Leem

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

Purpose: Recently, body typing, a long history phenotype-based classification, has been an emerging interest as a potential approach toward personalized medicine. We aim to use multi-dimension physio-biological information from healthy adults to derive the so-called physio-type and examine its reproducibility and clinical application.
Methods: Physio-type was derived using the information of 146 physio-biomarkers of 672 apparently healthy adults aged between 20 to 69 years by hierarchical clustering on principal component (HCPC). Validation of the physio-type was performed by latent class analysis (LCA) on the same data after a dimension reduction. The association of the physio-type and the 12-year incidence of type 2 diabetes (T2DM) and hypertension (HTN) was examined in a large cohort study.
Results: Three physio-types were derived that substantially differed in physio-biomarker patterns in both genders. The PS3 was likely heavy, fatty, upper-trunk developed, and having high erythrocytes, poor glucose metabolism, and low fitness, whereas the PS1 was on the opposite side. The PS2 was likely muscular, tall, and having strong airway ventilation, high energy expenditure, quick glucose metabolic response, and high fitness. The pattern of difference in physio-biomarkers among three physio-types was compatible independently to the data sets and clustering methods. In the validation study, Cohen's Kappa values range between 0.643 and 0.873. Cohort study shows that the PS3 had the highest risk of T2DM and HTN and the risk reduced in the PS1 and PS2, respectively. In comparison with the PS3, the mean T2DM hazard ratios of the PS1 and PS2 were 0.62~0.87 and 0.44~0.65, whereas the mean HTN hazard ratios of the PS1 and PS2 were 0.58~0.64 and 0.53~0.59, respectively.
Conclusions: The application of multidimensional physio-biological information could help to access the physio-types' traits holistically. These derived physio-types were distinctive with explainable interactions from external traits, inner functions, and clinically relevant. Further studies should be performed to consolidate the physio-type platform and extend its application. Funding number: 19172MFDS168

Keywords: Physio-type, Phenotype, Body type, Typology

P20-12-05

Lipid emulsion provides neuroprotection in an animal model of stroke.

Motomasa Tanioka[1,2], Wyun Kon Park[3], Joohyun Park[2,4], Jong Eun Lee[2,4], Bae Hwan Lee[1,2]

[1]Department of Physiology, [2]Brain Korea 21 PLUS Project for Medical Science, [3]Department of Anesthesiology and Pain Medicine, Anesthesia and Pain Research Institute, [4]Department of Anatomy, Yonsei University College of Medicine, Seoul, Korea

Purpose: Stroke is a life-threatening condition that leads to the death of many people around the world. Reperfusion injury after ischemia is a recurrent problem associated with various surgical procedures in brain arteries. Lipid emulsion was recently shown to attenuate ischemic reperfusion injury in the heart and to protect the brain from excitotoxicity. However, investigations on the protective mechanisms of lipid emulsion against ischemia in the brain are still lacking. This study aimed to determine the neuroprotective effects of lipid emulsion in an in vivo rat model of ischemic reperfusion injury through middle cerebral artery occlusion (MCAO).
Methods: Rats were subjected to MCAO surgery under sodium pentobarbital anesthesia and were administered with lipid emulsion through intra-arterial injection during reperfusion. The experimental animals were assessed for neurological deficit wherein the brains were extracted at 24 h after reperfusion for triphenyltetrazolium chloride staining, immunoblotting and qPCR.
Results: Neuroprotection was found to be dosage-dependent and the rats treated with 20% lipid emulsion had significantly decreased infarction volumes and lower Bederson scores. Phosphorylation of Akt and glycogen synthase kinase 3-β (GSK3-β) were increased in the 20% lipid emulsion-treated group. The Wnt-associated signals showed a marked increase with a concomitant decrease in signals of inflammatory markers in the group treated with 20% lipid emulsion. The protective effects of lipid emulsion and survival-related expression of genes such as Akt, GSK-3β, Wnt1 and β-catenin were reversed by the intra-peritoneal administration of XAV939 through the inhibition of the Wnt/β-catenin signaling pathway.
Conclusions: These results suggest that lipid emulsion has neuroprotective effects against ischemic reperfusion injury in the brain through the modulation of the Wnt signaling pathway and may provide potential insights for the development of therapeutic targets.

Keywords: Neuroprotection, Stroke, Ischemia, Middle cerebral artery occlusion, Reperfusion injury, Lipid emulsion, Excitotoxicity

P20-12-06

Dipeptide YA is responsible for the positive effect of oyster hydrolysates on alcohol metabolism in single ethanol binge rodent models

Adrian S. Siregar[1,2], Marie Merci Nyiramana[1,2], Eun-Jin Kim[1], Eui-Jung Shin[1,2], Min Seok Woo[1,2], Jin-Mok Jin-Mok[3], Jung Hwan Kim[4], Dong Kun Lee[1,2], Jong Ryeal Hahm[5], Hyun Joon Kim[2,6], Chang-Woon Kim[7], Nam-Gil Kim[8], Si-Hyang Park[9], Yeung Joon Choi[10], Sang Soo Kang[2,6], Seung-Geun Hong[1], Jaehee Han[1], Dawon Kang[1,2]

[1]Department of Physiology and Institute of Health Sciences, College of Medicine, Gyeongsang National University, [2]Department of Convergence Medical Science, Gyeongsang National University, Jinju, [3]Department of Clinical Laboratory Science, Masan University, Changwon, [4]Department of Premedicine, [5]Department of Internal Medicine, Hospital and Institute of Health Sciences, [6]Department of Anatomy and Institute of Health Sciences, College of Medicine, Gyeongsang National University, Jinju, [7]Department of Obstetrics and Gynecology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, [8]Department of Marine Biology and Aquaculture and Institute of Marine Industry, Gyeongsang National University, Tongyeong, [9]Sunmarin Biotech, [10]Ocean-Pep, Jinju Bioindustry Foundation, Jinju, Korea

Purpose: Accumulative alcohol hangovers cause liver damage through oxidative and inflammatory stress. Numerous antioxidant and anti-inflammatory reagents have been developed to reduce alcohol hangovers, but these reagents are still insignificant and have limitations in that they can cause liver toxicity. Oyster hydrolysate (OH), another reagent that has antioxidant and anti-inflammatory activity, is a product extracted through an enzymatic hydrolysis process from oysters (Crassostrea gigas), which can be easily eaten in meals. This study was aimed at determining the effects of OH and tyrosine-alanine (YA) peptide on alcohol metabolism, using a single high dose of ethanol (EtOH) administered to rodents.
Methods: The effects of OH and YA on alcohol metabolism were determined using a single high dose of ethanol (EtOH, 3 g/kg) administered to rodents, by monitoring alcohol metabolic enzymes, oxidative stress signals, and inflammatory mediators.
Results: In vitro experiments showed that OH pretreatment inhibited EtOH-induced cell death, oxidative stress, and inflammation in liver cells and macrophages. In vivo experiments showed that OH and YA pre-administration increased alcohol dehydrogenase, aldehyde dehydrogenase, and catalase activity in EtOH binge treatment. In addition, OH pre-administration alleviated CYP2E1 activity, ROS production, apoptotic signals, and inflammatory mediators in liver tissues.
Conclusions: These results showed that OH and YA enhanced EtOH metabolism and had a protective effect against acute alcohol liver damage. Our findings offer new insights into a single high dose of EtOH drinking and suggest that OH and YA could be used as potential marine functional foods to prevent acute alcohol-induced liver damage.

Keywords: Alcohol, Inflammation, Liver injury, Oxidative stress

P20-12-07

Effects of Cheonwangbosimdan (CBD) of cognitive function induced by Scopolamine

Sungyoung Cho, Changsu Na

Department of Korea Medicine, Dongshin University, Korea

Purpose: The incidence of AD (Alzheim's Disease), one of the main causes of dementia, is expected to reach 13 million in the U.S. alone by 2050 and more than 100 million worldwide, mainly due to cognitive impairment, accounting for 55 to 70 percent of all dementia.
AD is a chronic disease that causes daily life disorders as memory and other cognitive functions gradually deteriorate due to deterioration of brain cells, and various theories such as accumulation of amyloid proteins in the brain, death of cholinergic nerve cells, abnormalities in energy metabolism of mitochondria, abnormalities in neurons, other oxidative stress, genetic mutation, and neuroinflammatory reactions are being raised. However, degenerative brain diseases such as Alzheimer's have not clearly identified a single disease mechanism.
In this study, the Cheonwangbosimdan (CBD) was formed by combining raw paper, dansam, and maekmun-dong, which are known to help improve cognitive function, as well as white porcelain and hyeonsam, which are effective in protecting nerve cells, and the cognitive function of Cheonwangbo Simdan against cognitive models damaged by Scopolamine (Sco).
Methods: Morris Water Maze test, Reverse Transcription Polymerase Chain Reaction, ACh content and ACHE active measurements
Results: Morris water maze
Moriss tests were conducted five times during the experiment. In addition to observing significance in the control group (0.05) compared to the general group, significant differences were observed in the experimental group ($p<0.05$).
RT- PCR
We observed a decrease in the DNA expression of BDNF and CREB in comparators compared to the general group, and an increase in expression in experimental groups compared to comparators. However, no significant difference was seen.
Ach kit
Acetylcholine observed a significant decrease in control over the general population ($p<0.01$) (a significant increase in the control group was observed in the control group).
Acetylcholinesterase kit
The Acetylcholinesterase activity observed an increase in comparators compared to the general group, and a decrease in comparators was observed in the control group. However, no significant difference was seen.
Conclusions: AD accounts for the majority of dementia, and so far the clear mechanism for treatment is unclear. In this study, we studied the effects of CBD, a herbal medicine, on improving cognitive function. The results of this study showed the effects in the results of Ach,AchE in the experimental group, and the effects were observed in the BDNF-CREB mechanism. However, this suggests a possible effect on CBD's cognitive function and suggests the need for further research.

Keywords: Scopolamine, Cheonwangbosimdan (CBD), Acetylcholine (ACH), Acetylcholinesterase, BDNF (brain-derived neurophic factor), CREB (cAMP response element-binding), chAT (choline acetyltransferase)

P20-12-08

Generation of a gene edited Hemophilia A patient-derived iPSC line, YCMi001-B-1, by targeted insertion of coagulation factor FVIII using CRISPR/Cas9

Sanghyun Park[1,2], Jin Jea Sung[1,2,3], Sang-Hwi Choi[1,2], Jongwan Kim[4], Myung Soo Cho[4], Dong-Wook Kim[1,2,3]

[1]Department of Physiology, [2]Brain Korea 21 PLUS Program for Medical Science, [3]Severance Biomedical Research Institute, Yonsei University College of Medicine, [4]ES Team, S. Biomedics Co., Korea

Purpose: Hemophilia A is a genetic disease caused by mutations in the coagulation factor FVIII gene. Since a mild increase in FVIII improves symptoms in patients, Hemophilia A is an ideal target for cell or gene therapy. In this study, we describe a universal genome correction strategy to restore FVIII expression in induced pluripotent stem cells (iPSCs) derived from a patient with hemophilia A.
Methods: Previously, we generated an induced pluripotent stem cell (iPSC) line, Del-iPSC-Epi6, using adipose tissue-derived mesenchymal stem cells obtained from a hemophilia A patient with a gross deletion of exons 8-22 in FVIII locus. In this study, we inserted the B-domain deleted form of FVIII (BDD-FVIII) cDNA into exon 1 of FVIII locus in the Del-iPSC-Epi6 line using CRISRP/Cas9 to restore FVIII expression. In detail, Cas9/sgRNA and BDD-FVIII donor plasmid were introduced into patient-iPSCs via electroporation. Following G418 selection, NeoR in the gene-corrected iPSCs was removed by Cre recombinase expression. G418 resistant colonies were individually picked, expanded, and verified via PCR and Sanger sequencing to identify correctly targeted clones. Then, we confirmed that gene-corrected iPSC line

(YCMi001-B-1) maintains typical characteristics of pluripotent stem cell. Finally, YCMi001-B-1 was differentiated into endothelial cells and determined FVIII expression.
Results: We established gene-corrected hemophilia A patient-iPSC line (YCMi001-B-1). YCMi001-B-1 maintained pluripotency marker expression, exhibited the capacity to generate all 3 germ layers and a normal karyotype. YCMi001-B-1 also sucssessfully differentiated into endothelial cells and restored FVIII expression, which are deficient in patient-iPSC derivatives.
Conclusions: CRISPR/Cas9 mediated gene editing allowed site-specific integration of BDD-FVIII cassette into exon 1 of FVIII locus in the patient-iPSCs. Our gene correction strategy resulted in the expression of FVIII transcript from the gene-corrected iPSC line-derived endothelial cells. Importantly, It is significant that our strategy restores FVIII expression in patient-iPSCs under the FVIII endogenous promoter without introducing exogenous promoters.

Keywords: Hemophilia A, Coagulation factor FVIII, Induced pluripotent stem cell (iPSC), Gene editing, CRISPR/Cas9

P20-12-09

Echinochrome A increases mitochondrial mass and function by modulating mitochondrial biogenesis regulatory genes

Seung Hun Jeong[1,2,3], Hyoung Kyu Kim[1,2,3], In-Sung Song[1,2,3], Jubert Marquez[3], Amy H. Kim[3], Kyung Soo Ko[1,2,3], Byoung Doo Rhee[1,2,3], Natalia P. Mishchenko[4], Sergey A. Fedoreyev[4], Valentin A. Stonik[4], Jin Han[1,2,3]

[1]Cardiovascular and Metabolic Disease Center (CMDC), National Research Laboratory for Mitochondrial Signaling, Inje University, Busan, [2]Department of Physiology, College of Medicine, Inje University, Busan, [3]Department of Health Sciences and Technology, Graduate School of Inje University, Busan, Korea, [4]George B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Science, Vladivostok, Russia

Purpose: Echinochrome A (Ech A) is a natural pigment from sea urchins that has been reported to have antioxidant properties and a cardio-protective effect against ischemia reperfusion injury. In this study, we ascertained whether Ech A enhances mitochondrial biogenesis and oxidative phosphorylation in rat cardio myoblast H9c2 cells. To study the effects of Ech A on mitochondrial biogenesis, we measured mitochondrial mass, oxidative phosphorylation levels, and mitochondrial biogenesis regulatory gene expression.
Methods: Chemicals, cell culture, cell viability measurement, cytotoxicity measurement, mitochondrial membrane potential measurement, ROS measurement, mitochondrial ATP level measurement, OCR measurement, mitochondrial mass measurement, RT-PCT and real-time PCR, qPCR for mitochondrial DNA, Western blot
Results: Echinochrome A reduced reactive oxygen species (ROS) generation but did not interfere with cellular viability. Echinochrome A enhanced mitochondrial biogenesis function. Echinochrome A upregulated expression of mitochondrial biogenesis regulated gene and increased mitochondrial contents. Echinochrome A modulated proliferator-activated receptor gamma co-activator (PGC)-1α expression via phosphorylation of CREB, and Ech A treatment increased phosphorylation of CREB and PGC-1α.
Conclusions: Echinochrome A increased mitochondrial mass and OXPHOS function significantly, which enhanced mitochondrial energy efficiency by modulating major mitochondria biogenesis regulatory genes, including PGC-1α and NRF-1. Our results suggest that Ech A has the potential to enhance mitochondrial energy metabolism, which may be clinically beneficial for the treatment of various mitochondrial dysfunctions implicated in metabolic diseases.

Keywords: Echinochrome A, Mitochondrial biogenesis, Oxygen consumption rate

P20-12-10

Generation of a human induced pluripotent stem cell line, YCMi002-A, from a Factor VII deficiency patient carrying F7 mutations and an isogenic control line

Do-Hun Kim[1,2], Chul-Yong Park[3], Sung-Rae Cho[4], Dong-Wook Kim[1,2,5]

[1]Department of Physiology, [2]Brain Korea 21 PLUS Program for Medical Science, Yonsei University College of Medicine, Seoul, [3]Center for Genome Engineering, Institute for Basic Science, Daejeon, [4]Department and Research Institute of Rehabilitation Medicine, [5]Severance Biomedical Research Institute, Yonsei University College of Medicine, Seoul, Korea

Purpose: Factor VII (FVII) deficiency is the most common among the rare bleeding disorders, which is caused by mutations in coagulation factor VII. Clinical features caused by FVII deficiency vary from mild or asymptomatic to fatal cerebral hemorrhage. To establish a disease model for FVII deficiency, we generated an induced pluripotent stem cell (iPSC) line, YCMi002-A, from FVII deficiency patient-derived fibroblasts and its isogenic control cell line.
Methods: We reprogrammed patient-derived fibroblasts into iPSCs using integration-free episomal plasmids expressing five pluripotency factors (OCT4, SOX2, KLF4, L-MYC, and Lin28). After the induction of iPSC, we picked several iPSC colonies with a normal human embryonic stem-cell (hESC) like morphology. After selection, we checked the expressions of several pluripotency markers by immunostaining and quantitative-polymerase chain reactions. We identified heterozygous compound mutations in FVII locus by Sanger sequencing. To correct mutations in FVII loci, we used CRISPR/Cas9 system and single-stranded oligodeoxynucleotides as donor template.
Results: YCMi002-A highly expressed the pluripotency markers (OCT4, SOX2, and NANOG), and the surface markers (SSEA4, TRA-1- 81, and TRA-1-60). The expression level of pluripotency markers measured using quantitative-polymerase chain reactions (PCR) in YCMi002-A was similar to human embryonic stem cell H9. Pluripotency was further evaluated by differentiation of three germ layers. Using embryoid body (EB) formation assay, we demonstrated that YCMi002-A cells could be differentiated into the principal cells in three germ layers with expression of ectodermal marker Nestin, endodermal marker Sox17, and mesodermal marker α-SMA. We identified heterozygous compound mutations in YCMi002-A by Sanger sequencing. By transient screening with the CRISPR/Cas9 system and single-stranded oligodeoxynucleotides (ssODNs), the two mutations were sequentially corrected.
Conclusions: We established an iPSC line derived from an novel FVII compound heterozygous patient and its isogenic control line. These iPSC lines may be helpful to study the correlation between the severity of FVII deficiency and mutations.

Keywords: Human induced pluripotent stem cell (hiPSC), Type II clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), Factor VII deficiency, Gene correction

P20-12-11

Early cardiomyocyte differentiation from mouse embryonic stem cell is regulated by mitochondrial pyruvate dehydrogenase phosphatase 1

Sun Woo Kim[1], Hyoung Kyu Kim[1], Hye Jin Heo[1], Jae Boum Youm[1], Sung Woo Cho[2], In-Sung Song[1], Sun Young Lee[1], Tae Hee Ko[1], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], Jin Han[1]

[1]Department of Physiology, National Research Laboratory for Mitochondrial Signaling, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, [2]Laboratory of Vascular Biology and Stem Cell, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Purpose: To examine whether mitochondrial pyruvate dehydrogenase phosphatase 1 (PDP1) affect cardiomyocyte differentiation, we established PDP1 up- and down-regulated mESCs and investigated the relatively unre-

vealed mitochondrial function including energy metabolism.

Methods: Embryoid bodies formation, Measurement of Beating rate, Patch clamp, Microarrays, Immunocytochemistry, Quantitative Real-Time PCR, Western blotting, Flow cytometry, ATP quantitation assay, Oxygen consumption rate, PDH activity and pyruvate assay

Results: Differentiation of mESC-derived cardiomyocytes using EBs, Electrophysiological properties of mESC-derived cardiomyocytes in EBs, Mitochondrial function changes during differentiation, Gene expression changes during differentiation, Pdp1 overexpression and mitochondrial function in mESC-derived EBs, Pdp1 overexpression and changes in PDH complex activity in differentiating EBs, Pdp1 overexpression and cardiomyogenesis in mESC-derived EBs, Pdp1 suppression and mitochondrial function in mESCderived EBs, Downregulation of PDH complex activity via Pdp1 inhibition in feeder-free mESCs, Upregulation of cardiomyogenesis from Pdp1 inhibition in feeder-free conditions

Conclusions: Up-regulated PDP1 induce the elevated energy production following higher mitochondrial function but attenuate the cardiomyogenesis even under the same hypoxic condition of EBs differentiation. We suggest here that PDP1 acts as the mitochondrial regulator which modulates cardiomyogenesis from mESCs differentiation.

Keywords: Embryonic stem cells (ESCs), Mitochondrial pyruvate dehydrogenase phosphatase 1 (PDP1)

INDEX

Author Index

[I]

[J]

[K]

[L]

[M]

[N]

[O]

[P]

Keyword Index

[A]

[B]

[C]

[D]

[E]

[F]

[G]

[H]

[A]

[J]

[K]

[L]

[M]

[N]

[O]

[P]

[R]

[S]

[T]

[U]

[V]

[W]

[X]

[Y]

[Z]

[Etc.]