The 64th Annual Meeting of Korean Physiological Society in conjunction with KOJACH

KPS 2012

24-26 | 10 | 2012

INJE UNIVERSITY HAEUNDAE PAIK HOSPITAL BUSAN

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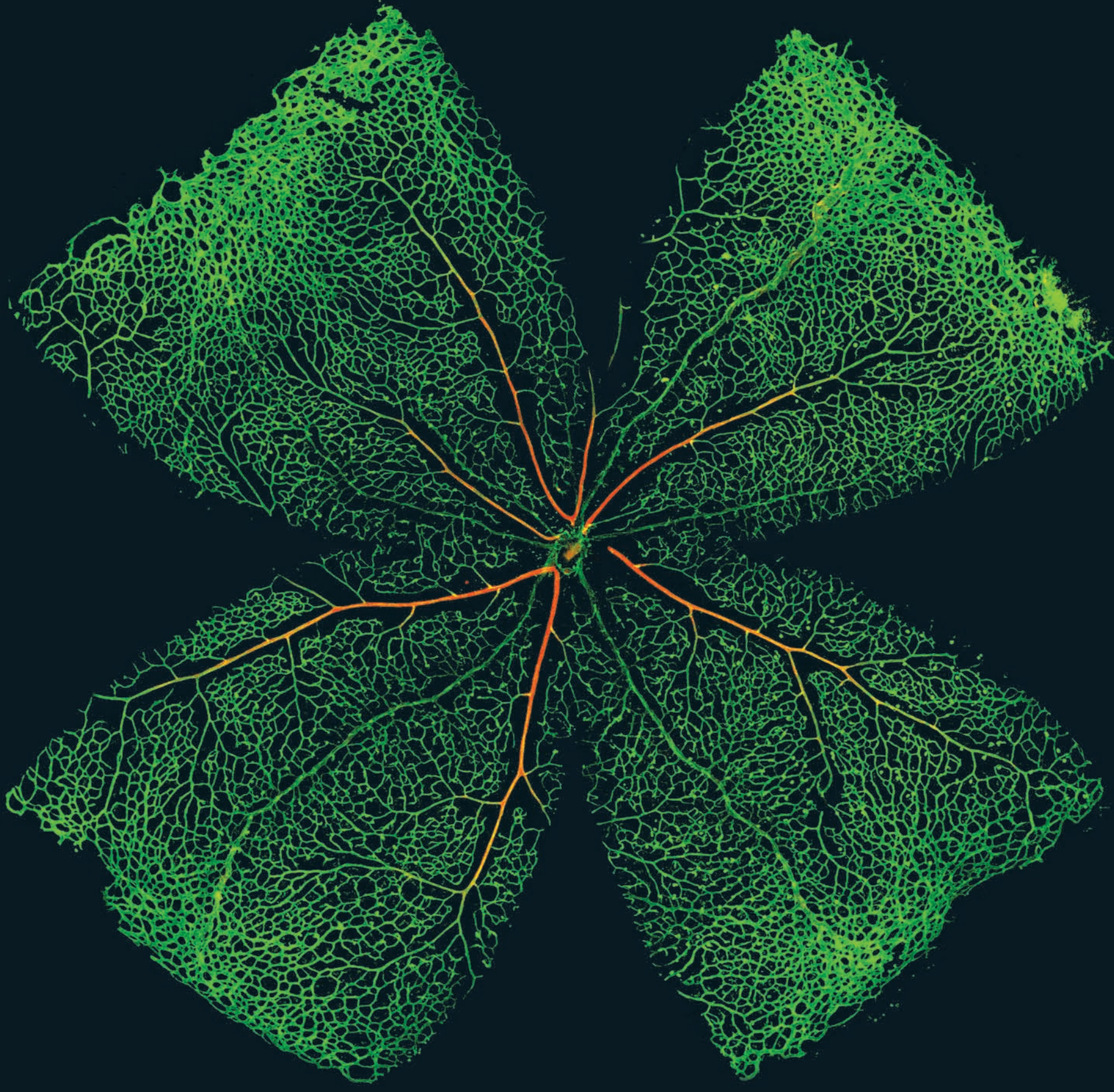
BEAUTY OF LIFE



Korean Physiological Society
Inje University

2012 대한생리학회 임원명단

고 문	**강두희 강복순 고일섭 권종국 길원식 김광진 김기순 김기환 김명석 김용근 김우겸 김종규 김종환 김중수 남숙현 박양생 박춘식 박형진 배선호 백광세 문창현 신홍기 양일석 엄대용 엄융의 윤평진 이상돈 이상호 이석강 이종흔 이진옥 이중우 조경우 채의업 최덕경 하종식 홍승길**
자문 위원	**김 전 나흥식 민병일 이승일 이원정 이종은 조양혁**

회 장	**이원정**	차기 회장	**민병일**
이 사 장	**조양혁**	차기이사장	**나흥식**
기금위원장	**문창현**	총무 이사	**이덕주**
교육 이사	**안덕선**	정보 이사	**김민선**
국제 이사	**정진섭**	기획 이사	**한재희**
편집 이사	**한희철**	학술 이사	**김상정**
부편집장	**강동묵 김상정 안동국 이지희**	학술 위원	**강동묵 김성준 배영민 전병화 진영호**

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

조직위원회

김나리 인제의대

김용운 영남의대

김재호 부산의대

박규상 연세원주의대

배재성 경북의대

배혜란 동아의대

서인석 서울의대

송대규 계명의대

안도환 고신의대

염재범 인제의대

임채헌 울산의대

정진섭 부산의대

한재희 경상의대

한 진 인제의대

Acknowledgement

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Ministry of Education & Science Technology

National Research Foundation of Korea

Korean Federation of Science and Technology Societies

Biomembrane Plasticity SRC, Seoul National University

Cardiovascular and Metabolic Disease Center, Inje University

Inje University

Exhibited by

고마바이오텍	글로케스
다이아텍코리아	대한과학
동남교역	라이프텍
브니엘 바이오	알파사이언스
인코사이언스	에스엠텍
제노믹원	팜텍
팔이합성	환은바이오텍
칼자이스 사이언스	태신바이오사이언스
싸이텍코리아	KDB
Parks System	YK 성형외과

Contents

Invitation (초대의 글)

대한생리학회 회원 여러분! 안녕하세요?

우리 학회는 이번 가을 해운대에 있는 인제의대 백병원에서 정기학술대회를 개최합니다. 세계적인 석학의 기조강연과 다양한 주제의 수준 높은 심포지엄, 그리고 포스터 세션의 풍성한 내용 등이 여러분을 기다리고 있습니다. 이번 학술대회는 학문적 토론이 꽃피고, 연구자들 우의가 무르익는 풍성한 잔치입니다. 또한 국내외 협력의 네트워크를 구축하는 뜻 깊은 자리입니다. 회원 여러분의 적극적인 참여와 협조로, 학술대회가 알찬 성과를 거두리라고 확신합니다.

이번 학술대회를 준비하기 위해서 많은 분들이 애쓰셨습니다. 이사님들을 비롯하여, 인제의대 관계자 여러분, 연구발표에 참여하신 여러분, 그 밖에 물심양면으로 지원해주신 여러분들의 노고에 깊이 감사드립니다. 회원 여러분! 가을 정취가 가득한 해운대 바닷가에 우리 모두 모여서, 풍요로운 학문의 성찬을 즐기며 우리들의 우의도 도탑게 합시다. 여러분의 건강과 눈부신 발전을 기원합니다.

감사합니다.

대한생리학회 회 장 **이 원 정**

대한생리학회 이사장 **조 양 혁**

Schedule (일정표)

Wednesday, October 24

장소: 인제대학교 의과대학(부산광역시 진구 개금동 소재)

Time	12th Floor, Lecture Hall, Cardiovascular and Metabolic Disease Center, Inje University
11:00～14:00	Registration for Satellite Symposium
14:00～14:30	Welcome address for Satellite Symposium, Vice President of Inje University
14:30～17:30	**Satellite Symposium:** Mitochondrial Biology (Co-organized by Cardiovascular and Metabolic Disease Center, Inje University)
17:30～18:00	Q&A
18:00～18:10	Photograph for Satellite Symposium
18:10	Closing Ceremony for Satellite Symposium

Thursday, October 25

장소: 인제대학교 해운대백병원(부산광역시 해운대구 좌동 소재)

Time	Hall A	Hall B	Time
11:40～12:00		Advisory Board Meeting	11:40～12:00
12:00～13:20		Board of Directors Meeting	12:00～13:20
12:30～13:20	Registration		12:30～13:20
13:20～13:30	Opening Ceremony		13:20～13:30
13:30～14:30	**Special Lecture I**	**Special Focus Session I:** Two-Photon Imaging Approaches in Physiology	13:30～15:10
14:30～15:30	**Special Lecture II**		
15:30～16:30	**Special Lecture III**	**Special Focus Session II:** Synapse Pathology in Neurological Diseases	15:10～16:30
16:30～18:00	Coffee Break and **Poster Presentation**		16:30～18:00
	Poster Oral Presentation I: Ion Channels and Transporters	**Poster Oral Presentation II:** Molecular Physiology	
18:00	Reception		18:00

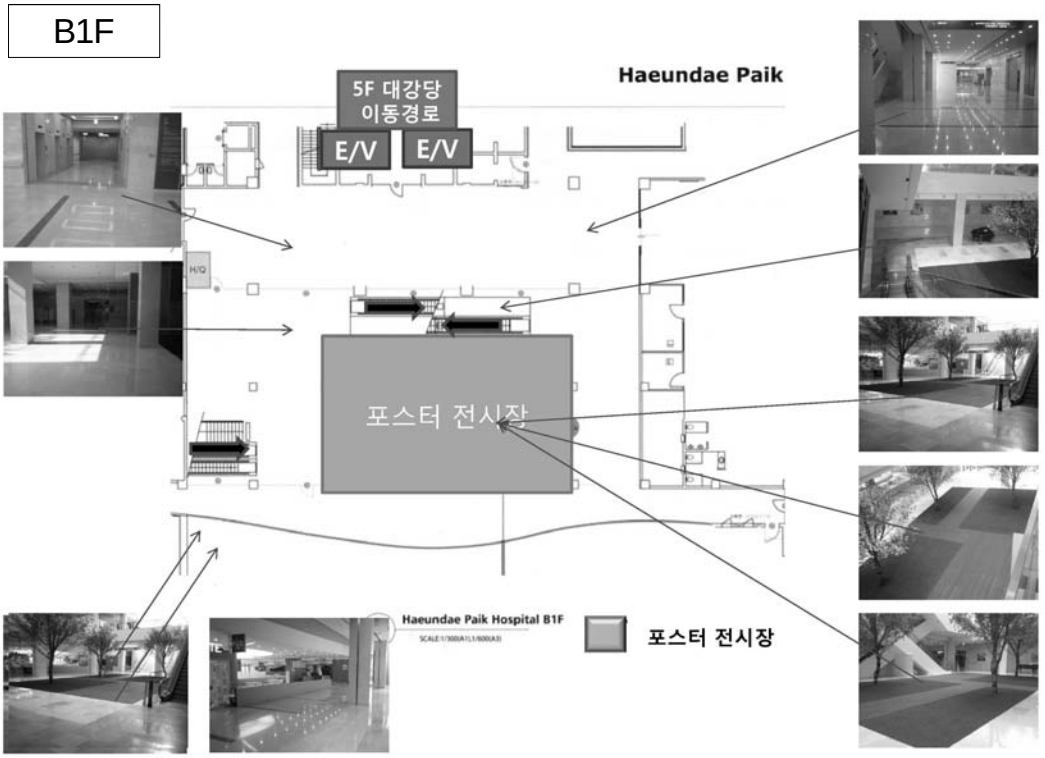
Friday, October 26

장소: 인제대학교 해운대백병원(부산광역시 해운대구 좌동 소재)

Time	Hall A	Hall B	Time
09:00～09:30	Youdang Scholarship Award Lecture		09:00～09:30
09:30～12:00	**Symposium I (KOJACH Symposium):** Ion Channel Mechanisms for Integration of Neural Signals (Sponsored by KOJACH and Biomembrane Plasticity SRC)	**Symposium II:** The Next Advance in Cardioprotection (Co-organized by Cardiovascular and Metabolic Disease Center, Inje University)	09:30～12:00
12:00～12:30	General Assembly		12:00～13:30
12:30～13:30	Photograph and Lunch		
13:30～15:00	Coffee Break and **Poster Presentation**		13:30～15:00
	Poster Oral Presentation III: Neuronal Cells and Muscle Cells	**Poster Oral Presentation IV:** Systemic or Integrative Physiology	
15:00～16:40	**Special Focus Session III:** Metabolism and Bioenergetics	**Special Focus Session IV:** Hypothalamic Neuroendocrine Regulation	15:00～16:40
17:00～17:20	Poster Prize Award and Gift Lucky Draw Closing Ceremony		17:00～17:20
17:20～17:30			17:20～17:30

Venue Guide (학술대회장 안내)

인제대학교 해운대백병원



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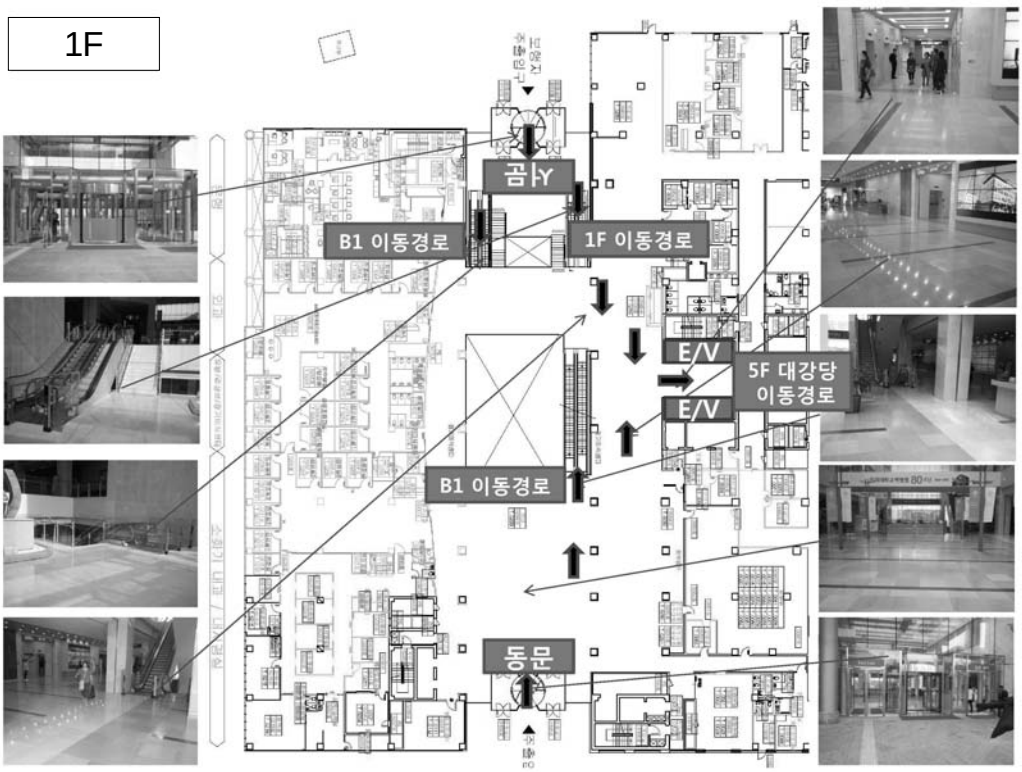
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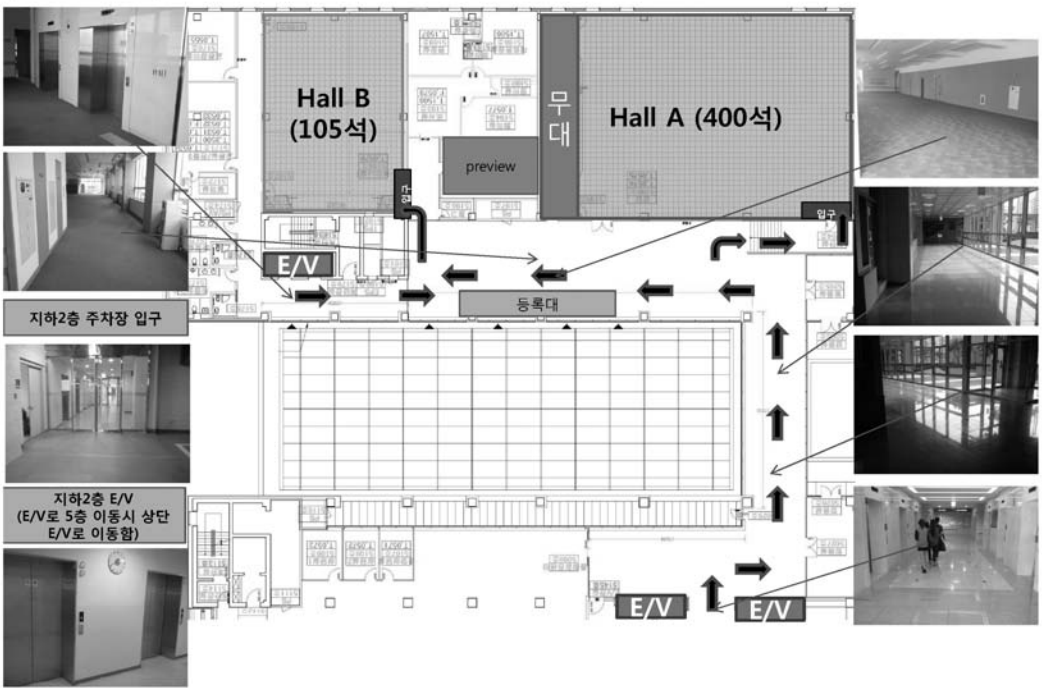
B1 이동경로
1F 이동경로
E/V
E/V
5F 대강당
이동경로
B1 이동경로
동문

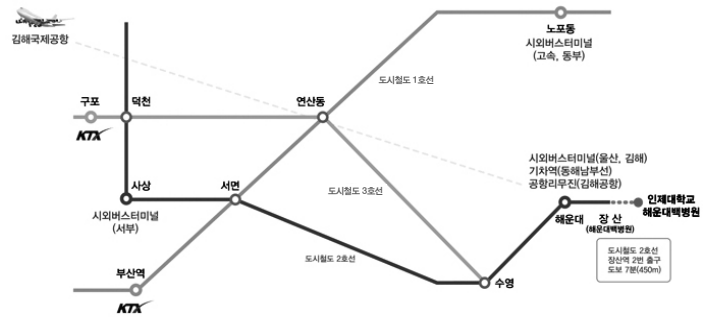
5F


Hall B
(105석)
preview
무대
Hall A (400석)
입구
E/V
등록대
지하2층 주차장 입구
지하2층 E/V
(E/V로 5층 이동시 상단
E/V로 이동함)
E/V
E/V

➜ 병원 부대시설

	- 신한은행: 1층 - 영업시간: 09:00～16:00 - 전화번호: 1577-8000		- 버들(전문식당가): 지하1층 - 영업시간: 07:30～20:30 - 전화번호: 797-0377
	- 카페 모카(커피전문점): 1층 - 영업시간: 08:00～20:30 - 전화번호: 797-0250		- 건강보조식품: 지하1층 - 영업시간: 09:00～18:00 - 전화번호: 797-0306
	- 세븐일레븐(편의점) : 지하1층 - 영업시간: 24시간 - 전화번호: 797-0378		- 여행사: 지하1층 - 영업시간: 09:30～18:00 - 전화번호: 797-0303

➜ 찾아오는 길



공항 리무진버스

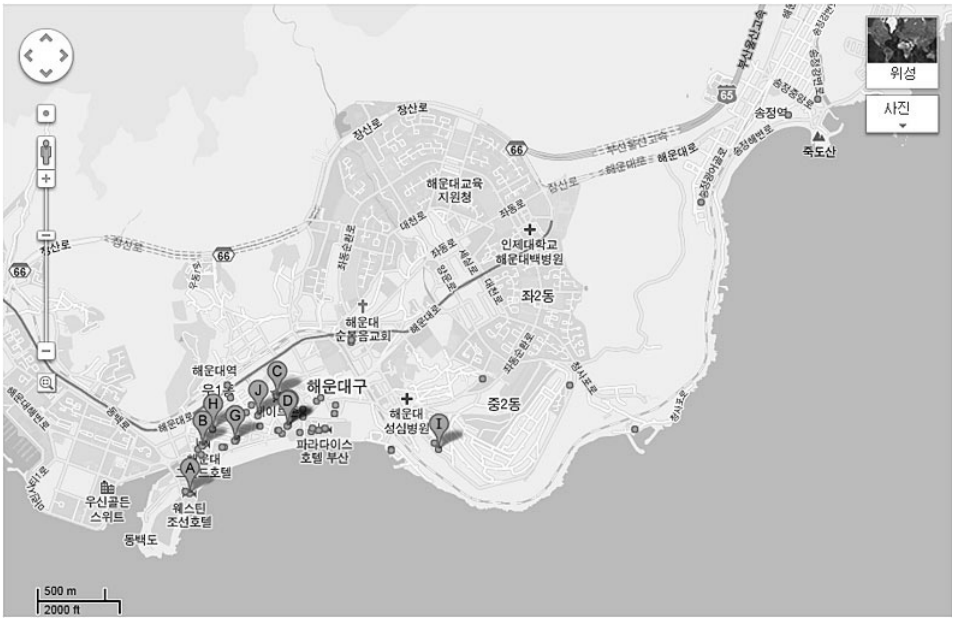
번 호	배 차	요 금	주요 경유지(호텔)
2	20분	6,000원	벡스코 센텀호텔 그랜드호텔 웨스틴 조선 노보텔 앰배서더 파라다이스 호텔

자가용 이용 시

- 공항에서 경전철-지하철 이용시
  - 사상역에서 환승
  - 총 29개 정거장
  - 소요시간 1시간

- KTX 부산역에서 지하철 이용시
  - 서면역에서 환승
  - 총 24개 정거장
  - 소요시간 47분

➜ 학회장소 및 인근 호텔 위치



➜ 티파니21 투어

- 다양한 컨텐츠와 이벤트를 즐길 수 있는 새로운 개념의 파티컨벤션 크루즈
- 8 km의 아름다운 해운대 해안선과 APEC 회의장이 있는 동백섬, 멋진 야경을 자랑하는 국내 최대 해상교량인 광안대교 감상
- 19:00～21:00 디너투어: 저녁식사(뷔페, 또는 코스 요리), 음료제공, 라이브공연



➜ 교통편과 약도

- **버스**
  - 버스
    * 100, 36, 5, 200-1, 63, 42, 302, 307번
      (약 22분 소요. 병원 바로 앞 부흥고 정류장에서 1003번을 타고 8정거장을 가서 동백섬입구 정류장에서 하차)

- **지하철**
  - 지하철 : 2호선 동백섬역 하차

- **자가용 이용안내**

해운대방면 → 동백역- 경부고속도로(대구, 울산)→ 원동IC → 해운대방면 → 동백역

- 티파니투어 소요시간 19～21시. 18시 30분부터 승선가능

Scientific Program (학술프로그램)

Wednesday, October 24

12th Floor, Lecture Hall, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan

14:30～17:30	**Satellite Symposium:** Mitochondrial Biology Co-organized by Cardiovascular and Metabolic Disease Center, Inje University **Chairs:** *Huangtian Yang, Shinya Minatoguchi*
14:30～14:50	▶ The Post-Translational Modifications of Mitochondrial Proteins in Heart Failure *Sung Ryul Lee (Inje University, Korea)*
14:50～15:30	▶ Atrial Natriuretic Peptide Prevents the Mitochondrial Permeability Transition Pore Opening by Inactivating Glycogen Synthase Kinase 3β via PKG and PI3K in Cardiac H9c2 Cells *Zhelong Xu (The University of Northth Carolina, USA)*
15:30～15:50	*Coffee Break*
15:50～16:30	▶ Transcription Factor-Driven Conversion of Quiescent Cardiomyocytes to Pacemaker Cells *Hee Cheol Cho (Cedars-Sinai Medical Center, USA)*
16:30～16:50	▶ The Novel Action Mechanism of Angiotensin II Receptor Antagonist *Jin Han (Inje University, Korea)*
16:50～17:30	▶ Mitochondrial DNA That Escapes from Autophagy Causes Inflammation and Heart Failure *Issei Komuro (The University of Tokyo, Japan)*

Thursday, October 25

Hall A

13:30～14:30	**Special Lectures I** **Chair:** *Yang Hyeok Cho*
	▶ Molecular Mechanisms and Novel Treatments of Heart Failure *Issei Komuro (The University of Tokyo, Japan)*
14:30～15:30	**Special Lecture II** **Chair:** *Won-Jung Lee*
	▶ Organotypic Angiogenesis and Vascular Remodeling *Gou Young Koh (KAIST, Korea)*
15:30～16:30	**Special Lecture III** **Chair:** *Sang Jeong Kim*
	▶ Biological Therapies for Cardiac Arrhythmias: Can Genes and Cells Replace Drugs and Devices? *Hee Cheol Cho (Cedars-Sinai Medical Center, USA)*
16:30～17:30	**Poster Oral Presentation I (PO-1~6):** Ion Channels and Transporters **Chairs:** *Dong Mook Kang, Jin Han*

Hall B

13:30～15:10	**Special Focus Sessions I:** Two-Photon Imaging Approaches in Physiology **Chair:** *Sun Kwang Kim*
13:30～13:55	▶ Combined Two-Photon Microscopy and Optical Coherence Tomography for *In vivo* Tissue Study *Ki Hean Kim (POSTECH, Korea)*
13:55～14:20	▶ Video-Rate In Vivo Microscopy Approaches for the Real-Time Visualization of Dynamic Phenomena in Living Mouse *Pilhan Kim (KAIST, Korea)*
14:20～14:45	▶ *In vivo* Two-Photon and Intravital Imaging Study on Neurovascular Coupling and Cerebral Hemodynamics in Neurodegenerative Disease *Yong Jeong (KAIST, Korea)*
14:45～15:10	▶ *In vivo* Two-Photon Imaging Study on Synaptic Structure and Function in the Mouse Somatosensory Cortex during Chronic Pain *Sun Kwang Kim (Kyung Hee University, Korea)*
15:10～16:30	**Special Focus Session II:** Synapse Pathology in Neurological Diseases **Chair:** *Joo-Min Park*
15:10～15:35	▶ Molecular Basis of Neurotrophin and Cocaine Action in the Brain's Reward Circuitry *Joo-Min Park (Jeju National University, Korea)*
15:35～16:00	▶ Aminopeptidase P1 Deficiency and Brain Disorder *Myoung-Hwan Kim (Seoul National University, Korea)*
16:00～16:25	▶ Regulation of Glutamate Receptor Trafficking in the Excitatory Synapses *Young Ho Suh (Ajou University, Korea)*
16:30～17:30	**Poster Oral Presentation II (PO-7~12):** Molecular Physiology **Chairs:** *Byeong-Hwa Jeon, Jae-Ho Kim*

Friday, October 26

Hall A

09:00～09:30	**Youdang Scholarship Award Lecture** **Chair:** *Byung-Il Min*
	▶ Transient Receptor Potential Vanilloid Subtype 1 (TRPV1): A Critical Gateway for the Pain Mechanism and Pain Therapeutics *Seog Bae Oh (Seoul National University, Korea)*
09:30～12:00	**Symposium I (KOJACH Symposium 2012):** Ion Channel Mechanisms for Integration of Neuron Signals (Sponsored by KOJACH and Biomembrane Plasticity SRC) **Chair:** *Won Kyung Ho*
09:30～09:35	*Introduction by Chairperson*
09:35～10:15	▶ Activity-Dependent Regulation of Ion Channel Distribution in an Auditory Circuit *Hiroshi Kuba (Nagoya University, Japan)*
10:15～10:45	▶ Molecular Mechanisms for EPSP-Spike Potentiation of Synaptic Inputs to the Distal Dendrite of Hippocampal CA3 Pyramidal Neurons *Suk-Ho Lee (Seoul National University, Korea)*
10:45～11:25	▶ Distinct Dynamic Switch of GABA Release in Fast-Spiking and Non-Fast-Spiking GABAergic Interneurons in the Hippocampus *Cheng-Chang Lien (National Yang-Ming University, Taiwan)*
11:25～11:55	▶ A-type K^+ Channel Trafficking for Somatic Processing with Given Synaptic Inputs *Sung-Cherl Jung (Jeju National University, Korea)*
11:55～12:00	*Closing*
13:30～14:30	**Poster Oral Presentation III (PO-13~18):** Neuronal Cells and Muscle Cells **Chairs:** *Young-Ho Jin, Youngmin Bae*
15:00～16:40	**Special Focus Session III:** Metabolism and Bioenergetics **Chair:** *Kyu Sang Park*
15:00～15:25	▶ Role of SREBP-1a in High Sucrose Diet-mediated Metabolic Disease *Seung-Soon Lim (Keimyung University, Korea)*
15:25～15:50	▶ Modulation of Mitochondrial ATP Export by Phosphate Uptake in Insulin-Secreting Cells *Kyu-Sang Park (Yonsei University, Korea)*
15:50～16:15	▶ Extracellular ATP and P2Y2 Receptors Mediate Intercellular Ca^{2+} Waves Induced by Mechanical Stimulation in Submandibular Gland Cells: Role of Mitochondrial Regulation of Store Operated Ca^{2+} Entry *Shin-Young Ryu (Seoul National University, Korea)*
16:15～16:40	▶ Tetrahydrobiopterin is an Essential Cofactor for Mitochondrial Biogenesis and Oxidative Phosphorylation *Hyoung Kyu Kim (Inje University, Korea)*

Hall B

09:30～12:00	**Symposium II:** The Next Advance in Cardioprotection (Co-organized by Cardiovascular and Metabolic Disease Center, Inje University) **Chairs:** *Zhelong Xu*, *Dae Kyu Song*
09:30～10:00	▶ Cardioprotection via Adaptation to Intermittent Hypoxia: a Relative Simple Intervention Ischemia/Reperfusion Injury *Huang-Tian Yang (Chinese Academy of Science, China)*
10:00～10:30	▶ Alpha-Glucosidase Inhibitor and Cardioprotection *Shinya Minatoguchi (Gifu University, Japan)*
10:30～11:00	▶ Mitochondria and Cardioprotection *Tetsuji Miura (Sappro Medical University, Japan)*
11:00～11:30	▶ The SDF-1α/CXCR4 axis Contributes to the Cardioprotective Effect of Ischemic Postconditioning in Isolated Rat Hearts *Jeong-Su Kim (Pusan National University, Korea)*
11:30～12:00	*Q & A*
13:30～14:40	**Poster Oral Presentation IV (PO-19-25):** Systemic or Integrative Physiology **Chairs:** *Sung-Joon Kim, Joo Hyun Nam*
15:00～16:40	**Special Focus Session IV:** Hypothalamic Neuroendocrine Regulation **Chair:** *Sung Kyu Han*
15:00～15:25	▶ Neuroendocrine Regulation of Body Energy Balance: Role of Inflammatory Signals *Byung Joo Lee (Ulsan University, Korea)*
15:25～15:50	▶ Physiological Significance of $GABA_A$ Tonic Inhibition in Magnocelluar Neurosecretory System *Jin Bong Park (Chungnam National University, Korea)*
15:50～16:15	▶ Non-Classical Estrogen Action on GnRH Neurons: Mechanism and Role *Istvan M Abraham (University of Otago, New Zealand)*
16:15～16:40	▶ Circadian Waves and Long-Range Neural Connections in Biological Master Clock, SCN *Kyoung Jin Lee (Korea University, Korea)*

Satellite Symposium

Youdang Scholarship Award Lecture

Special Lectures

Symposia

Special Focus Session

Poster Presentation (Poster Oral Presentation)

Session I : Ion Channels & Transporters

Thursday, October 25

Friday, October 26

Session II : Molecular Physiology

Thursday, October 25

Friday, October 26

Session III : Neuronal Cells

Thursday, October 25

Friday, October 26

Session IV : Muscle Cells

Thursday, October 25

Friday, October 26

MU-13 Effects of Bisphosphonates on Rat Cardiac Left-Ventricular Pressure (LVP) ····· S 109
Jong-Tak Han, Hyang-Ae Lee, Sung-Gurl Park, Ki-Suk Kim
Next-generation pharmaceutical research center, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, Daejeon 305-600, Korea

MU-14 The Potential Role of Cortisol on Cardiac Contractility: Activation of PKC and Ouabain-Targeted Na^+/K^+ ATPase ····· S 109
Sung-Ryul Lee, Hyung-Kyu Kim, Sae-Eun Lim, Sun-Young Lee, Tae-Hee Ko, Na-Ri Kim, Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan 614-735, Korea

MU-15(PO-16) Myogenic Response in Rat Posterior Cerebral Arteries: Role of Endogenous ENaC and TRP Channels ····· S 110
Eok-Cheon Kim, Mihwa Lim, Soo-In Yeon, Duck-Sun Ahn, Young-Ho Lee
Department of Physiology, College of Medicine, Brain Korea 21 Project for Medical Science, Yonsei University, Seoul 120-752, Korea

MU-16 Metabolic Substrates Diminish the Anti-Adrenergic Effect of Insulin on Rat Left Ventricular Myocyte Contractility and Induce Arrhythmias ····· S 110
Zai Hao Zhao, Sung Joon Kim, Yin Hua Zhang
Department of Physiology, Seoul National University, College of Medicine, Seoul, Korea

MU-17 Protective Effect of Melatonin against TNF-α Toxicity in the L6 Myotubes ····· S 111
Hae-Jung Kwon, Jae-Hyung Park, Yung E. Earm, Jae Hoon Bae, Dae-Kyu Song
Department of Physiology, Keimyung University School of Medicine, Daegu, Korea

MU-18(PO-17) SR Ca^{2+} Channels are Altered in Cultured Vascular Smooth Muscle Cells ····· S 111
Eun Ji Kim, Hyung Seo Park, Dong Kwan Kim, Shin Hye Kim, Ji Hun Cha, Yu Eun Ji Kim, Hyung Seo Park, Dong Kwan Kim, Shin Hye Kim, Ji Hun Cha, Yu Jin Jang, Se Hoon Kim
Department of Physiology, College of Medicine, Konyang University

MU-19 Hypertrophy in Skeletal Myotubes Induced by Junctophilin-2 Mutant, Y141H, Involves an Increase in Store-operated Calcium Entry via Orai1 ····· S 111
Jin Seok Woo[1], Chung-Hyun Cho[2], Keon Jin Lee[1], Do Han Kim[3], Jianjie Ma[4], Eun Hui Lee[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, [2]Department of Pharmacology and Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, [3]Department of Life Science, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea, [4]Department of Physiology and Biophysics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

MU-20 Impaired Endurance Exercise Capacity in AQP3 Knock-out Mice ····· S 112
Mi-Ja Kim[1], Dong-Hwan Kim[1], Young-Jun Kim[1], Ju-Hyun Lim[2], Hae-Rahn Bae[2]
[1]Department of Coaching, College of Sports Science, Dong-A University and
[2]Department of Physiology, Dong-A University College of Medicine, Busan 602-714, Korea

MU-21(PO-18) Mutual Regulation between STIM1 and NFATc3 to Induce C2C12 Myoblast Differentiation ····· S 112
Thi Thanh Tam Phuong, Tong Mook Kang
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon 440-746, Korea

MU-22 Oleanolic Acid Accentuates Atrial Natriuretic Peptide Secretion in Beating Rat Atria ····· S 112
Hye Yoom Kim[1,2], Oh Jeong Kwon[1,2], Joung Hyun Kho[1,2], Tan Rui[1,2], Hye Rim Yoo[1,2], Kyung Woo Cho[2], Dae Gill Kang[1,2], Ho Sub Lee[1,2]
[1]Department of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Researh Center, Wonkwang University, Iksan 570-749, Korea

MU-23 Mechanism of the 5-HT2A Receptor-Mediated Vasoconstriction in Rat Mesenteric Artery: Receptor-Specific Roles of Caveolae, Src Tyrosine Kinase, and Kv Channels ····· S 113
Dong Jun Sung[1],Hyun Ju Noh[2], Jae Gon Kim[2], Sang Woong Park[2], Hana Cho[1], Young Min Bae[2]
[1]Department of Physiology, School of Medicine, Sungkyunkwan Univsersity, Suwon, Korea, [2]Department of Physiology, School of Medicine, Konkuk University, Chungju, Korea

Session V : Secretory Cells

Thursday, October 25

SC-1 Effects of DA-6034 on Induction of Ca^{2+} Signaling in Exocrine Gland Cells ····· S 114
Hye Won Ji, Soonhong Park, Yu-Mi Yang, Dong Min Shin
Department of Oral Biology, College of Dentistry Yonsei University, Seoul 120-752, Korea

Friday, October 26

SC-2(PO-19) Role of Mitochondrial Ca^{2+} Uniporter in Mitochondrial pH Gradient and Metabolism-Secretion Coupling in INS-1E Cells ····· S 114
Xianglan Quan, Ranjan Das, Shanhua Xu, Tuyet Nguyen Thi, Seong-Woo Jeong, In Deok Kong, Seung-Kuy Cha, Kyu-Sang Park
Department of Physiology and Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju 220-701, Korea

Session VI : Systemic and Intergrative Physiology

Thursday, October 25

SY-1 Cilostazol Attenuated Catecholamine Mediated Myocardial Remodeling against Restrained Stress Following Myocardial Infarction Model in Rat ····· S 115
Sam Youn Lee[2], Nam-ho Kim[2], Ko Jum Suk[2], Hyang Jeong Jo[3], Eul Sig Choi[1], Moon Young Lee[1]
[1]Department of Physiology, School of Medicine Wonkwang University, [2]Cardiocerebrovascular Center, Wonkwang University Hospital, [3]Department of Pathology, Wonkwang University, Iksan, Korea

SY-2 Repeated Stimulation of Peripheral P2Y1 Receptors Facilitate Capsaicin and Acidic pH-Induced Thermal Nociception via Phosphorylation of TRPV1 in Rats ····· S 115
Soon-Gu Kwon, Suk-Yun Kang, Ji-Young Moon, Sheu-Ran Choi, Hoon-Seong Choi, Jang-Hern Lee
Department of Veterinary Physiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Korea

SY-3 Migration in CD3+ T Cells Isolated from DJ-1 Deficient Mice is Involved in the SDF-1/CXCR4-Mediated ERK1/2 Pathway ····· S 115
Seung Hyo Jung, Kang Pa Lee, Sehyung Pak, Dong-Youb Lee, Eun-Seok Park, Kyung-Jong Won, Bokyung Kim
Department of Physiology, School of Medicine, Konkuk University, Chungju 380-701, Korea

SY-4(PO-23) Angiotensin III Stimulates High Atrial Stretch-Induced Atrial Natriuretic Peptide Secretion via AT2R/PI3K/Akt/eNOS/ GC/PKG ····· S 116
Byung Mun Park, Young-Bin Oh, Shan Gao, Seung Ah Cha ,Suhn Hee Kim
Department of Physiology, Diabetic Research Center, Chonbuk National University Medical School

SY-5 Odor Discrimination in the Main Olfactory Bulb of Anesthetized Dogs ····· S 116
Chin Su Koh[1], Kyung-Jin You[2], Changkyun Im[1], In Seok Seo[1], Jaewoo Shin[1], Jae-hong Park[1], Hwan Gon Lee[1], Hyun-Chool Shin[2], Hyung-Cheul Shin[1]
[1]Department of Physiology, College of Medicine, Hallym University, Chuncheon, [2]Department of Information & Telecommunication, School of IT, Soongsil University, Seoul, Korea

SY-6 Angiotensin II Type 2 Receptor Agonist Stimulates Stretch-Induced ANP Secretion via PI 3K/PKG/NO Pathway ····· S 116
Shan Gao, Byung Mun Park, Seung Ah Cha, Woo Hyun Park, Suhn Hee Kim
Department of Physiology, Diabetic Research Center, Chonbuk National University Medical School, Jeonju, Korea

SY-7 Sustained Hypertension Causes Insulin Resistance Mediated by Oxidative Stress and ROS ····· S 117
Shan Gao[1], Bung Mun Park[1], Ui Jin Bae[2], Woo Hyun Park[1], Byung Hyun Park[2], Suhn Hee Kim[1]
Departments of [1]Physiology and [2]Biochemistry, Diabetic Research Center, Chonbuk National University Medical School, Jeonju, Korea

Friday, October 26

Satellite S-1

The Post-Translational Modification of Mitochondrial Proteins in Heart Failure

Sung Ryul Lee

National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University

Heart failure, the leading cause of death worldwide, is a pathophysiological state wherein the heart is unable to provide sufficient blood flow to the entire body. Mitochondrial dysfunction is a hallmark of numerous diseases, including heart failure. Posttranslational modification (PTM) is the chemical modification of a protein after its translation, which regulates activity of protein and serves as a molecular On/Off switch. Our hypothesis is that disturbance of PTM in mitochondrial proteins might play a role in mitochondrial malfunction and lead to heart failure. The present study aims to investigate the optimized recovery mechanisms against heart dysfunction or heart failure through modulating affected post-translational modifications of mitochondrial proteins.
Specific aims are:
1. to identify the post-translational modifications of mitochondrial proteins using phosphoproteomic technique following heart dysfunction or failure using ischemia/reperfusion model.
2. to elucidate how post-translational modification of mitochondrial proteins are caused in heart failure and what are the effects of their changes on mitochondrial functions.
3. to provide therapeutic potentials for recovery from mitochondrial malfunction and thereby revitalizing failing heart.
This research goal will be achieved through establishment of animal model of heart failure, analysis of post-translational modification in mitochondrial proteins and then finding an underlying mechanism involved in post-translational modification of mitochondrial proteins on heart failure.

Satellite S-2

Morphine Prevents Mitochondrial Oxidative Stress at Reperfusion by Activating Mitochondrial Src Tyrosine Kinase and Induces Cardioprotection against Ischemia/reperfusion Injury in Rat Hearts

Zhelong Xu

Department of Anesthesiology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Morphine may protect the heart from ischemia/reperfusion injury by targeting mitochondria, but little is known about the exact mitochondrial events that mediate morphine's protection. We aimed to address the role of the mitochondrial Src tyrosine kinase and mitochondrial oxidative stress in the cardioprotective action of morphine. Morphine given prior to ischemia reduced infarct size in isolated rat hearts subjected to 30 min ischemia followed by 2 h of reperfusion, an effect that was aborted by the selective Src tyrosine kinase inhibitor PP2, suggesting a role of Src tyrosine kinase in the action of morphine. Morphine also attenuated LDH release in a Src tyrosine kinase dependent manner in HL-1 cells subjected to 90 min simulated ischemia followed by 4 h of reperfusion. However, morphine failed to reduce LDH release in HL-1 cells transfected with Src siRNA. Morphine increased mitochondrial Src phosphorylation at reperfusion in rat hearts and this was abrogated by PP2, indicating an activation of mitochondrial Src tyrosine kinase by morphine. Morphine attenuated mitochondrial protein carbonylation at reperfusion through Src tyrosine kinase, implying that morphine can prevent mitochondrial oxidative stress. Finally, the inhibitory effect of morphine on the mitochondrial complex I activity was reversed by PP2. In conclusion, morphine induces cardioprotection against ischemia/reperfusion injury by preventing mitochondrial oxidative stress through mitochondrial Src tyrosine kinase. Inhibition of mitochondrial complex I at reperfusion by Src tyrosine kinase may account for the prevention of mitochondrial oxidative stress by morphine.

Satellite S-3

Transcription Factor-Driven Conversion of Quiescent Cardiomyocytes to Pacemaker Cells

Hee Cheol Cho

Cedars-Sinai Medical Center, USA

The heartbeat originates within the sinoatrial node (SAN), a small highly-specialized structure containing ＜10,000 genuine pacemaker cells. The ～5 billion working cardiomyocytes downstream of the SAN remain quiescent when it fails, leading to circulatory collapse and fueling a $6B/year electronic pacemaker industry. To engineer faithful biological replicas of rare SAN cells as an alternative therapeutic strategy, we expressed a gene critical for early SAN specification in working cardiomyocytes *in vitro*, and *in vivo* in a model of bradycardia. Within days of transduction with Tbx18, ventricular cardiomyocytes in culture developed spontaneous electrical firing physiologically indistinguishable from that of SAN cells, along with morphological and epigenetic features characteristic of SAN cells. Focal Tbx18 gene transfer in the guinea-pig ventricle yielded ectopic pacemaker activity *in vivo*, correcting a bradycardic disease phenotype. Myocytes transduced *in vivo* acquired the cardinal tapering morphology and physiological automaticity of native SAN pacemaker cells, while controls remained rectangular and quiescent. The creation of induced SAN-like pacemaker (iSAN) cells by Tbx18 gene transfer opens new prospects for bioengineered pacemakers.

Satellite S-4

The Novel Action Mechanism of Angiotensin II Receptor Antagonist

Jin Han

National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University

Objectives: The aim of this study was to investigate the cardioprotective effect of fimasartan, a newly developed angiotensin II receptor blockers (ARBs) in Korea, against myocardial ischemia/reperfusion (I/R) injury and find its underlying effects on reducing mitochondrial damage.
Backgrounds: ARBs have cardioprotective effects through anti-apoptosis, anti-atherosclerosis, and target organ protection beyond blood pressure lowering. Disturbance in mitochondrial homeostasis following reperfusion is a critical determinant for cardiac necrotic/apoptotic death.
Methods: Fimasartan was administrated to SD rats (3 mg/kg, intravenously), cardiomyocytes (50 μmol/L) and H9c2 (50 μmol/L) before ischemia or hypoxia. Preparation of MI, echocardiograms, DNA fragmentation, TUNEL assay, immunoblotting, oxygen consumption, confocal microscopy, and L-type Ca^{2+} current (ICa, L) were assessed.
Results: Fimasartan pretreatment remarkably reduced myocardial infarction and highly recovered cardiac performance after I/R. Fimasartan treatment also reduced apoptotic cell death both *in vivo* and in hypoxia/reoxygenation treated H9c2 cells. In fimasartan-treated cardiomyocytes, hypoxia/reoxygenation induced-mitochondrial $O2^-$ production and collapse of membrane potential were markedly attenuated. Additionally, mitochondrial Ca^{2+} overload during reoxygenation was suppressed by fimasartan and this may be related to inhibition of the L-type Ca^{2+} current (ICa, L) and mitochondrial calcium uniporter (MCU). Fimasartan pretreatment increased phosphorylation of Akt and glycogen synthase kinase-3β at reperfusion and decreased pro-apoptotic p53 protein level and increased anti-apoptotic Bcl-2 protein level.
Conclusions: Fimasartan preconditioning has the potential to modulate Bcl-2 and suppress I/R induced-Ca^{2+} overload by inhibiting ICa, L and MCU. These effects would be beneficial in preventing mitochondrial dysfunction and apoptosis accompanied by I/R.

Satellite S-4

Mitochondrial DNA That Escapes from Autophagy Causes Inflammation and Heart Failure

Issei Komuro

Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Department of Cardiovascular Medicine, The University of Tokyo Graduate School of Medicine, Japan

Heart failure is a leading cause of morbidity and mortality in industrialized countries. Although infection with microorganisms is not involved in the development of heart failure in most cases, inflammation has been implicated in the pathogenesis of heart failure. However, the mechanisms responsible for initiating and integrating inflammatory responses within the heart remain poorly defined. Mitochondria are evolutionary endosymbionts derived from bacteria and contain DNA similar to bacterial DNA. Mitochondria damaged by external haemodynamic stress are degraded by the autophagy/lysosome system in cardiomyocytes. Here we show that mitochondrial DNA that escapes from autophagy cell-autonomously leads to Toll-like receptor (TLR) 9-mediated inflammatory responses in cardiomyocytes and is capable of inducing myocarditis and dilated cardiomyopathy. Cardiac-specific deletion of lysosomal deoxyribonuclease (DNase) II showed no cardiac phenotypes under baseline conditions, but increased mortality and caused severe myocarditis and dilated cardiomyopathy 10 days after treatment with pressure overload. Early in the pathogenesis, DNase II-deficient hearts showed infiltration of inflammatory cells and increased messenger RNA expression of inflammatory cytokines, with accumulation of mitochondrial DNA deposits in autolysosomes in the myocardium. Administration of inhibitory oligodeoxynucleotides against TLR9, which is known to be activated by bacterial DNA, or ablation of Tlr9 attenuated the development of cardiomyopathy in DNase II-deficient mice. Furthermore, Tlr9 ablation improved pressure overload-induced cardiac dysfunction and inflammation even in mice with wild-type Dnase2a alleles. These data provide new perspectives on the mechanism of genesis of chronic inflammation in failing hearts (Nature 2012).

Youdang Scholarship

Transient Receptor Potential Vanilloid Subtype 1 (TRPV1): A Critical Gateway for the Pain Mechanism and Pain Therapeutics

Seog Bae OH

National Research Laboratory for Pain, Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Korea

TRPV1 in peripheral sensory terminals is a well-known molecular transducer of pain. In this presentation, I will discuss a critical role of spinal TRPV1 for pain information processing and a potential role of peripheral TRPV1 for producing pain-specific local anesthesia as the route of the permanently charged lidocaine derivative QX-314 into nociceptors.

Neuropathic pain and allodynia may arise from sensitization of central circuits. I will present a novel mechanism of disinhibition-based central sensitization resulting from long-term depression (LTD) of GABAergic interneurons as a consequence of TRPV1 activation in the spinal cord. Intrathecal administration of TRPV1 agonists led to mechanical allodynia that was not dependent on peripheral TRPV1 neurons. TRPV1 was functionally expressed in GABAergic spinal interneurons and activation of spinal TRPV1 resulted in LTD of excitatory inputs and a reduction of inhibitory signaling to spinothalamic tract (STT) projection neurons. Mechanical hypersensitivity after peripheral nerve injury was attenuated in $TRPV1^{-/-}$ mice but not in mice lacking TRPV1-expressing peripheral neurons. Mechanical pain was reversed by a spinally applied TRPV1 antagonist while avoiding the hyperthermic side effect of systemic treatment. Our results demonstrate that spinal TRPV1 plays a critical role as a synaptic regulator and suggest the utility of CNS-specific TRPV1 antagonists for treating neuropathic pain.

Lidocaine is a widely used local anesthetic agent that works by blocking the activation of voltage-gated sodium channels. However, its use as a pain-killer is limited since lidocaine anesthetizes *all* nerve fibers, blocking signals conveying innocuous sensation, motor and autonomic control, as well as nociception. A new approach of pain-selective local anesthesia targeting TRPV1 was published in 2007. Binshtok et al. showed that selective silencing of nociceptive nerve fibers can be achieved by delivering the permanently charged lidocaine derivative QX-314 into nociceptors via TPRV1 channels. I will present evidences that this approach could have potential utility in selectively treating dental and orofacial pain, while leaving both proprioceptive sensation and motor function untouched, in the trigeminal system. One drawback of this approach is the pain immediately following the injection of capsaicin. As an attempt to circumvent this problem, we are currently searching for less-irritable TRPV1 agonists while allowing QX-314 to enter TRPV1 when co-administered. We are also examining supplemental transporting routes for delivering QX-314 in the population of nociceptive neurons that do not express TRPV1.

SL I

Molecular Mechanisms and Novel Treatments of Heart Failure

Issei Komuro

Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Department of Cardiovascular Medicine, The University of Tokyo Graduate School of Medicine, Japan

Prolonged cardiac hypertrophy causes heart failure, but its mechanisms are largely unknown. Pressure overload, which is produced by constricting transverse aorta of mice, induced cardiac hypertrophy without cardiac dysfunction until 14 days and initially promoted vascular growth in the heart by hypoxia-inducible factor-1 (Hif-1)- dependent induction of angiogenic factors. After 14 days, however, there was no further cardiac hypertrophy but cardiac function was impaired, and the vascular density was reduced with downregulation of Hif-1 and angiogenic growth factors. Inhibition of angiogenesis prevented the development of cardiac hypertrophy and induced systolic dysfunction. Sustained pressure overload induced an accumulation of p53 that inhibited Hif-1 activity and thereby impaired cardiac angiogenesis and systolic function. Conversely, promoting cardiac angiogenesis by introducing angiogenic factors or by inhibiting p53 accumulation developed hypertrophy further and restored cardiac dysfunction under chronic pressure overload. These results suggest that the anti-angiogenic property of p53 has a crucial function in the transition from cardiac hypertrophy to heart failure (Nature 2007). Cell therapy using peripheral mononuclear cells was very effective for ~70% of patients with limb ischemia. Implanted cells stimulate muscle cells to produce angiogenic factors, thereby promoting neovascularization in ischemic tissues. We have recently found that this therapy is also effective for cardiac ischemia.

Wnt signaling plays critical roles in development of various organs and pathogenesis of many diseases, and augmented Wnt signaling has recently been implicated in mammalian aging and aging-related phenotypes. We have found that complement C1q activates canonical Wnt signaling, which is involved in aging-associated less regenerative activity and fibrosis. Serum C1q concentration is increased with aging, and Wnt signaling activity is augmented during aging in the serum and in multiple tissues of wild-type mice, but not in those of C1qa-deficient mice. C1q activates canonical Wnt signaling by binding to Frizzled receptors and subsequently inducing C1s-dependent cleavage of the ectodomain of Wnt coreceptor LRP6. Skeletal muscle regeneration in young mice is inhibited by exogenous C1q treatment, whereas aging-associated impairment of muscle regeneration is restored by C1s inhibition or C1qa gene disruption. Recently we found that the C1q-wnt signaling pathway also plays a critical role in the development of heart failure and arteriosclerosis. Our findings therefore suggest that the C1q -wnt pathway causes various aging-related diseases (Cell 2012).

CURRICULUM VITAE

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Education:

1978-1982	M.D., Faculty of Medicine, University of Tokyo

Professional Experience:

1982-1984	Resident in Internal Medicine, Tokyo University Hospital
1984-1989	Clinical and Research Fellow in Cardiology, Department of Medicine III, University of Tokyo School of Medicine
1989-1993	Research Fellow and instructor, Molecular Medicine Unit and Cardiovascular Division, Beth Israel Hospital and Harvard Medical School
1993-1998	Instructor in Medicine, Chief of Molecular Cardiology Division, Department of Medicine III, University of Tokyo School of Medicine
1998-2000	Assistant Professor in Medicine, Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine
2001-2010	Professor and chairman, Department of Cardiovascular Science and Medicine Chiba University Graduate School of Medicine
2009-	Professor and chairman, Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine
2010-	Visiting Professor in Medicine, Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine
2012-	Professor and chairman, Department of Cardiovascular Medicine, Tokyo University Graduate School of Medicine

SL II

Organotypic Angiogenesis and Vascular Remodeling

Gou Young Koh and LVBSC Members

Laboratory of Vascular Biology and Stem Cells, Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea

Angiogenesis and vascular remodeling are fundamental and dynamic processes that are essential not only for the development and maintenance of every organ, but also for the regeneration of failing organs. In addition, angiogenesis and vascular remodeling are versatile processes, which actively and differently respond to the micro-environment of each organ. Here, we report three instances of organ specific angiogenesis and vascular remodeling. First, we show that intravitreal supplementation of angiopoietin-1 (Ang1) induced ordered retinal angiogenesis into central avascular retina presumably mediated through the integrin ?v?5 signaling pathway, leading to reduced avascular regions, hypoxia and neovascular tuft formation by improving blood perfusion in the mouse oxygen-induced retinopathy model. Moreover, role and source of Ang2 for postnatal and pathologic angiogenesis in the retina are finely clarified using Ang2-reporter and KO mice and anti-Ang2-blocking antibody. Second, uterine angiogenesis is an essential process not only for uterine growth but also for embryonic growth during pregnancy. Accordingly, blood vessels in the uterus were dynamically and markedly remodeled during post-implantation, pregnancy, and after delivery. We observed that VEGF-A-VEGFR2, rather than Ang-Tie2, actively participate in uterine angiogenesis during post-implantation period. Most importantly, progesterone rather than estrogen significantly influences the decidual angiogenesis in uterus by regulating the expression of VEGF-VEGFR2 system during post-implantation period. Thus, progesterone secreted from ovary governs decidual angiogenesis in uterus during pregnancy. Third, the vasculatures of endocrine organs have distinguishable characteristics such as endothelial fenestrations, and it also serves as a conduit for hormonal transport between endocrine gland microenvironment and systemic circulation. Here, we show unique patterns of gene expressions for angiogenic growth factors and distinctive features and responsiveness of vascular remodeling upon various stimuli in the thyroid gland, parathyroid gland and adrenal glands.

CURRICULUM VITAE

Name: Gou Young Koh

Education:

1977-1983	Chonbuk National University Medical School M.D.
1983-1990	Chonbuk National University Medical School Ph.D.

Professional Career:

1983-1987	Research Assistant (Prof. Kyung Woo Cho) Chonbuk National University Medical School
1987-1990	Flight Surgeon, Korean Air Force Academy (Military duty)
1990-1991	Research Fellow (Prof. Thomas Maack) Department of Physiology, Cornell University Medical College
1992-1994	Research Fellow and Associate (Prof. Loren J. Field) Indiana University, Krannert Institute of Cardiology
1995-2001	Assistant Professor/Associate Professor Department of Physiology, Chonbuk National University Medical School
1997-2003	Director, National Creative Research Initiatives for Endothelial Cells Korean Minister of Science and Technology
2001-2003	Associate Professor, Department of Life Sciences Pohang University of Science and Technology (POSTECH)
2003-2010	Professor
2011-	Distinguished Professor Graduate School of Medical Science and Engineering Korea Advanced Institute of Science and Technology (KAIST)

SL III

Biological Therapies for Cardiac Arrhythmias: Can Genes and Cells Replace Drugs and Devices?

Hee Cheol Cho

Cedars-Sinai Medical Center, USA

The heartbeat originates within the sinoatrial node (SAN), a small highly-specialized structure containing ＜10,000 genuine pacemaker cells. The ～5 billion working cardiomyocytes downstream of the SAN remain quiescent when it fails, leading to circulatory collapse and fueling a $6B/year electronic pacemaker industry. With the exception of ablation methods that yield selective endocardial destruction, present therapies are nonspecific and/or palliative. Progress in understanding the underlying biology opens up prospects for new alternatives. Here, state of the art in gene- and cell-based therapies to correct cardiac rhythm disturbances is presented. Included in the topic are the rationale for such approaches, efforts to address aspects of tachyarrhythmia, and advances in creating a biological pacemaker to cure bradyarrhythmia. In particular, emphasis is given to our new work of direct cell reprogramming strategies to engineer faithful biological replicas of rare SAN cells from ordinary cardiomyocytes. Insights gained bring the field closer to a paradigm shift away from devices and drugs, and toward biologics, in the treatment of rhythm disorders.

CURRICULUM VITAE

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Education:

1991-1995	B.Sc (Biochemistry). McMaster University (Hamilton, Ontario)
1997-2003	Ph.D (Physiology). University of Toronto (Toronto, Ontario). Supervisor: Peter H. Backx
2003-2006	Post-doctoral fellowship, Johns Hopkins University, Division of Cardiology. Supervisor: Eduardo Marbán

Professional Appointments:

July, 2012	Merit increase to Step III, Assistant Professor-in-Residence, UCLA (effective July 1, 2012)
2011-present	Assistant Professor, Department of Medicine, Cedars-Sinai Medical Center
2011-present	Assistant Professor-in-Residence, Step II, UCLA
2009-present	Director of Cellular Electrophysiology Research Scientist II-Faculty The Heart Institute, Cedars-Sinai Medical Center
2006-2009	Scientific Program Director, Excigen Inc., Baltimore, MD

S-I-1

Activity-Dependent Regulation of Ion Channel Distribution in an Auditory Circuit

Hiroshi Kuba

Nagoya University, Japan

Axon initial segment (AIS) that separates axonal and somato-dendritic compartments is a highly specialized neuronal structure enriched with voltage-gated Na^+ channels and functions as the site of spike initiation in neurons. Conventionally, AIS was thought to be an uniform and static structure. However, our recent studies in avian auditory circuits have revealed that the distribution of Na^+ channels at AIS is organized in a manner specific to the function of individual neurons and to exhibit plasticity with changes in synaptic inputs. In nucleus magnocellularis (NM), which is involved in a precise relay of timing information, the length of AIS, defined as the distribution of Na^+ channels, differs depending on sound frequency and increases with decreasing frequencies to accommodate frequency-specific variations in synaptic inputs. In nucleus laminaris, which integrates the timing information from both NMs for sound localization, the length and the location of AIS vary depending on sound frequency; it is shorter and more remote for higher frequency. Furthermore, AIS of NM neurons elongates to increase its excitability when synaptic inputs are removed by cochlea ablation, suggesting their contribution to the homeostatic control of neural activity. In this symposium, I will summarize these newly found aspects of AIS, and emphasize the importance of Na^+ channel distribution in determining the function of neural circuits.

S-I-2

Molecular Mechanisms for EPSP-Spike Potentiation of Synaptic Inputs to the Distal Dendrite of Hippocampal CA3 Pyramidal Neurons

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Proximal and distal dendrites of a hippocampal CA3 pyramidal cell (CA3-PC) are innervated by mossy fibers (MF) from dentate gyrus and temporoammonic (TA) pathway from entorhinal cortex, respectively. Regulation of distal dendritic excitability is of crucial importance in dendritic integration of cortical inputs in CA3-PCs. We studied the intrinsic excitability change induced by somatic repetitive firing at the frequency that can be elicited by MF stimulation. Conditioning of a CA3-PC with somatic firing caused a sustained decrease of input conductance and action potential onset time. The same conditioning enhanced the TA excitatory post-synaptic potentials (EPSPs) but not of MF-EPSPs. We show that these excitability changes are induced by Ca^{2+}- and protein tyrosine kinase-dependent downregulation of D-type K^+ current subunit Kv1.2 that displays higher activity in the distal apical dendrites. Our results imply that MF-induced somatic firing can gate direct cortical inputs to CA3-PCs.

S-I-3

Distinct Dynamic Switch of GABA Release in Fast-Spiking and Non-Fast-Spiking GABAergic Interneurons in the Hippocampus

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GABAergic interneurons regulate excitability of neurons through local inhibition at different subcellular domains of target neurons. However, how distinct subtype of GABAergic interneurons exerts dynamic GABA release is largely unknown. Here we showed that GABAergic transmission at non-fast-spiking interneuron-dentate granule cell (DGC) synapses is rapidly switched from the low release mode to the high release mode when driven by a burst action potential pattern in a presynaptic population, whereas the dynamics of fast-spiking interneuron-DGC synapses is relatively independent of presynaptic activity. Detailed anatomical analysis of recorded interneurons revealed that non-fast-spiking interneurons are dendritic inhibitory interneurons (DIs) consisting of cholecystokinin-expressing basket cells (CCK^+-BCs), hilar interneurons with commissural-associational pathway-associated cells (HICAP cells) and hilar interneuron with perforant pathway-associated axon terminals (HIPP cells). By contrast, fast-spiking interneurons are perisomatic inhibitory interneurons (PIs), including parvalbumin-expressing (PV^+-BCs) and axo-axonic cells (AACs). Overall, our study reveals a general view of distinct activity-dependent regulation of dendritic and perisomatic inhibition in the hippocampus.

S-I-4

A-Type K^+ Channel Trafficking for Somatic Processing with Given Synaptic Inputs

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Since its original description, the induction of synaptic plasticity has been known to be accompanied by a lasting modulation in the intrinsic excitability (intrinsic plasticity) of hippocampal neurons. Likely to the activity-dependent down-regulation of synaptic or dendritic A-type K^+ channels (I_A channels) during long-term potentiation (LTP), somatic I_A channels are also sensitive to synaptic modification, showing a two-phased change of I_A channel activity in rat hippocampi. In our previous paper, induction of LTP resulted in an immediate but short lasting hyperpolarization of the voltage-dependence of steady-state I_A channel inactivation along with a progressive, long-lasting decrease in peak I_A density. These biphasic changes of somatic I_A channels directly enhanced the intrinsic excitability of CA1 neurons.

Then, how can synaptic-dependent LTP modulate the kinetics and density of somatic I_A channels? From our preliminary data, we hypothesized that Ca^{2+}-induced Ca^{2+} release (CICR) from endoplasmic reticulum (ER), which is activated by synaptic Ca^{2+} influx, may be required for the synapse-induced somatic modification. In primary hippocampal neurons dissociated from rat (E20) brains, we confirmed that the increment of Ca^{2+} influx induced the significant reduction of peak amplitudes of somatic I_A. This responsiveness of neurons was critically dependent on both NMDARs and VDCCs, providing evidence that the internalization of somatic I_A channels by Ca^{2+} influx may require the activation of presynaptic VDCCs to release endogenous glutamate as well as postsynaptic NMDARs activation. Moreover, ryanodine receptors (RyRs) but not IP_3 receptors in ER, contributed to the internalization of somatic I_A channels, evidenced by pharmacological approaches with Ryanodine (RyR blocker) and 4CMC (RyR opener). This indicates that CICR through RyRs from ER is crucial to regulate somatic excitability via I_A channels trafficking during synaptic modification. In particular, the activation of Ca^{2+} signaling by CICR suggests the possible involvement of PKA pathway for voltage-dependent ion channels trafficking. Consequently, the trafficking of somatic I_A channels, which is sensitive to synaptic modification, can provide a physiological model to explain not only the memory formation in synapses but also the information storage in soma (This work is supported by the National Research Foundation of Korea [NRF] grant funded by the Korea government [MEST] [No. 2010-0013821]).

S-II-1

Cardioprotection via Adaptation to Intermittent Hypoxia: a Relative Simple Intervention Ischemia/Reperfusion Injury

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Ischemic heart disease and lethal reperfusion injury upon reestablishment of blood flow remain a big challenge for clinical cardiologists and basic researchers. The discoveries of ischemic preconditioning (IPC) and postconditioning (IPoC), repetitive episodes of short-term I/R before or after ischemia conferring cardioprotection, have revealed an endogenous defensive mechanism in the heart. Therefore, the approaches aimed at ultimate protection of the heart against ischemia/reperfusion (I/R) insult via triggering or mimicking intrinsic defensive mechanisms might be of therapeutic significance. Ischemic tolerance of the heart can also be induced by intermittent hypobaric hypoxia (IHH), which is characterized by attenuation of I/R-induced contractile dysfunction, ventricular arrhythmias and infarct size, but the underlying mechanisms are far from clear. Our studies revealed that IHH strongly protects the heart against I/R-induced cytosolic and mitochondrial Ca^{2+} overload. We then analyzed the involvement of sarcolemmal membrane receptors and Ca^{2+} transport, sarcoplasmic reticulum Ca^{2+} handing proteins, mitochondrial effectors and signal pathways. Reactive oxygen species (ROS) are paradoxically regarded as a major cause of myocardial I/R injury and a trigger of cardioprotection. We demonstrated that an elevated ROS generation during early reperfusion is critical for triggering the cardioprotection and also contributes to IHH-afforded cardioprotection against I/R injury. These results further support the view that proper IHH training is beneficial to the ischemic heart. Recently, we also found that exposure to IHH 7 days after the onset of myocardial infarction (MI) significantly reduced the scar area, and improved myocardial viability and left ventricular function. Our findings indicate that long-lasting IHH might provide a unique and promising preventive and therapeutic approach for treating ischemic heart disease

Key Words: Intermittent hypobaric hypoxia, Ischemia/reperfusion injury, Reactive oxygen species

S-II-2

Alpha-Glucosidase Inhibitor and Cardioprotection

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Patients with type 2 diabetes mellitus are at substantially increased risk of coronary artery diseases such as angina pectoris and myocardial infarction. The Funagata Diabetes Study reported that postprandial glucose levels, not fasting glucose levels, were associated with cardiovascular disease. Therefore, alpha-glucosidase inhibitors, which inhibit glucose absorption from the intestine, thereby reducing postprandial hyperglycemia, are useful for the treatment of type 2 diabetes mellitus. Among alpha-glucosidase inhibitors, miglitol is a drug that can be absorbed from the intestine, while acarbose and voglibose are unabsorbable drugs. We have reported that intravenous administration of miglitol significantly reduces myocardial infarct size through inhibiting glycogenolysis during ischaemia and this is associated with inhibition of hydroxyl radical production during ischaemia and reperfusion. On the other hand, it has been reported that chronic oral treatment with miglitol increases plasma glucagon-like peptide 1 (GLP-1) levels in humans. GLP-1 is one of two physiological hormones that meet the criteria of “incretin” that is released from the intestine in response to nutrients and exerts a potent insulin-releasing effect on pancreatic beta-cells. It is now well established that GLP-1 induced insulin secretion leads to significant postprandial glucose lowering in both diabetic animal models and patients with type 2 diabetes. It has also been reported that stimulation of GLP-1 receptors protects the heart against ischaemia-reperfusion injury. We therefore, in the present session, show that oral administration of miglitol may have cardioprotective effects not only through the inhibition of glycogenolysis during ischaemia but also through the stimulation of GLP-1 receptors by increased plasma GLP-1 due to oral administration of miglitol. Our findings may thus provide new insight into therapeutic strategies for the treatment of patients with diabetes mellitus combined with coronary artery disease.

S-II-3

Mitochondria and Cardioprotection

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Mitochondria have multiple functions for maintenance of cell viability and also for inducing cell death. Accumulating evidence indicates that opening of the mitochondrial permeability transition pore (mPTP) is a major mechanism directly leading to cell necrosis after ischemia/reperfusion. Ischemic preconditioning and its mimetics activate multiple signaling pathways that converge on glycogen synthase kinase-3beta (GSK-3beta) in mitochondria, and phosphorylation of mitochondrial GSK-3beta at Ser9 elevates threshold for opening of the mPTP. Although mechanism by which phosphorylated GSK-3beta inhibits mPTP opening is still unclear, our recent studies indicate that inhibition of interaction of cyclophilin-D (CypD), a matrix protein, with mitochondrial inorganic phosphate carrier (PiC), a putative subunit of the mPTP, is involved in the elevation of threshold for mPTP opening. Interestingly, increased myocardial susceptibility to ischemia/reperfusion injury in diabetic hearts is associated with lowered threshold for mPTP opening by increased non-phosphorylated GSK-3beta in mitochondria and enhanced CypD-PiC interaction during ischemia. These findings support the notion that mitochondrial kinase signaling pathways play major roles in regulation of the mPTP and thus in cardioprotection from ischemia/reperfusion injury.

S-II-4

The SDF-1α/CXCR4 axis Contributes to the Cardioprotective Effect of Ischemic Postconditioning in Isolated Rat Hearts

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Purpose: There is limited information about the role of stromal cell derived factor-1α (SDF-1α)/CXCR4 axis in ischemic postconditioning (IPOC). We hypothesized that the SDF-1α/CXCR4 signaling pathway is directly involved in the cardioprotective effect of IPOC.
Method: Isolated rat hearts were subjected to 30-min of regional ischemia and 2-hour of reperfusion. IPOC was induced by 6 cycles each of 10-sec reperfusion and 10-sec global ischemia. CXCR4 antagonist AMD3100 was applied in IPOC-induced hearts. Infarct size was assessed with tetrazolium and the functional recovery of hearts and their cardiac enzymes leakage were determined. The phosphorylation states of extracellular signal regulated kinase 1/2 (ERK1/2) and Akt was determined by Western blots.
Results: IPOC significantly reduced infarct size from 29.3 ± 8.4% to 17.8 ± 7.0% of the risk area (p=0.022 vs. CON). AMD3100 attenuated the infarct reducing effect by IPOC (28.5 ± 8.9 %, p=0.045 vs. IPOC). Lactate dehydrogenase and creatine kinase were significantly lower in the IPOC group (p=0.02 vs. CON and p=0.048 vs. CON, respectively) and these effects were reversed by AMD3100 (p= 0.047 vs. IPOC and 0.042 vs. IPOC, respectively). ERK1/2 and Akt phosphorylation was increased by IPOC (226.1 ± 71.8%; p=0.01 vs. CON and 296.4 ± 93.1%; p=0.02 vs. CON, respectively) and this totally blocked by AMD3100 (83.8 ± 28.0%; p=0.02 vs. IPOC and 138.3 ± 28.5%; p=0.009 vs. IPOC, respectively).
Conclusion: The present study demonstrates that the SDF-1α/CXCR4 signaling is involved in the cardioprotection by IPOC and this signaling pathway couples to the ERK1/2 and Akt pathways.

SFS-I-1

Combined Two-Photon Microscopy and Optical Coherence Tomography for *In vivo* Tissue Study

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In vivo tissue imaging is important in both biology and medicine. We developed a combined two-photon microscopy (TPM) and optical coherence tomography (OCT) which provides complementary information of tissues *in vivo*. TPM is a 3D fluorescence microscopic technique with high imaging depths down to a few hundred microns and provide molecular and cellular information in the superficial region. OCT is another 3D imaging technique based on light back reflection and can provide information of structure, vasculature, and birefringence down to a few millimeters. This combined system provides both information in the same tissue region. This combined system was applied to the imaging of various tissues such as the skin, bladder, and plant. Preliminary study results will be presented.

SFS-I-2

Video-Rate *In vivo* Microscopy Approaches for the Real-Time Visualization of Dynamic Phenomena in Living Mouse

Pilhan Kim

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Small animal, particularly mouse, has been an important test bed for basic and translational biomedical study preceding clinical application. Recent advances in genomic technology have allowed a creation of animal model for human disease with genetically encoded biomarkers, notably such as green fluorescent protein (GFP). Combined with mature fluorescence-based probes, it has opened up new avenue to investigate complex pathophysiology of human disease in animal model with much greater details at cellular and molecular level. Accordingly, novel fluorescence imaging methods that can visualize anatomical structure with functional and molecular information provided by fluorescent probes in animal model *in vivo* have drawn great attentions.

While all of major clinical imaging modality such as ultrasound, CT, MRI and PET has been modified and adapted, optical imaging, especially laser scanning confocal and multiphoton fluorescence microscopy, are the only one readily provides cellular resolution of sub-micrometer in live animal. Over the recent years, these technologies enabled dynamic 3D visualization of various biological processes unfold in real-time in the living subject, which provides unprecedented insights those were impossible to obtain by traditional static 2D snapshots (e.g. histopathology and cytometry). It has been utilized to monitor gene expression, protein activity, drug delivery, cell trafficking, cell interaction, physiological response under external stimuli in live animal *in vivo*, which provides new insights unobtainable by conventional ex vivo and in-vitro study. However, for most viscera and thoracic organs deeply placed inside animal, application of these techniques has been challenging due to the limited imaging depth and lack of non-invasive technique for access.

In this presentation, a custom-built video-rate confocal/ multi-photon microscopy optimized for *in vivo* visualization will be presented. Based on the system, three *in vivo* microscopy approaches to analyze 1) Behaviors of flowing T/B Lymphocytes in the high endothelial venules of lymph node, 2) Transport of lipid and drug molecule by enterocyte in villi of small intestine, 3) Time-dependent behavior of blood circulating metastatic tumor cells, will be introduced.

SFS-I-3

In vivo Two-Photon and Intravital Imaging Study on Neurovascular Coupling and Cerebral Hemodynamics in Neurodegenerative Disease

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Early diagnosis of Alzheimer's disease (AD) is crucial for the development of its prevention or treatment. Recently, based on the observation that increased task-induced blood oxygen level-dependent (BOLD) signal in cognitively normal AD-risk subjects, neuronal hyperactivation has been proposed as an early event in AD. However, there has been no evidence of hyperactive hemodynamic response in animal models of AD. In this talk, I will provide evidences of augmented hemodynamic response pattern in a mouse model of AD through *in vivo* imaging methods such as intrinsic optical signal imaging, multi-photon laser scanning microscopy, and laser Doppler flowmetry. Sensory stimulation induced augmented and prolonged hemodynamic responses depicted by changes in total, oxy-, and deoxy-hemoglobin concentration. The difference between transgenic AD model mice (Tg) and wild type littermates (Wt) was significant at 7 months of age when amyloid plaques and cerebral amyloid angiopathy had developed, but not at earlier ages. Correspondingly, pial arteriole diameter change induced by sensory stimulation was also augmented and prolonged in Tg at this age. Cerebral blood flow response was also augmented, but not prolonged. Of note, hypercapnia-induced vasodilatation was not different between Tg and Wt. Collectively, these data support that the BOLD signal hyperactivation in non-demented AD-risk human subject might have been a result from enhanced neurovascular signaling pathway. I will also cover experimental techniques to delineate roles of neurovascular units in AD progression, mainly using multi-photon laser scanning microscopy - observing neuronal and astrocyte Ca^{2+} signal response pattern and simultaneous observation of penetrating arteriole dilatation and astrocyte endfeet Ca^{2+} signal response.

SFS-I-4

In vivo Two-Photon Imaging Study on Synaptic Structure and Function in the Mouse Somatosensory Cortex during Chronic Pain

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Peripheral nerve injury triggers maladaptive plastic changes along the somatosensory nervous system so that altered nociceptive signal processing, represented by neuropathic pain hypersensitivity (e.g. tactile allodynia, a painful response to innocuous mechanical stimuli), occurs. Previous studies have suggested that structural and functional plastic changes in the primary somatosensory cortex (S1) following peripheral nerve injury contribute to neuropathic pain. However, remodeling of cortical connections following injury has been believed to take months or years, based on macroscopic imaging or static measurement; this is not temporally correlated with the rapid development of allodynia and S1 hyperexcitability. Here, we reported, by using long-term two-photon imaging of postsynaptic dendritic spines in living adult mice, that synaptic connections in the S1 are rewired within days following sciatic nerve ligation injury through phase-specific and size-dependent spine survival/growth. Spine turnover in the S1 area corresponding to the injured paw markedly increased during an early phase of neuropathic pain and restored in a late phase of neuropathic pain, which was prevented by immediate local blockade of the injured nerve throughout the early phase. New spines that generated before nerve injury showed volume decrease after injury, whereas more new spines that formed in the early phase of neuropathic pain became persistent and substantially increased their volume during the late phase. Further, pre-existing stable spines survived less following injury than controls and such lost persistent spines were smaller in size than the survived ones that displayed long-term potentiation (LTP)-like enlargement over weeks. We also found that formation and elimination of presynaptic axonal boutons slightly increased and decreased, respectively, only in the early phase of neuropathic pain. These results suggest that peripheral nerve injury induce rapid and selective remodeling of cortical synapses, which is associated with neuropathic pain development, probably underlying, at least partially, long-lasting sensory changes in neuropathic subjects.

SFS-II-1

Molecular Basis of Neurotrophin and Cocaine Action in the Brain's Reward Circuitry

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Cocaine blocks reuptake of dopamine and evokes euphoria by amplifying reward pathways. Cocaine addiction is linked to learning-related changes of synapses, and requires the activation of both NMDAR and group 1 metabotropic glutamate receptors (mGluR). The molecular basis of coupling between reward pathways and glutamate receptors remains largely unknown. Here, we report a signaling pathway essential for cocaine-evoked plasticity that dynamically regulates the coupling of group 1 metabotropic receptor mGluR5 with the NMDA receptor channel. Two coordinate events are required; dynamic phosphorylation of the Homer binding site in the C-terminus of mGluR5, and induction of the immediate early gene Homer 1a. Phosphorylation of mGluR5 is required for binding of a prolyl isomerase, Pin1, while Homer 1a is required for Pin1 to compete with cross-linking forms of Homer to bind mGluR5. Pin1 accelerates the isomerization of the pS-P bond within the Homer binding site of mGluR5, and this is necessary to increase an mGluR5 activated slow inward current (SIC) mediated by NMDA receptors. Mouse genetic models that prevent dynamic increase of the mGluR-SIC, share the phenotype that cocaine fails to induce normal motor sensitization. The mGluR5-Pin1 mechanism is conditional on coupled events that occur at the synapse and the nucleus, and serves as a robust molecular switch to link effects of neuromodulators and growth factors important for reward, with glutamate pathways important for plasticity.

SFS-II-2

Aminopeptidase P1 deficiency and brain disorder

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Many biologically active peptides in mammals have a highly conserved proline residue in the penultimate N-terminal position. Thus far, 23 such peptides have been identified, including cytokines, growth hormones, and neuropeptides. Metabolic degradation of these peptides is mediated by a family of proline-dependent proteases known as aminopeptidase Ps (designated APP1-3). The deficiency of APP activity in humans leads to excretion of undigested imino-oligopeptides in the urine. In addition to this peptiduria, developmental retardation and microcephaly were observed in an APP-deficient human subject. However, a direct causal relationship between APP-deficiency and neurodevelopmental disorders, and APP isoform associated with peptiduria remain unknown. Importantly, it is still not known if a deficiency in APP is associated with neuropsychiatric disorders. To address these questions, I genetically disrupted APP1 function in mice and investigated the consequences *in vivo*. In this talk I will discuss the physiological role of APP1 in brain function and how APP1 deficiency causes brain disorders.

SFS-II-3

Regulation of Glutamate Receptor Trafficking in the Excitatory Synapses

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Glutamate is a major neurotransmitter in the excitatory synapses, and ionotropic glutamate receptors are a major class of heterotetrameric ligand-gated ion channels in the brain. Three classes of ionotropic glutamate receptors, namely AMPA-, Kainate-, NMDA-sensitive glutamate receptors have been identified since the early 1990s. Among the three classes of glutamate receptors, AMPA receptors (AMPARs) mediate fast synaptic transmission in the synapses by gating rapidly upon binding to glutamate. The ability of AMPA receptors to undergo rapid activity-dependent recruitment to synapses is believed to underlie learning and memory formation. This AMPA subtype of glutamate receptor assembles directly with auxiliary subunits known as tetraspanning transmembrane protein TARPs (transmembrane AMPAR regulatory proteins) that regulate AMPAR trafficking and kinetics such as gating, permeability, and pharmacology. Six transmembrane AMPA receptor has been classified as Type I [γ-2(stargazin), γ-3, γ-4, γ-8] and Type II (γ-5, γ-7). Recently, proteomic analyses have identified cornichon proteins (CNIH2/3) as a new class of AMPAR binding proteins and it was proposed that they also function as auxiliary subunits of AMPARs. Functional characterization has shown that cornichons, like TARPs, affect AMPAR trafficking and kinetics in heterologous cells. However the role of cornichons in neurons is less clear.
Here we characterize the expression, the interaction with AMPAR, and regulation of AMPARs trafficking by CNIH2 in hippocampal neurons. We will discuss further the recent advance on the role of cornichons in AMPAR trafficking and kinetics of synaptic AMPAR-mediated transmission.

SFS-III-1

Role of SREBP-1a in High Sucrose Diet-mediated Metabolic Disease

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The sterol regulatory element binding proteins (SREBPs) activate expression of key genes required for lipid metabolism and regulation. We established a line of mice that selectively reduces expression of SREBP-1a (SREBP-1aDF) leaving expression of the overlapping SREBP-1c and unlinked SREBP-2 transcripts unaffected. Our current study demonstrates that SREBP-1a is involved in high sucrose diet-mediated metabolic disease. We show SREBP-1aDF mice become glucose intolerant and insulin resistant on high sucrose. Both GLUT4 protein and mRNA expression are decreased in muscle and adipose tissue of high sucrose fed SREBP-1aDF mice. However, SREBP-1aDF mice prevent high sucrose dependent hepatic steatosis even though mutant mice induce insulin resistance. We further find SREBP-1aDF mice also reduce serum free fatty acid on high sucrose diet. High sucrose diet does not activate adipose triglyceride lipase and hormone-sensitive lipase mRNA expression in adipose tissue from SREBP-1aDF mice. Moreover, expression of tumor necrosis factor-α is decreased in high sucrose fed mutant adipose tissue compared to wild-type mice. Tumor necrosis factor-α has known to be a regulator of lipolysis in adipose tissue. Those data indicate adipose lipolysis is reduced due to decrease of tumor necrosis factor-a in SREBP-1aDF mice, resulting in decreased serum free fatty acid. In a previous study, we already demonstrated that fatty acid oxidation was increased in SREBP-1aDF mice due to reduction of acetyl-coA carboxylase 2 in liver. Thus, those data suggest that SREBP-1aDF mice are more susceptible to high sucrose diet-mediated insulin resistance owing to decreases of both GLUT4 protein and mRNA expression. In addition, SREBP-1aDF mice are protected from hepatic steatosis on high sucrose diet through altered lipolysis and fatty acid oxidation.

SFS-III-2

Modulation of Mitochondrial ATP Export by Phosphate Uptake in Insulin-Secreting Cells

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In pancreatic β-cells, cytosolic ATP/ADP ratio, which is regulated by mitochondrial ATP generation and its translocation, is a dominant signal for insulin exocytosis upon nutrient stimulation. Inorganic phosphate (P_i) is a primary substrate for ATP synthesis and also known to regulate oxidative phosphorylation. In this study, we investigated the role of P_i uptake on mitochondrial bioenergetics and ATP export in rat clonal β-cell INS-1E. Application of succinate or glycerol-3-phosphate increased ATP levels in incubating solutions as well as lysates of α-toxin-permeabilized INS-1E cells. Substrate-induced ATP increases in incubating solutions, but not in lysates, were completely abrogated by an inhibitor of adenine nucleotide translocase (ANT) or the absence of ADP, which implies that mitochondrial ATP release was mediated by ANT exclusively. Extramitochondrial P_i markedly accelerated the rate of ATP export compared to ATP production. Addition of P_i dose-dependently acidified mitochondrial matrix pH in the presence of metabolic substrates. Conversely, P_i hyperpolarized mitochondrial membrane potential which could augment the driving force for ANT activity. Inorganic arsenate exactly mimicked the effects of P_i on electrochemical gradient and ATP export, but not on ATP synthesis. Mersalyl, a P_i uptake inhibitor, selectively blocked the mitochondrial hyperpolarization and ATP translocation stimulated by P_i. Dissipation of mitochondrial pH gradient by the pretreatment with nigericin or monensin completely abolished P_i-induced changes. We suggest that P_i uptake into mitochondria driven by proton gradient accelerates ATP/ADP exchange in insulin-releasing cells, which may augment the efficiency of signal generation for metabolism-secretion coupling.

SFS-III-3

Extracellular ATP and P2Y2 Receptors Mediate Intercellular Ca^{2+} Waves Induced by Mechanical Stimulation in Submandibular Gland Cells: Role of Mitochondrial Regulation of Store Operated Ca^{2+} Entry

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Coordination of Ca^{2+} signaling among cells contributes to synchronization of salivary gland cell function. However, mechanisms that underlie this signaling remain elusive. Here, intercellular Ca^{2+} waves (ICW) in submandibular gland cells were investigated using Fura-2 fluorescence imaging. Mechanical stimulation of single cells induced ICW propagation from the stimulated cells through approximately 7 layers of cells or approximately 120 microm. Our findings indicate that an extracellular ATP-dependent pathway is involved because the purinergic receptor antagonist suramin and the ATP hydrolyzing enzyme apyrase blocked ICW propagation. However, the gap junction uncoupler oleamide had no effect. ATP is released from mechanically stimulated cells possibly through opening of mechanosensitive maxi-anion channels, and does not appear to be directly linked to cytosolic Ca^{2+}. The ICW is propagated by diffusing ATP, which activates purinergic receptors in neighboring cells. This purinergic signaling induces a Ca^{2+} transient that is dependent on Ca^{2+} release via IP_3 receptors in the ER and store operated Ca^{2+} entry (SOCE). Finally, inhibition of mitochondrial Ca^{2+} uptake modified ICW indicating an important role of these organelles in this phenomenon. These studies increase our understanding of purinergic receptor signaling in salivary gland cells, and its role as a coordination mechanism of Ca^{2+} signals induced by mechanical stimulation.

SFS-III-4

Tetrahydrobiopterin is an Essential Cofactor for Mitochondrial Biogenesis and Oxidative Phosphorylation

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The multifunctional cofactor tetrahydrobiopterin (BH_4) has antioxidant effects, and low BH_4 concentration is implicated in various cardiovascular diseases that involve mitochondrial dysfunction. We investigated the role of BH_4 in the regulation of cardiac mitochondrial function using sepiapterin reductase knockout (*Spr*-) mice as a model of BH_4 deficiency. We observed that BH_4 deficiency induced cardiac damage and systolic dysfunction that resulted in shortened life span. Based on systematic and integrative analysis of the mitochondrial proteome, we found that BH_4 deficiency resulted in significant oxidative phosphorylation remodeling at the protein level. On the mitochondrial level, BH_4 deficiency reduced mitochondrial number, impaired mitochondrial inner membrane integrity and oxidative phosphorylation, and increased reactive oxygen species (ROS) generation and oxidative stress on mitochondrial DNA (mtDNA). Exogenous BH_4 supplementation rescued mitochondrial and cardiac dysfunction in *Spr*- mice. BH_4 deficiency also reduced expression of major regulators of mitochondrial biogenesis and respiration, such as peroxisome proliferator-activated receptor γ coactivator-1 α (PGC1-α) and mitochondrial transcription factor A (mtTFA), which was rescued by BH_4 supplementation. In addition, we found that these effects were independent of nitric oxide (NO). Collectively, these results indicate that BH_4 is essential for mitochondrial biogenesis and oxidative phosphorylation via its regulation of PGC1-α and mtTFA expression.

SFS-IV-1

Neuroendocrine Regulation of Body Energy Balance: Role of Inflammatory Signals

Byung Ju Lee

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Inflammatory signaling in the hypothalamus is an essential mediator of the acute sickness response such as the anorexia, cachexia, fever and inactivity that are triggered by systemic inflammatory stimuli and promote negative energy balance. On the other hand, recent studies implicate low-grade chronic inflammation within the hypothalamus as a key factor whereby high-fat diets (HFD) cause central leptin and insulin resistance and thereby promote the positive energy balance (weight gain). Toll-like receptors (TLRs) are family members of pattern-recognition receptors (PRRs) recognizing a wide array of pathogens as well as some endogenous molecules. We have recently found that TLR2 activation in the hypothalamic microglial cells plays an important role in the acute inflammation-induced sickness response by stimulating cyclooxygenase 2 and proopiomelanocortin (POMC) expression. Though NFkB signaling in neurons has recently been known necessary in the generation of HFD-associated weight gain, our recent findings suggest that signaling to other transcriptional regulators such as NFAT family members is also important for generation of this positive energy balance.

SFS-IV-2

Physiological Significance of $GABA_A$ Tonic Inhibition in Magnocelluar Neurosecretory System

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$GABA_A$ receptors ($GABA_AR$) are responsible for generating the sustained tonic form of inhibition (I_{tonic}) as well as conventional fast inhibitory postsynaptic currents (IPSCs; referred to here as I_{phasic}) in the magnocellular neurosecretory system comprised of oxytocin and vasopressin neurons. Besides blocking GAB_AA-mediated IPSCs, the $GABA_AR$ blockers uncovered I_{tonic} both in oxytocin and vasopressin neurons in supraoptic nucleus (SON). 4,5,6,7-tetrahydroisoxazolo[5,4-c]-pyridin-3-ol (THIP) facilitated I_{tonic} without affecting the main characteristics of IPSCs, while DS-2, a relatively selective modulator of $GABA_AR$ δ-subunits, caused minimal changes in I_{tonic} of SON magnocellular neurosecretory cells (MNCs). Quantitative RT-PCR analysis showed much lesser expression of $GABA_AR$ δ-subunit than the α_5 or γ_2-subunit in the SON. Facilitation of I_{tonic} by benzodiazepines further supported the role of $GABA_AR$ γ_2-subunit in I_{tonic} of SON MNCs. Under basal conditions, I_{tonic} was strongly modulated by the activity GABA transporters (GATs), mostly the GAT3 isoform localized in SON glial cells/processes. Extracellular activation of GABAergic afferents evoked a small gabazine-insensitive, bicuculline-sensitive current, which was enhanced by GAT blockade. Blockade of I_{tonic} increased input resistance, induced membrane depolarization and firing activity, and enhanced the input-output function of SON neurons.

Neurosteroids (e.g. allopregnanolone and 3α,5α-tetrahydrodeoxycorticosterone) as well as a general anesthetic, propofol, increased both I_{tonic} and I_{phasic} in SON MNCs, although more than 90% of the current increase was mediated by I_{tonic}. L-655,708, a relatively selective $GABA_AR$ α_5-subunit inverse agonist, attenuated the neurosteroid facilitation of I_{tonic} but not of I_{phasic}. In consistent with the enhancement of the currents, propofol attenuated ongoing firing activities of SON MNCs. Selective inhibition of I_{phasic} failed to block the propofol suppression of the firing activities, while inhibition of I_{tonic} and I_{phasic} efficiently inhibited the propofol-induced neurodepression in SON MNCs.

In summary, $GABA_A$ receptors of different molecular configuration mediate phasic and tonic inhibition in SON MNCs. The latter inhibitory modality under the tight control of glial GATs activity plays a major role in modulating SON neuronal excitability, and is an effective target of clinically used drugs as well as endogenous neurosteroids in the neurons.

SFS-IV-3

Non-Classical Estrogen Action on GnRH Neurons: Mechanism and Role

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The gonadal steroid estrogen exerts an important modulatory influence on the activity of multiple neuronal networks. In addition to classical genomic mechanisms of action, estrogen also exerts poorly understood rapid, non-classical effects on membrane receptors and signalling molecules in neurons. In these experiments first we examined the mechanism of estrogen-induced rapid actions on electrical activity and intracellular signalling within gonadotropin-releasing hormone (GnRH) neurons. We investigated the effect of 17-beta-estradiol (E2) on membrane currents, Ca^{2+} homeostasis and cAMP response element-binding protein (CREB) related signalling pathway in GnRH neurons using variety of methods such as single cell electrophysiology, Ca^{2+} imaging, transgenic technology and immunohistochemistry. These results showed that E2 rapidly induces Ca2+ transients via estrogen receptor (ER) alpha related trans-synaptic GABAergic signalling. Furthermore E2 increases CREB phosphorylation via indirect and direct ER beta dependent activation of CaM/PKA/ERK1/2 signaling pathway. In the second series of experiments we investigated the physiological role of rapid E2 action on CREB in GnRH neurons. In these investigations we have examined the estrogen negative feedback on mice with GnRH neuron specific CREB deletion (GnRH-CREB KO) by means of immunohistochemistry. The GnRH-CREB KO mice exhibited disordered estrous cycles and estrogen negative feedback and premature reproductive senescence. Taken together our result suggest that E2 activates CREB via multiple signalling pathways in GnRH neurons in and this non-classical action plays critical role in the regulation of estrogen negative feedback on these neurons.

SFS-IV-4

Circadian Waves and Long-Range Neural Connections in Biological Master Clock, SCN

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The suprachiasmatic nucleus (SCN) is the master clock in mammals governing the daily physiological and behavioral rhythms. It is composed of thousands of clock cells with their own intrinsic periods varying over a wide range (20～28 h). Despite this heterogeneity, an intact SCN maintains a coherent 24 h periodic rhythm through some cell-to-cell coupling mechanisms. This lecture will discuss our recent experiments and analyses examining how the clock cells are connected to each other and how their phases are organized in space by monitoring the cytosolic free calcium ion concentration of clock cells using the calcium binding fluorescent protein, cameleon. Extensive analyses of 18 different organotypic slice cultures of SCN show that the SCN calcium dynamics is coordinated by phase-synchronizing networks of long-range neurites as well as by diffusively propagating phase waves. The networks appear quite extensive and far-reaching, and the clock cells connected by them exhibit heterogeneous responses in their amplitudes and periods of oscillation to TTX treatments. Taken together, our study suggests that the network of long-range cellular connectivity has an important role for SCN achieving its phase and period coherence.

IC-1(PO-1)

Allopregnanolone Attenuate Glutamate Release from Central Terminals of the Visceral Afferent Vagus Nerve in the Nucleus Tractus Solitarii (NTS)

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Pregnancy often accompanies several symptoms that have bad effects for pregnant women and fetus. Such symptoms include toxemia, diabetes, and hypertension. Hypertension is the most common medical problem encounterd during pregnancy, complication 2-3% of pregnancies. Pregnancy not only includes hypertension but also exasperate pre-existing hypertension symptoms. However, the exact pathogenesis pregnancy related hypertension has not been established yet. Plasma concentration of the many pregnancy related hormones change during pregnancy and some of them directly modulate neuronal activity by changing membrane conductance of the neuronal cells. Some of the pregnancy hormones and their metabolites modulate activity of ion channels in the brain. Therefore there is high probability that those hormones action in the CNS many relate with pregnancy-induced hypertension. Cranial visceral afferent vagus nerve transfer activity information of visceral organs to neurons of the nucleus tractus solitarii (NTS). Activation of vagus nerve or its nerve bundle in the NTS, solitary tract (ST), increase excitatory neurotransmitter glutamate and inhibit autonomic reflex responses including cardiovascular activity. Therefore, we tested allopregnanolone effects on glutamatergic synaptic transmission on 2nd order NTS neurons, which directly connected visceral afferent nerve via glutamatergic synapses. Allopregnanolone significantly attenuated amplitude of evoked EPSCs. Also allopregnanolone decreased spontaneous EPSC frequency. $GABA_A$ receptor selective antagonist, gabazine (100, 1000 nM), did not affect allopregnanolone-induced decreases in evoked EPSC amplitude and spontaneous EPSCs frequency decrease. There results imply that progesterone may induce hypertension during pregnancy by suppressing visceral afferent signal transmission in NTS. **Acknowledgements:** This study was supported by a grant from the Korea Food Research Institute (Project No. E0121402).
Key Words: Nucleus tractus solitarii (NTS), Pregnancy, Glutamatergic excitatory synaptic response (EPSC)

IC-2

mGluR5-dependent Enhancement of Dendritic Persistent Na^+ Currents Induces Short-term Potentiation of E-S Coupling in CA1 Pyramidal Neurons

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The group I metabotropic glutamate receptors (mGluRs) play important roles in regulating intrinsic excitability and synaptic plasticity. We discovered that high frequency stimulation (50 stimuli at 100 Hz) to the Shaffer collateral pathway near proximal apical dendrites of CA1 pyramidal neurons, did not affect EPSC, but induced potentiation of EPSP-spike coupling (E-S potentiation) which lasted for about 2 min. This type of short-term E-S potentiation had never been described, so we investigated underlying mechanisms. We found that same stimulation induced mGluR5- and ryanodine receptor-dependent Ca^{2+} release, and that inhibition of Ca^{2+} release using mGluR5 blocker (MPEP), cADP ribose blockers (8-NH2-cADPR and nicotinamide), or ryanodine, abolished E-S potentiation. We further investigate ion channel mechanism and found that inhibition of persistent Na^+ current ($I_{Na,P}$) using riluzole abolished E-S potentiation. Voltage clamp analysis confirmed that $I_{Na,P}$ was potentiated by group I mGluR agonist, DHPG, especially when DHPG was applied locally to the apical dendrites. This $I_{Na,P}$ potentiation was abolished by inhibiting mGluR5-dependent Ca^{2+} release or calmodulin. In CA1 pyramidal neurons transfected with Nav1.6 siRNA, same stimulation failed to induce E-S potentiation. Taken together, we concluded that synaptic stimulation to apical dendrites of CA1 pyramidal neurons induces mGluR5-dependent Ca^{2+} release to activate calmodulin, which in turn potentiates dendritic $I_{Na,P}$ and induces E-S potentiation.
Key Words: mGluR5, Persistent sodium currents

IC-3

Inhibitory Mechanism of BIM (I) [GF 109203X], on L-type Ca^{2+} Channels in Rat Ventricular Cells

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We investigated the effect of a specific protein kinase C (PKC) inhibitor, bisindolylmaleimide I [BIM (I), GF 109203X], on L-type Ca^{2+} channels in rat ventricular myocytes. In the whole-cell configuration, BIM (I) alone inhibited the L-type Ca^{2+} current in a concentration-dependent manner, with a K_d value of 3.31±0.25 μM, and a Hill coefficient of 2.34± 0.23. Inhibition was immediate after applying BIM (I) in the bath solution and then it partially washed out. The steady-state activation curve was not altered by applying 3 μM BIM (I), but the steady-state inactivation curve shifted to a more negative potential with a change in the slope factor. Other PKC inhibitors, PKC-IP and chelerythrine, showed no significant effects either on the L-type Ca^{2+} current or on the inhibitory effect of BIM (I) on the L-type Ca^{2+} current. The results suggest that the inhibitory effect of BIM (I) on the L-type Ca^{2+} current is direct and independent of the PKC pathway. Thus, our results should be considered in studies using BIM (I) to inhibit PKC activity and ion channel modulation.
Key Words: Bisindolylmaleimide I, Protein kinase C, L-type Ca^{2+} current, Ventricular myocytes

IC-4(PO-2)

An Essential role of PI(4,5)P_2 for Maintaining the Activity of the TRPC4β

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The Transient Receptor Potential Canonical 4 (TRPC4) channel is a Ca^{2+}-permeable, non-selective cation channel in mammalian cells and mediates a number of cellular functions. Many studies show that TRPC channels are activated by stimulation of $G\alpha_q$-PLC-coupled receptors. However, our previous study showed that the TRPC4 current was inhibited by co-expression of a constitutively active form of $G\alpha_q$ ($G\alpha_q^{Q209L}$). It may have caused a shortage of phosphoinositide phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) because a constitutively active $G\alpha_q$ would have persistently activated PLCβ. Therefore, we used an inducible system to regulate PI(4,5)P_2 specifically and acutely. The TRPC4β current was reduced by inducible $G\alpha_q^{Q209L}$ but not by the mutants whose binding ability to PLCβ is impaired. If the aforementioned phenomenon resulted from desensitization of TRPC4 by PKC, the current of TRPC4β (T877A) mutant which is not phosphorylated by protein kinase C (PKC) would not be inhibited by co-expression of a $G\alpha_q$ ($G\alpha_q^{Q209L}$). Contrary to the expectation, however, we detected the inhibitory action of GαqQ209L. Depletion of PI(4,5)P_2 using the inositol polyphosphate 5-phosphatase (Inp54p) inducible system led to an irreversible inhibition of TRPC4 currents after application of rapamycin to HEK293 cells that were co-expressing TRPC4 with Inp54p. On the other hand, phosphatidylinositol 4-phosphate 5-kinase (PIP5K) inducible system did not activate the initial gating of TRPC4β channel. Even in the case of $G\alpha i_2$-activated TRPC4β currents, the acute depletion of PI(4,5)P_2 led to reduced TRPC4β currents. A PI(4,5)P_2 increase, however, did not induce any changes in TRPC4β activation. Therefore, we suggested that PI(4,5)P_2 is not the activator for TRPC4 activation but it is still necessary for regulating TRPC4 activation. Especially, TRPC4 desensitization might be a result of hydrolysis of PI(4,5)P_2 since TRPC4 desensitization through muscarinic receptor 3 which activates $G\alpha_q$-PLC pathway disappeared by adding PI(4,5)P_2 and nonhydrolysis PI(4,5)P_2. These findings indicate an essential role of PI(4,5)P_2 for maintaining the activity of TRPC4β.

Key Words: TRPC4, GPCR, PI(4,5)P_2, Desensitization, Inducible system

IC-5

Decreased Expression of ATP-Sensitive K^+ Channel in Aortic Smooth Muscle During Isoproterenol-Induced Left Ventricular Hypertrophy

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We investigated the impairment of K_{ATP} channels in aortic smooth muscle cells (ASMCs) from hypertrophied rabbits. The amplitude of K_{ATP} channels induced by the K_{ATP} channel opener pinacidil was greater in ASMCs from control than from hypertrophied animals. In phenylephrine pre-constricted aortic rings, pinacidil induced relaxation in a dose-dependent manner. The dose-dependent curve was shifted to the right in the hypertrophied compared with the control model. Although the level of Kir6.2 subtype expression did not differ between ASMCs from the control and hypertrophied models, those of the Kir6.1 and SUR2B subtypes were decreased in the hypertrophied model. Application of the CGRP and forskolin induced a K_{ATP} current in both control and hypertrophied animals; however, the K_{ATP} current amplitude did not differ between the two groups. Furthermore, PKA expression was not altered. These results suggests that the decreased K_{ATP} current amplitude and K_{ATP} channel-induced vasorelaxation in the hypertrophied animals were attributable to the reduction in K_{ATP} channel expression, but not to changes in the intracellular signaling mechanism that activates the K_{ATP} current.

Key Words: Aortic smooth muscle cell, Hypertrophy, ATP-sensitive K^+ channel

IC-6

Inhibition of CRAC Channel by Curcumin and Caffeic Acid Phenethyl Ester Via Electrophilic Addition to a Cysteine Residue of Orai1

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Ca^{2+} influx through Ca^{2+}-release activated Ca^{2+} channels (CRAC) is critical for activating immune cells. Orai and STIM proteins comprise the molecular components of CRAC. We previously observed that curcumin and caffeic acid phenethyl ester (CAPE) inhibit CRAC current in Orai1/TIM1-co-expressing HEK293 cells. Both compounds contain electrophilic α,β-unsaturated carbonyl groups that potentially form Michael addition with cysteine residues. We investigated the sensitivity of cysteine mutated Orai1 to curcumin and CAPE to delineate their inhibitory mechanism. Replacing the 195 cysteine residue with serine (C195S) reversed the effect of CAPE from inhibition to facilitation and significantly weakened the inhibitory effect of curcumin. Tetrahydrocurcumin, a curcumin metabolite, showed a less potent inhibitory effect on I_{CRAC}, and this effect was abolished in C195S Orai1. Additive mutation of other cysteines (C143S and C126S) had no further influence on the effects of CAPE and curcumin. These results indicate that the electrophilic addition to the Orai1 195 Cysteine was responsible for the inhibitory effect of I_{CRAC} by curcumin and CAPE.

Key Words: Ion channel, CRAC channel, Lymphocytes

IC-7

Large-conductance Ca^{2+}-activated K^+ Channel Alpha-subunits in Mouse Cardiomyocytes

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Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels are widely distributed in cellular membranes of various tissues, but have not previously been found in cardiomyocytes. In this study, we cloned a gene encoding the mouse cardiac BK_{Ca} channel α-subunit (mCardBKa). Sequence analysis of the cDNA revealed an open reading frame encoding 1154 amino acids. Another cDNA variant, identical in amino acid sequence, was also identified by sequence analysis. The nucleotide sequences of the two mCardBKa cDNAs, type 1 (*mCardBKa1*) and type 2 (*mCardBKa2*), differed by three nucleotide insertions and one nucleotide substitution in the N-terminal sequence. The amino acid sequence demonstrated that mCardBKa was a unique BK_{Ca} channel α-subunit in mouse cardiomyocytes, with amino acids 41-1153 being identical to mouse Slo1 and amino acids 1-40 corresponding to Kcnma1. These findings suggest that a unique BK_{Ca} channel α-subunit is expressed in mouse cardiomyocytes.

Key Words Large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel α-subunit, Cardiomyocytes, Cloning, Mouse

IC-8(PO-3)

Insulin Regulates Cell Surface Abundance of Orai1 Channel Via VAMP2-dependent Pathway in Mouse Podocytes

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Diabetic nephropathy (DN) correlates with insulin resistance that occurs in type 2 diabetes patients. Hyperinsulinemia causes podocytes dysfunction along with apoptosis, thickening of the glomerular basement membrane and effacement of the podocyte foot process - all histological features typical of DN. In podocyte, intracellular Ca^{2+} regulation is critical for maintaining foot process. Here, we examined molecular mechanism by which insulin increase intracellular Ca^{2+} via store-operated Ca^{2+} entry (SOCE) in cultured mouse podocytes. SOCE is a major route of Ca^{2+} influx in nonexcitable cells. All Orai proteins which are pore-forming unit of SOCE channels were expressed in mouse podocytes. SOCE was significantly decreased by siRNA Orai1 in mouse podocytes, but not by siRNA Orai2 or Orai3. Insulin stimulated SOCE in a time-dependent manner. Insulin stimulation of Orai channels was detectable in 10min. Insulin-mediated SOCE activation was inhibited by a PI3K or Erk1/2 inhibitors indicating that both Akt and Erk1/2 signaling cascades are critical for channel regulation. Cell surface biotinylation studies showed that insulin increased cell surface abundance of Orai1 channel. Insulin does not affect gating and activation kinetics of Orai1 channel. Orai1 physically interacts with VAMP2 and treatment of brefeldin A or cleavage of siRNA-mediated VAMP2 knock-down significantly decreased SOCE. Taken together, these results suggest that insulin increases cell surface expression of Orai1 channels via activating VAMP2-dependent exocytotic pathway. This mechanism of regulation may contribute to understanding of Ca^{2+} regulation mediated by insulin signaling in mouse podocyte [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0024789)].

Key Words: Store-operated Calcium Channel, Orai1, Pococyte, Insulin, VAMP2

IC-9

Modulation of N-type Ca^{2+} Current by Agmatine Via Imidazoline I_2 Receptor Activation in Rat SCG

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Agmatine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_2 receptors (IR_2) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_2-induced inhibition of noradrenaline (NA) release is still unknown. We therefore, investigated the effect of IR_2 activation on voltage-dependent Ca^{2+} channels which is known to play an pivotal role in regulating NA in rat superior cervical ganglion (SCG) neurons, using the conventional whole-cell patch-clamp method. In the presence of rauwolscine (3 μM), which blocks α_2-adrenoceptor ($R\alpha_2$), agmatine inhibited voltage-dependent Ca^{2+} current (I_{Ca}) by about 30%. This agmatine-induced inhibition was almost completely prevented by BU224 (10 μM) which blocks IR_2. In addition, φ-conotoxin (CgTx) GVIA (1 μM) occluded agmatine-induced inhibition of I_{Ca}, but, agmatine-induced I_{Ca} inhibition was not affected by pertussis toxin (PTX) nor shows any characteristics of voltage-dependent inhibition. These data suggest that agmatine inhibit voltage-dependent N-type Ca^{2+} current ($I_{Ca\text{-}N}$) via activating IR_2. Finally, agmatine significantly decreased the frequency of AP firing in a partially reversible manner. This inhibition of AP firing was almost completely occluded in the presence of φ-CgTx. Taken together, our results suggest that activation of IR_2 in SCG neurons reduced $I_{Ca\text{-}N}$ in a PTX-and voltage-insensitive pathway, and this in-

hibition attenuated repetitive AP firing in SCG neurons.
Key Words: Agmatine, Imidazoline receptors, SCG

IC-10

Fluid Pressure Activates a Non-selective Cation Current and a Cl^- Current in Rat Atrial Myocytes

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Regurgitant blood jets in mitral valve incompetence are known to predispose to atrial fibrillation. To understand cellular basis for this arrhythmia we examined a fluid pressure (FP)-gated currents (I_{FP}) in single rat atrial myocytes using the whole-cell patch clamp. FP was applied onto an entire cell area using a fluid micro-jet system. The application of FP of ~16 dyn/cm^2 with a normal bath solution elicited a transient inward current (~1 pF/pA^{-1} at -80 mV) in a pressure-dependent manner. The removal of extracellular Ca^{2+} largely enhanced the I_{FP} and eliminated the current adaptation. Under physiological conditions, the I_{FP} displayed an inwardly- and outwardly-rectifying current-voltage relationship with a reversal potential (E_{rev}) of approximately -52 mV. The Cl^- channel blockers, DIDS and 9-AC, suppressed inward and outward I_{FP} by ~50% and 70-80%, respectively. In symmetrical Cl^- solutions, the E_{rev} was shifted rightward (≅-18 mV) and the outwardly rectifying I_{FP} was attenuated. In the symmetrical Cl^- conditions, removal of extracellular Na^+ largely reduced inward I_{FP}, and produced a left shift of E_{rev} (≅-64 mV). In addition, the elimination of internal K^+ shifted E_{rev} to ≅+8.4 mV and decreased outward I_{FP}. Although low concentrations of extracellular Ca^{2+} blocked I_{FP} with a negative shift of E_{rev}, high concentrations of extracellular Ca^{2+} produced a right shift of E_{rev}. Gadolinium ion (Gd^{3+}), the stretch-activated channel blocker, partially blocked the inward I_{FP}. FP of the same magnitude elicited spontaneous membrane depolarization with repetitive action potentials (APs) and prolongation of APs. We conclude that FP activates an outwardly rectifying Cl^- channel and a Gd^{3+}- and Ca^{2+}-sensitive non-selective cation channel, carrying Na^+, K^+, and Ca^{2+}, which may, at least in part, explain the blood-jet induced atrial arrhythmias.
Key Words: Fluid pressure, Atrial myocytes, Non-selective cation current, Cl^- current, Arrhythmia

IC-11

Ryanodine but Not IP_3 Receptors Participate in the Internalization of Somatic A-type K^+ Channels in Hippocampal Neurons

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Internalization and insertion of ion channels in plasma membrane of neurons are targeted by Ca^{2+} signaling cascades. We previously reported that A-type channel (I_A channel) expression in soma and dendrites is dependent on Ca^{2+} influx via NMDA receptors (NMDARs). Now, we tested if Ca^{2+} influx via NMDARs is independently working for activating Ca^{2+} signaling and if Ca^{2+} release from endoplasmic reticulum (ER) in pre- and postsynaptic neurons participates in I_A channel trafficking in soma of pyramidal neurons of hippocampi. Using the primary hippocampal neurons dissociated from rat (E20) brains, changes of I_A amplitude were electrophysiologically measured under the condition of high Ca^{2+} (3.6 mM, for 24 hours to culture media) and several agents to modulate Ca^{2+} release from ER. High Ca^{2+} condition induced the significant reduction of peak amplitudes of I_A. These neuronal responses were dependent on both NMDARs and VDCCs, showing sensitivities to APV and nimodipine treatment. This provides evidence that the internalization of I_A channels by Ca^{2+} influx may require activation of presynaptic VDCCs to release endogenous glutamate as well as postsynaptic NMDARs activation. Moreover, Ca^{2+}-induced reduction of I_A amplitude was abolished by Ryanodine (10 μM) to block ryanodine receptors of ER, while 4 CmC (50 μM) treatment to open Ca^{2+} store alone reduced I_A peaks without extracellular Ca^{2+} addition. This indicates that Ca^{2+} induced Ca^{2+} release (CICR) from ER is crucial to regulate somatic excitability via I_A channels trafficking, which is targeted by synaptic modulation. In particular, the activation of Ca^{2+} signaling by CICR suggests possible involvement of PKA and IP_3 pathways for modulating somatic excitability via voltage-dependent ion channels trafficking. The research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2010-0013821).
Key Words: A-type K^+ channel, Ryanodine receptor, Endoplasmic reticulum, Hippocampus, Voltage-dependent channel

IC-12(PO-4)

Dual Mechanisms Diminishing Tonic $GABA_A$ Inhibition of Dentate Gyrus Granule Cells in Noda Epileptic Rats

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The Noda epileptic rat (NER), a mutant found in a Wistar colony, spontaneously shows tonic-clonic convulsion with paroxysmal discharges. In the present study, we measured phasic (I_{phasic}) and tonic $GABA_A$ current (I_{tonic}) in NER hippocampal dentate gyrus granule cells (DGGCs), and compared the results with those of normal parent strain Wistar rats (WIS). I_{tonic}, uncovered by a bicuculline-induced outward shift in holding current, was significantly smaller in NER than in WIS ($p < 0.01$). The frequency of IPSCs was also significantly less in NER than in WIS ($p < 0.05$) with-

out significant difference in the amplitude and decay time of IPSCs between WIS and NER. Attenuation of I_{tonic} in NER was further confirmed in the presence of GABA transporter blockers, NO-711 and nipecotic acid, with no difference in neuronal GABA transporter expression between WIS and NER. I_{tonic} responses to extrasynaptic GABAA receptor agonists (THIP and DS-2) were significantly reduced in NER in relation to WIS ($p < 0.05$). Allopregnalone caused much less I_{tonic} increase in NER than in WIS, while it prolonged the IPSC decay time to a similar rate in the two groups. Expression of $GABA_A$ receptor δ but not γ_2 subunit was decreased in the dentate gyrus of NER in relation to that of WIS. Taken together, our results showed a combination of attenuated presynaptic GABA release and extrasynaptic $GABA_A$ receptor expression reduced I_{tonic} amplitude and its sensitivity to neurosteroids, which is likely to diminish the gating function of DGGCs and render NER more susceptible to seizure generation.

Key Words: $GABA_A$ receptors, Noda epileptic rat, Dentate gyrus granule cells

IC-13

Cellular Mechanisms of Mechanical Allodynia and Thermal Hyperalgesia

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The present study investigated underlying cellular mechanisms of mechanical allodynia and thermal hyperalgesia produced by IL-1β injection. Experiments were carried out on Sprague-Dawley rats weighing between 230-280 g. Under anesthesia, a polyethylene tube 10 (PE10) was implanted in a subcutaneous area of one vibrissa pad, which enabled us to inject IL-1β or other chemicals. After subcutaneous administration of IL-1β (1 ng), we examined air-puff thresholds (mechanical allodynia) and head withdrawal latency time (thermal hyperalgesia). Subcutaneous administration of IL-1β produced mechanical allodynia and thermal hyperalgesia. Both behavioral responses were completely blocked by pretreatment with IL-1 receptor antagonist. Subcutaneous administration of IRTX, a TRPV1 antagonist, 10 min prior to the injection of IL-1β blocked IL-1β-induced thermal hyperalgesia, but not mechanical allodynia. On the contrary, pretreatment with D-AP5, an NMDA receptor antagonist, or NBQX, an AMPA receptor antagonist blocked IL-1β-induced mechanical allodynia, but not thermal hyperalgesia. Pretreatment with H89, a PKA inhibitor, significantly blocked IL-1β-induced mechanical allodynia, while the same dose of H89 failed to restore the IL-1β-induced thermal hyperalgesia. On the other hand, pretreatment with chelerythrine, a PKC inhibitor, dose-dependently inhibited IL-1β-induced thermal hyperalgesia, while it did not affect IL-1β-induced mechanical allodynia. Systemically administration of resiniferatoxin (RTX), which is an ultrapotent analog of capsaicin and can deplete capsaicin-sensitive C fibers, blocked IL-1β-induced thermal hyperalgesia, but not mechanical allodynia. Besides, analgesia produced by lidocaine, a local anesthetics, solely appears to result from suppression of IL-1β-induced thermal hyperalgesia. These results suggest that IL-1β-induced mechanical allodynia and thermal hyperalgesia are mediated by different cellular mechanisms associated with the peripheral NMDA, AMPA and TRPV1 receptors. Moreover, peripheral PKA/NMDA or AMPA signaling pathway mediates IL-1β- induced mechanical allodynia through large diameter A-β fibers and peripheral PKC/TRPV1 signaling pathway mediates IL-1β-induced thermal hyperalgesia through small diameter C-fibers, respectively.

Key Words: Mechanical allodynia, Thermal hyperalgesia, IL-1β, TRPV1, NMDA

IC-14

Modulation of hERG Channel Kinetics by Caffeic Acid Phenethylester (CAPE): Involvement of Cysteine 723

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Caffeic acid phenethyl ester (CAPE) is a plant-derived polyphenolic compound, and is a major component of propolis. Our previous study showed that CAPE and curcumin inhibits Ca^{2+}-release activated Ca^{2+} channel (CRAC) and K^+ channels in T-lymphocytes. Human ether-a-go-go related gene (hERG) K^+ channel in cardiomyocyte is inhibited by numerous natural and pharmaceutical compounds, potentially resulting cardiac arrhythmia. A recent study reported that curcumin inhibits hERG channel. Since the structures of two compounds are similar, we studied the effect of CAPE on hERG channels in overexpressed HEK-293 cells. CAPE dose dependently inhibited repolarization tail current of hERG (IC_{50} = 10.6 ± 0.5 μM). However, hERG current during depolarization phase (between -40 and 0 mV) was increased, and voltage-dependence of activation curve was left-shifted by 10 mV ($V_{1/2}$: from -17.5 to -26.5). Both activation and deactivation kinetics were accelerated by CAPE. Tyr652 and Phe656 are known as specific sites for the variety of hERG blockers. However, the inhibitory effect of CAPE was not attenuated in the mutated hERG (Y652A and F656A). A recent study showed that oxidative stress inhibits hERG and Cys723 residue confers the sensitivity to oxidative stress. Interestingly, a point mutation of Cys723 (C723S) significantly attenuated the inhibitory effect of CAPE. Curcumin induced similar changes of hERG current, which was also showed attenuated in C723S mutant. The complex effects of CAPE and curcumin indicate that some natural polyphenolic compounds could differently modulate hERG current depending on the phase of cardiac action potential. The critical role of reactive cysteine residue (C723) suggested that CAPE and curcumin might form chemical binding (Michael adduction) with thiol group.

Key Words: hERG, Polyphenol, Caffeic acid phenethylester

IC-15

A Neuroprotective Role of Hyperpolarization Activated Cation Channels in Early Developmental CA1 Neurons

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Immature hippocampal neurons showing higher input resistance (R_{in}) are very vulnerable to hyperexcitable or epileptogenetic conditions. This phenomenon has been often mentioned for explaining the neuroprotective roles of hyperpolarization-activated cation channels (I_h channels) to regulate membrane R_{in}. In this study, we tried to electrophysiologically clarify the relationship between membrane R_{in} and I_h channels and their neuroprotective roles specific for developmental neurons. For this, we first classified developmental CA1 neurons of rats (within postnatal 3 weeks) as two groups by measuring the onset time (less or more 20 ms) to fire the first action potential (AP) by a current pulse injection (100 pA, 800 ms). As expected, neurons showing shorter onset time (short-OsT), exhibited higher R_{in}, while longer onset time (Long-OsT) neurons revealed lower R_{in}. However, the amplitude of voltage sags induced by negative current injections (-200 ~ -50 pA with 50 pA step, 800 ms) was significantly larger in Short-OsT than in Long-OsT neurons. Interestingly, suprathreshold excitabilities of repetitive APs were significantly enhanced by temporal depolarization stimulation (TDS, -14 mV holding for 150 sec) in Long-OsT but not in Short-OsT neurons, suggesting the existence of I_h roles for preventing overexcitation in high R_{in} condition. This has then been evidenced in experiments to test ZD7288 effects, revealing TDS-induced enhancement in suprathreshold excitabilities of Short-OsT neurons. These results indicate that, although they are not critical factors to determine R_{in} during at least an early developmental stage, the expression level of I_h channels might be regulated by young CA1 neurons to compensate high R_{in} for a neuroprotective modulation from epileptogenetic conditions. The research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2010-0013821).

Key Words: Hyperpolarization-activated cation channel, Neuronal hyperexcitability, Membrane input resistance, CA1 development

IC-16(PO-5)

Characteristics of Mitochondrial Ca^{2+} Efflux Pathways in Single Ventricular Myocytes of Rat

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Mitochondria play a role in Ca^{2+} buffering and they have Ca^{2+} influx and efflux pathways. It has been known that the main Ca^{2+} influx pathway was Ca^{2+} uniporter and the Ca^{2+} efflux pathways were Na^+/Ca^{2+} exchanger (NCX) and H^+/Ca^{2+} exchanger. We used the permeabilized cardiac myocytes with saponin and studied the characteristics of Ca^{2+} efflux mechanisms in mitochondria. We used Fura-2FF to monitor the change of Ca^{2+} and measured NADH and mitochondrial membrane potential with (TMRE) simultaneously. After Ca^{2+} was loaded into the mitochondria, we tested the Ca^{2+} efflux dynamics in various conditions. We reported previously the half activation concentration of Na^+ (Kh) of Na^+-dependent Ca^{2+}-efflux (mitoNCX) was about 1.66 mM and Na^+-dependent Ca^{2+}-efflux was greatly affected by the change of extra-mitochondrial K^+ concentration with Kh of K^+ of about 7.01 mM. The absence of K^+, Ca^{2+}-efflux was inhibited initially and then gradually occurred. Ca^{2+}-efflux at 0 mM K^+ with Na^+ and Ca^{2+}-efflux in the absence of Na^+ were similar. Mitochondrial pH was not changed during Ca^{2+}-influx and efflux. However, Na^+-independent Ca^{2+}-efflux was greatly affected by the change of extra-mitochondrial pH and became faster when pH became basic. These results were contradictory to the previously suggested H^+/Ca^{2+} exchanger. And more, Na^+-independent Ca^{2+}-efflux was inhibited by the application of 1 μM Ru360, which was known as the Ca^{2+} uniporter blocker. The blocking effect of Ru360 only occurred in the initial phase of Ca^{2+}-efflux and could not completely block Ca^{2+}-efflux. From those results suggested that mitochondrial Ca^{2+} efflux was occurred through Na^+-dependent and Na^+-independent pathways and Na^+-independent pathways were composed of at least two different pathways, RU360-senstive and RU360-insenstive. Extramitochondrial pH affected Na^+-independent pathways. The exact nature of Ca^{2+} efflux from the mitochondria needs to be identified. This work was supported by the grant (No.2012-0446) from NRF.

Key Words: Mitochondria, Calcium, NCX, Fura2-FF, TMRE

IC-17

Fluid Pressure Triggers Action Potential and a Subsequent Transverse Ca^{2+} Wave via Local Ca^{2+} Wave-dependent Na^+-Ca^{2+} Exchange Activation in Rat Atrial Myocytes

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There is clinical evidence for a predisposition to atrial fibrillation by regurgitant blood-jets in patients with mitral regurgitation. To understand cellular basis for this atrial arrhythmia we investigated local and global Ca^{2+} signals in single rat atrial myocytes using micro fluid-jet system that

approximates the regurgitant jet, combined with 2-D confocal Ca^{2+} imaging. Atrial myocytes, lacking t-tubules, have peripheral junctional Ca^{2+} release sites and non-junctional (central) release sites in the cell interior. Pressurized fluid flow (fluid pressure, FP) of ~12 dyn/cm^2 elicited local Ca^{2+} waves: peripheral local waves and local waves crossing a cell. FP of ~16 dyn/cm^2 induced two different types of global Ca^{2+} waves: longitudinal wave ("L-wave") and transverse wave ("T-wave"). The speed of L-wave propagation was similar to that of peripheral local wave (~90 μm/s), but lower than that of T-wave (~170 μm/s). The FP-induced T-wave was characterized by a rapid synchronous peripheral Ca^{2+} release followed by delayed slower central $Ca2^{+}$ release. This was similar to the action potential (AP)-triggered Ca^{2+} waves. Interestingly, however, most (~80%) of the FP-induced T-waves contained preceding Ca^{2+} increases. These preceding Ca^{2+} signals were observed as 1) homogenous small Ca^{2+} increase, 2) crossing local wave or 3) immature L-wave. The magnitudes of preceding Ca^{2+} increases shown as the crossing local wave and the L-wave correlated with the rate of initiation of the T-waves. Local Ca^{2+} wave-associated T-waves had larger local Ca^{2+} transients with a strong central Ca^{2+} release compared with the T-waves accompanying the homogenous preceding Ca^{2+} signal. Pre-treatment of tetrodotoxin (10 μM, 3 s) or clamping the membrane voltage eliminated the FP-induced T-waves, but not the preceding Ca^{2+} signals. Pre-exposure to the Na^{+}-Ca^{2+} exchange (NCX) inhibitor, KB-R7943 (0.2 μM, 8 min), suppressed FP-induced T-waves that were preceded by the crossing local wave or the immature L-wave. These results suggest that the crossing local Ca^{2+} wave and immature L-wave may activate NCX to trigger action potential during FP exposure, thereby producing T-wave.

Key Words: Arrhythmia, Atrial myocytes, Fluid pressure, Ca^{2+} waves, Na^{+}-Ca^{2+} exchange

IC-18

Time-dependent Modulation of K^{+} Currents in PMA and LPS-stimulated THP-1 Cells

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THP-1 human monocytic leukemia cells were induced to differentiate into macrophage or activate to produce cytokines when treated with Phorbol ester, phorbol 12-myristate 13-acetate (PMA) or Lipopolysaccharide (LPS), a cell membrane component of gram-negative bacteria. Aim of the present study was to investigate the difference of K^{+} channel expression on short-term stimulation (2, 4, 6, 24 hr) of PMA compared with LPS in THP-1 monocyte. Also, we have searched for the regulatory mechanisms and the physiological function of the channels in PMA/LPS-stimulated cells. Using molecular and electrophysiological technique, we examined the K^{+} channels expression by both PMA and LPS in THP-1 cells. Almost all un-stimulated THP-1 cells were present a delayed rectifier K^{+} channel (I_{DR}), was activated by depolarization to potential positive to -40 mV. Furthermore, 1-EBIO, an activator of intermediate conductance Ca^{2+} activated K^{+} channel, -sensitive currents (I_{SK}) were observed in about 50% of tested cells. Whereas the amplitude of I_{SK} was partially increased by PMA or LPS treatment within 24 hours, I_{SK} was found in above 75% of tested cells. Interestingly, I_{DR} was mostly absent from both PMA- and LPS-stimulated cells within 2, 24 hours, respectively. Also, inwardly rectifying K^{+} current (I_{IR}) was markedly observed in PMA-/LPS-treated cells. Although the patterns of K^{+} channel expression on PMA-stimulated cells were similar with these of LPS-treated cells, the modulation of each K^{+} channels was had different time-dependency. In particular, Iir induced by LPS but not by PMA appeared in early time (2 hr) and reached peak amplitude within 4 hour due to trafficking of this channel to the cell surface. Putative changes in K^{+} channel repression by treatment of PMA or LPS might correlate the initial response of physiological function. Also, the observed correlation between ion channel expression and function might be clue to studies of mechanisms of regulation in K^{+} channel expression.

Key Words: THP-1, LPS, PMA, K^{+} channel

IC-19

Regulation of Basal Autophagy by TRPM7 Channel

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Autophagy is well known cellular catabolic process for degradation and recycling of proteins, cytoplasmic components and organelles through the lysosomal machinery. Autophagy studies are mostly performed under starvation-induced conditions. Only few studies are reported regarding the basal autophagy, which is needed for basic cellular maintenance. We found that basal autophagy is regulated by the ubiquitously expressed transient receptor potential melastatin 7 (TRPM7) channel. TRPM7 channel is unique for having extra kinase domain in its sequence, and is Ca^{2+}- and Mg^{2+}- permeable. When TRPM7 channel expression is increased using tetracycline-inducible expression system, basal autophagy and AMPK phosphorylation (a main regulator for autophagy) are increased. Blocker for CamKKbeta, which is upstream regulator of AMPK, is able to inhibit the effect of TRPM7 channel expression. The expression of TRPM7-deltaK, which lacks the kinase domain, also induced autophagy and AMPK activity, indicating that kinase domain is not necessary. In contrast, basal autophagy and phosphorylated AMPK level are decreased when TRPM7 channel expression is down-regulated by siRNA. Consistent with this result, basal autophagy, AMPK phosphorylation, are decreased by a specific TRPM7 blocker. These results indicate that TRPM7 channel regulates basal autophagy via CamKKbeta-AMPK signaling pathway.

Key Words: TRPM7, Autophagy

IC-20(PO-6)

Arginine Methylation of Voltage-gated KCNQ Potassium Channels Regulates Neuronal Excitability

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Neural M-type K^+ channels control somatic excitability, bursting and neurotransmitter release throughout the nervous system. M channels are known to be composed of heteromeric assembly of KCNQ2/KCNQ3 subunits. Post-translational modifications are rapidly emerging as the important determinants in the regulation of KCNQ channels. In this study, we demonstrate that protein arginine methyltransferase PRMT1 directly binds to and methylates KCNQ2 at four arginine residues, R333, R345, R353, and R435 of proximal C-terminus. Suppression of arginine methylation by PRMT1 knockdown or mutation of methylation sites strongly reduced KCNQ2 channel activity. Genetic deletion and pharmacologic block of PRMT1 led to an increase of the excitability of hippocampal neurons. Because the kinetics of activation and inactivation of KCNQ2/KCNQ3 channels are slow compared with Na^+ channels, they are expected to influence firing rates rather than action potential duration. Indeed, methylation of KCNQ2 channels is found to modify hippocampal neuron input resistance, action potential threshold, and firing frequency, but not action potential duration. PRMT1$^+$/- mice showed signs of neuronal persistent hyperexcitability including frequent interictal spiking and spontaneous seizures. The reduction of KCNQ channel activity seems to underlie this neuronal hyperexcitability, because mutant cells showed a substantial increase of input resistance and AP numbers generated during depolarizing current steps whereas subsequent addition of 10 μM XE991 did not have any effect on input resistance and action potential firing. Further, our voltage clamp study showed the M channel function was reduced by 30% in mutant cells. Together, PRMT1-dependent methylation set KCNQ channel activity and indirectly the responsiveness of the neurons to physiological stimuli.

Key Words: KCNQ2, Methylation, Potassium channel

IC-21

Activation of TRPC4 by $G\alpha_i$ Subunit Increases Calcium Selectivity and Controls Neurite Morphology in Cultured Hippocampal Neuron

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Transient receptor potential canonical (TRPC) channels are receptor-operated Calcium permeable cation channels involved in many physiological processes. All TRPC channels can be activated by G-protein-coupled receptor (GPCR)-$G\alpha_q$-PLC pathway even though the exact mechanism by which the channels are activated remains largely unknown. Recently we showed that TRPC4 and TRPC5 are activated primarily by selective $G\alpha_i$ subunits rather than $G\alpha_q$. TRPC4 is activated by several $G\alpha_i$ subunits, most prominently by $G\alpha_{i2}$, and TRPC5 by $G\alpha_{i3}$. Traditionally, ionic selectivity and conductance are considered as fundamental properties of ion channels. However several studies showed that pore properties can be changed by activation through agonist stimulation or protein interaction. Among TRP channels, TRPV1 and TRPA1 were shown to undergo pore dilation and selectivity change subsequent to activation with ligands stimulation. These results raise the possibility that activation of TRPC4 by interaction with $G\alpha_i$ subunits may cause changes in pore properties of TRPC4 and consequently affect cellular functions. In visceral smooth muscle cells, TRPC4 is one of the molecular identities with TRPC6 for the non-selective cation (NSC) current activated by muscarinic receptor stimulation (mICAT). However, little is known about the physiological role of TRPC4 in brain. Although TRPC4 is implicated in neurite extension in post-mitotic neurons, the role of TRPC4 in dendrite morphogenesis remains controversial. Since a novel TRPC4 activation mechanism by direct interaction with Gαi protein has been recently revealed, it is necessary to investigate the functional roles of TRPC4 and TRPC4-$G\alpha_i$ signaling complex in this aspect. We report here that interaction with Gαi subunits not only activates TRPC4 but also changes channel properties to increase the calcium permeability. Moreover, we present evidences that TRPC4 controls neurite morphogenesis in cultured hippocampal neuron and this function is augmented when activated by $G\alpha_i$ subunits.

Key Words: TRPC4, TRPC5, G-protein

IC-22

The Role of Novel Neurotransmitter Agmatine in Synaptic Plasticity

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Agmatine, an intermediate of L-arginine and putrescine, is considered as a novel putative neurotransmitter. It is synthesized in the brain, stored in synaptic vesicles, accumulated by uptake, released by membrane depolarization, and inactivated by agmatinase. Agmatine binds to α_2-

adrenergic receptor and imidazoline receptors, and blocks NMDA receptors. Agmatine administered intrathecally, locally or systemically, reduces the neuronal injury produced by excitotoxins, global/focal ischemia, spinal cord injury, and hypoxic ischemic injury. It also has notable effects on antidepressant-like effects in depression models. However, little is known about the role of agmatine on hippocampal synaptic plasticity. In the present study, we investigated the effect of agmatine on long-term potentiation (LTP, which is widely considered one of the major cellular mechanisms that underlies learning and memory) by theta burst stimulation (TBS) in the induction, maintenance and depotentiation in the CA1 stratum radiatum of mouse hippocampus slice. We found agmatine dose-dependently depresses LTP both in the induction and maintenance, and it also promotes depotentiation dose-dependently. The effect of agmatine on LTP was not blocked by doxaxosin (α_1-adrenergic receptor antagonist) and BU224 (imidazoline type 2 receptor antagonist). However, it was blocked by efaroxan (α_2-adrenergic receptor and imidazoline type 1 receptor antagonist) and partly blocked by MK-912 (selective α_2-adrenergic receptor antagonist). The effect of agmatine was also blocked by bicuculline ($GABA_A$ receptor antagonist). These results suggest that agmatine may exert its effect via α_2-adrenergic receptor and/or imidazoline type 1 receptor and $GABA_A$ receptor.

Key Words: Agmatine, α_2-adrenergic receptor, Imidazoline receptor, LTP, Hippocampus

IC-23

Characterization of ANO6-induced Chloride Currents Activated by Calcium in Mammalian Cells

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Ca^{2+}-activated Cl^- channels (CaCCs) control diverse functions in several physiological processes. Recently, anoctamins (ANOs) have been identified as a family of putative Cl^- channels, and ANO1 and ANO2 have been identified as essential components of the CaCCs. However, the role of other members of the ANO family in generating Cl^- currents via an increase in intracellular Ca^{2+} concentration is still controversial. Recent findings have indicated that ANO6 plays an important role in the Ca^{2+}-dependent exposure of phosphatidyl serine on the surface of platelet cells. However, ANO6 has also been reported to be an essential component of outward-rectifying Cl^- channels (ORCC) or small conductance calcium-activated non-selective cation channels. It is therefore unclear whether ANO6 constitutes a family of CaCCs or whether it is a protein with heterogeneous functions. Here, we examined the functional properties of human ANO6, which is over-expressed in human embryonic kidney (HEK) 293T cells and the human pancreatic duct cell line PANC-1, using whole-cell patch clamping, and also in excised inside-out patches. With both whole-cell and inside-out patch clamps, intracellular calcium ($[Ca^{2+}]_i$) induced a Cl^- current and displayed only strong outward rectification properties, even when we increased $[Ca^{2+}]_i$ to 1 mM. We also compared the biophysical properties of ANO6, such as calcium sensitivity, activation duration, and tail currents, with those of ANO1. The pharmacological properties of ANO6 currents showed low sensitivity to the CaCC blockers such as niflumic acid, DIDS, NPPB, and CaCCinh-AO1. Our results demonstrated that ANO6 functions as a Ca^{2+}-activated Cl^- channel with characteristics differing from those of ANO1.

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Key Words: Anoctamins, ANO6, Cl^- channel, CaCCs

IC-24

Murrayafoline-A Enhances Ca^{2+}-induced Ca^{2+} Release and Contractility in Rat Ventricular Myocytes

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Murrayafoline-A is a carbazole alkaloid isolated from a Vietnamease plant *Miliusa Balansae*. Here we demonstrate this compound as a novel positive inotropic agent and its action mechanism in ventricular myocytes. Murrayafoline-A increased cell shortenings dose-dependently in rat ventricular myocytes (EC_{50} = ~20 μM). The magnitudes of Ca^{2+} transients were significantly increased with no change in the kinetics of the Ca^{2+} transients. The Ca^{2+} spark frequency in resting cells was significantly enhanced by murrayafoline-A, while there was no change in the ryanodine binding of ryanodine receptors in the presence of this compound. The Ca^{2+} loading in the sarcoplasmic reticulum (SR), estimated as the magnitude of caffeine (10 mM)-induced Ca^{2+} transients, was significantly increased by murrayafoline-A. Interestingly, murrayafoline-A transiently enhanced Ca^{2+} current and Ba^{2+} current through the L-type Ca^{2+} channels in a voltage-dependent manner. These results suggest that murryafoline-A increases Ca^{2+} influx through the Ca^{2+} channels, and thereby enhancing Ca^{2+}-induced Ca^{2+} releases and SR Ca^{2+} loading. This mechanism may underlie the murrayafoline-A-induced positive inotropy.

Key Words: Murrayafoline-A, Positive inotropy, Ventricular myocytes, L-type Ca^{2+} channel, Ca^{2+}-induced Ca^{2+} release

IC-25

Estrogen Modulation of the Voltage-gated Potassium Channel Subunit Kv4.2 in Rat Presympathetic PVN Neurons

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The hypothalamic paraventricular nucleus (PVN) is an important site in regulating the autonomic nervous system. Among those, the neurons projecting the rostral ventrolateral medulla (PVN-RVLM) have a role of regulation of sympathetic outflow. It has been demonstrated that PVN-RVLM neurons express an abundant estrogen receptor subtype β (ER-β) and the A-type potassium current (I_A), known to be regulated by estrogen, is involved in the determination of biophysical properties of the PVN-RVLM neurons. Thus we investigated the effects of estrogen on the I_A and the expression of Kv4.2 and Kv4.3 subunits at the single cell level in the PVN-RVLM neurons. The ovariectomized female rats were divided into two groups, oil- and 17β-estradiol-treated (E2) groups. Single cell real-time RT-PCR and whole cell patch clamp recording were performed with the PVN-RVLM neurons identified by retrograde label tracing. The densities of I_A as well as 4-AP sensitive currents were diminished in the E2-groups compared to the oil-group. In addition, Kv4.2 mRNA expression, not Kv4.3, was decreased significantly in the E2 group. These results suggest that estrogen attenuates the I_A via Kv4.2 subunit in PVN-RVLM neurons.

Key Words: Estrogen, Kv4.2, A-type potassium current, PVN-RVLM neurons

IC-26

Effects of Amlodipine on Cardiac Action Potentials and Ion Channels

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Background: Calcium channel blockers (CCBs) have been widely used for treating hypertension, angina, and abnormal heart rhythms (e.g., atrial fibrillation, paroxysmal supraventricular tachycardia). Amlodipine belongs to the third-generation of dihydropyridine CCBs. The purpose of this study was to investigate the effect of amlodipine on cardiac action potentials and ion channels. **Method:** In these experiments, we examined the value of the isolated rabbit Purkinje fiber as an in vitro action potential (AP) assay to predict the potential of amlodipine to induce these undesirable adverse effects on the heart. Furthermore, we examined the effects of amlodipine on the major cardiac ion channels transiently transfected in HEK293 cells and the native voltage-gated Ca^{2+} channels in rat ventricular myocytes using the whole-cell patch clamp technique. **Results:** According to the results, amlodipine at 30 μM significantly shortened the APD_{50} from 160.4±7.9 to 102.1± 10.6 ms (n = 4) and showed a triangulated AP. However, amlodipine at concentrations up to 30 μM did not affect any other AP parameters, the APD_{90}, the resting membrane potential (RMP), maximum velocity of phase 0 (V_{max}) or the total amplitude (TA). In the ion channel studies, the half-maximum inhibiting concentration (IC_{50}) value for calcium channel of amlodipine is 0.23 μM. Amlodipine is also a potent blocker of cardiac K^+ channels, such as *hERG*, I_{Ks} and I_{K1}. The rank order of inhibitory potency was I_{Ks} > *hERG* > I_{K1} with IC_{50} values of 5.81, 6.78, and 9.78 μM, respectively. In addition to K^+ channels and Ca^{2+} channel, amlodipine also inhibited I_{Na} with the IC_{50} value of 6.38 μM. **Conclusion:** Our data indicate that the clinical use of amlodipine is safe within therapeutic plasma concentration (approximately 50 nM), but all patients taking this drug should be closely monitored because the amlodipine may lead to the triangulated AP and the inhibition of ion channels involved on cardiac action potentials.

Key Words: Amlodipine, Calcium channel blocker, Cardiac action potential, Ion channel

IC-27

Screening Marine Natural Products for Aquaporin Modulators

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Aquaporins (AQPs) are a family of integral membrane proteins that transport water and small solutes such as glycerol and perform many physiologic functions in human body. The implication of AQPs in several pathologic conditions makes AQP-based modulators attractive targets for novel drug therapies. Until now, no AQP inhibitors have yet been developed as a suitable candidate for clinical implication. We have screened extracts from marine natural products for AQP modulators by stopped-flow light scattering. All the 30 compounds we tested had little effects on osmotic water permeability when mouse red blood cells were exposed a 300 mM sucrose gradient. However, some of them affected the glycerol permeability significantly. Az and STC increased erythrocytes glycerol permeability whereas Glabrugui-none A, Rhz and AcAglRhz decreased it at a micromolar concentration when dissolved in either PBS or DMSO. The high selectivity together with its high water solubility makes the compound a suitable drug lead as aquaglyceroporin modulators. These results suggest possibility of extracts of marine natural products as AQP drug development.

Key Words: AQP, AQP-based modulators, Marine natural products

IC-28

Effect of Zactima, Antineoplastic Agent on Cardiac Repolarization

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The inhibition of the potassium current IKr and QT prolongation has been known to be associated with drug-induced torsades de pointes arrhythmias (TdP) and sudden cardiac death. In this study, we investigated the cardiac electrophysiological effects of zactima, a tyrosine kinase inhibitor with antiangiogenic and antitumor activity, that has been reported to prolong the QT interval by using the conventional microelectrode recording techniques in isolated rabbit purkinje fiber and whole-cell patch clamp techniques in human *ether-à-go-go* related gene (hERG)-stably transfected CHO cells. Zactima at 10 μM significantly decreased the Vmax of phase 0 depolarization ($p < 0.05$) and significantly prolonged the action potential duration at 90% repolarization (APD_{90}) ($p < 0.01$) whereas the action potential duration at 30% repolarization (APD_{30}) was not prolonged. For I_{hERG}, the IC_{50} value was 28.04±1.81 mM. For I_{hERG}, the IC_{50} value was 0.62±0.30 mM. For I_{K1}, the IC_{50} value was about 300 mM. Zactima was found to have no effect on sodium channel currents. When these results were compared with Cmax (41 nM) of clinical dosage (10 mg/kg, p.o), it can be suggested that zactima is not safe at the clinical dosage of 10 mg/kg from the electrophysiological aspect. These findings indicate that zactima, a tyrosine kinase inhibitor, may have torsadogenic potency.
Key Words: Action potential, Zactima, I_{hERG}, I_{Na}, I_{Ks}, I_{K1}, QT interval prolongation

IC-29

Telmisartan Delayed Inactivation of Voltage Gated Sodium Channel in Rat Heart

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Background: Telmisartan, known as Angiotensin II receptor blocker (ARB) and PPAR-gamma stimulator is a group of pharmaceuticals which modulate the renin-angiotensin- aldosterone system. It is mainly used for the management of hypertension, diabetic nephropathy and congestive heart failure. However, recently side effects of high dose Telmisartan on specific potassium and calcium ion channel were reported. **Methods and Results:** We evaluated the effect of high dose telmisartan on heart, cardiac myocytes and cardiac sarcolemmal ion channels. Hearts of 8 weeks SD rats were perfused with normal Tyrode's solution (control), telmisartan or losartan, another AngII antagonist, at the doses of 3, 10, 30 and 100 μM for 3 hours, respectively. Telmisartan treatment, significantly induced myocardial infarction by 21% at 30 μM ($P < 0.0001$) and 63% at 100 μM ($P < 0.001$), but not in the control and losartan group. In the cardiac performance analysis and M-mode echocardiography, Telmisartan treatment induced cardiac dysfunction including decreases in heart rate and coronary flow, hyper-contraction and arrhythmia. Confocal microscopy demonstrated that Telmisartan significantly elevated intracellular Ca^{2+} level leading to hyper contracture and cell death. Patch clamp analysis revealed that telmisartan induced Na^+ overload via slowing in the inactivation of voltage-gated Na^+ current (I_{Na}) which leads to activation of reverse mode of NCX activity and following Ca^{2+} overload in isolated cardiac myocytes. **Conclusion:** Telmisartan significantly delayed inactivation of voltage-gated Na^+ channel resulting cytosolic Na^+ overload and subsequent Ca^{2+} overload which potentially lead to cardiac dysfunction and myocardial infarction.
Key Words: Telmisartan, Myocardial Infarction, Cardiac dysfunction, Voltage gated Na^+ channel inactivation, Na^+/Ca^{2+} exchanger

IC-30

Closely Spatio-association of TRPC4 with Gαi in TRPC4 Activation Process

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Canonical transient receptor potential (TPRC) channels are Ca^{2+}-permeable nonselective cation channels that are widely expressed in numerous cell types. Seven different members of TRPC channels are isolated and canonical type of TRP channel family transduces signals of GPCR with various external stimuli. TRPC4 channels are known to be regulated by $G\alpha_i$ proteins. However, the molecular mechanism how $G\alpha_i$ proteins activate TRPC4 still remains to be questionable. To investigate the mechanism, we used whole patch clamp and FRET (Föster Resonance Energy transfer). We tagged mTRPC4 and G protein with CFP and YFP, respectively, and transiently transfected HEK293 cells with FRET pair. FRET efficiency between TRPC4 and Gα was nearly 8% and was greater than those between TRPC4 and Gβγ (nearly 4%). And QL mutant of Ga has nearly 18% of FRET efficiency. At the HEK293 cell transfected with M2 muscarinic receptor, application of carbachol (CCh) increased FRET efficieny from 9.66 4.64 % (n = 7) to 20 % (n = 7). In conclusion, we suggest that Gαi closely locates near TRPC4 and regulates TRPC4 channel activity.
Key Words: TRPC4, Gai, FRET

IC-31

Electrophysiological Properties of Novel Mutations in ClC-1 Chloride Channel of Korean Patients with Myotonia Congenita

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Myotonia Congenita is a genetic disease that displays the

symptom of muscle stiffness and impaired muscle relaxation caused by hyperexcitability of the plasma membrane. There are two types of Myotonia Congenita; Autosomal dominant Myotonia Congenital (Thomsen's disease) and autosomal recessive generalized Myotonia (Becker's myotonia) are caused by mutations in the skeletal muscle chloride channel, ClC-1. ClC-1, the member of a large family of anion channels, is voltage-gated chloride channel. It is abundantly expressed in human skeletal muscle. In skeletal muscle, the voltage-gated chloride channels contribute to stabilize the resting membrane potential and control electrical excitability. ClC-1 channels contain double-barreled structure which consists of two identical protopores. Each pore is voltage dependent and functions independently. When mutations in the gene for ClC-1 underlie Myotonia Congenita, it can affect normal function of the channel and damage the specialized property of independently working double pores. Since Mytonia Congenital was first diagnosed, a number of mutations widely displayed in the protein have been revealed so far. Here, nine mutants (p.M128I, p.S189C, p.M373L, p.P480S, p.G523D, p.M609K, p.T310M, p.R317X, p.R47W, A298T and p.G355R) from Korean patients who suffer from Myotonia Congenita were reported in 2009. We studied the functional changes of each mutant by using patch clamp method. We observed remarkably reduced chloride conductance from most of mutants. Mutants, p.M128I, and p.G523D showed steady-opened current pattern compared to WT. Furthermore, open probability of mutants was slightly or markedly altered and this clearly indicates the modification of pore property. The two significant mutants have also been tested under physiological condition. Low chloride concentration results in significant modification in the whole cell current and gating properties of mutations, p.M128I and p.G523D.

Key Words: Myotonia Congenita, Chloride Channel, ClC-1

IC-32

Regulation of Calcium Influx and Signaling Pathway in Cancer Cells Via TRPV6-Numb Interaction

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Ca^{2+} is a critical factor in the regulation of signal transduction and Ca^{2+} homeostasis is altered in different human diseases. The level of Ca^{2+} in cells is highly regulated through a diverse class of regulators. Among them is the Transient Receptor Potential Vanilloid 6 (TRPV6), which is a Ca^{2+} selective channel that absorbs Ca^{2+} in the small intestine. TRPV6 is overexpressed in some cancers and exhibits oncogenic potential, but its exact mechanism is still poorly understood. The Numb protein is a cell fate determinant that functions in endocytosis and as a tumor suppressor via the stabilization of p53. Numb protein consisted of four isoforms. Here, we showed a novel function of Numb1, which negatively regulates TRPV6 activity. The expression of Numb1 decreased cytosolic Ca^{2+} concentrations in TRPV6-transfected HEK293 cells. When all isoform of Numb were depleted using siRNA in a TRPV6 stable cell line, the levels of cytosolic Ca^{2+} increased. We observed an interaction between Numb1 and TRPV6 using co-immunoprecipitation. We confirmed this interaction using Fluorescence Resolution Energy Transfer (FRET). We identified the TRPV6 and Numb1 binding site using TRPV6 C-terminal truncation mutants and Numb1 deletion mutants. The binding site in TRPV6 was an aspartic acid at amino acid residue 716, and that binding site in Numb1 was arginine at amino acid residue 434. A Numb1 mutant, lacking TRPV6 binding activity, failed to inhibit TRPV6 activity. All isoform of Numb knockdown, using an siRNA-based approach in MCF-7 breast cancer cells, not only showed enhanced TRPV6 expression but also both the cytosolic Ca^{2+} concentration and cell proliferation were increased. The down-regulated expression of TRPV6 using siRNA increased Numb protein expression; however, the cytosolic influx of Ca^{2+} and proliferation of the cell were decreased. To examine downstream signaling during Ca^{2+} influx, we performed western blotting analysis on TRPV6 upregulated cancer cells (MCF-7, PC-3, HUTU- 80). Taken together, these results demonstrated that Numb1 interacts with TRPV6 through charged residues and inhibits its activity via the regulation of protein expression. Moreover, we provided evidence for a Ca^{2+}-regulated cancer cell signaling pathway and that the Ca^{2+} channel is a target of cancer cells.

Key Words: Numb, TRPV6, Cancer, Calcium

IC-33

EEA1-enriched Endosome-mediated Lysosomal Degradation of Endothelial $K_{Ca}3.1$

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It is known that globotriaosylceramide (Gb3)-induced $K_{Ca}3.1$ downregulation is associated with endothelial dysfunction in Fabry disease. We hypothesized that Gb3 induces $K_{Ca}3.1$ degradation and aimed to clarify the underlying mechanisms. $K_{Ca}3.1$, especially plasma membrane-localized $K_{Ca}3.1$, was downregulated in both Gb3-treated mouse aortic endothelial cells (MAECs) and human umbilical vein endothelial cells (HUVECs), and the $K_{Ca}3.1$ current was significantly reduced in Gb3-treated MAECs. Gb3-induced $K_{Ca}3.1$ downregulation was prevented by the lysosomal inhibitor bafilomycin A1 but not by the proteosomal inhibitor lactacystin or the endoplasmic reticulum stress-inducing agents tunicamycin and 2,5-di-t-butyl-1,4-benzohydroquinone. Further, Gb3 upregulated the levels of EEA1 and LAMP2 proteins in MAECs and the level of LAMP2 mRNA in HUVECs. Compared to aortic tissues and MAECs from age-matched wild-type mice, those from aged α-galactosidase A (*Gla*)-knockout mice, an animal model of Fabry disease, showed downregulated $K_{Ca}3.1$ expression and upregulated EEA and LAMP2 expression. In

contrast, no significant difference was found in EEA and LAMP2 expression between young *Gla*-knockout and wild-type MAECs. In aged *Gla*-knockout MAECs, $K_{Ca}3.1$ and EEA1 were found to be colocalized especially along the cell border; Rab5 was upregulated, and Rab5C knockdown restored $K_{Ca}3.1$ expression and the current. In addition, in aged *Gla*-knockout MAECs, clathrin was translocated close to the cell border and clathrin knockdown recovered $K_{Ca}3.1$ expression. Collectively, the results suggest that Gb3 accelerates $K_{Ca}3.1$ endocytosis and lysosomal degradation of internalized $K_{Ca}3.1$ in endothelial cells via clathrin/EEA1-mediated processes, leading to the endothelial dysfunction that contributes to vasculopathy in Fabry disease.

Key Words: Sphingolipids, Fabry disease, Endothelial dysfunction, Ca^{2+}-activated K^+ channel, Endocytic trafficking

IC-34

Effects Telmisartan on Voltage-gated Na^+ Channel

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Telmisartan is known as a type 1-selective angiotensin II receptor antagonist and a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ). It is effective against essential hypertension. However, there was a finding that high dose of telmisartan could induce a myocardial infarction in rat heart. In order to elucidate how telmisartan induces myocardial infarction, we examined if there is any ion channel modulation using patch clamp technique in rat ventricular myocytes. Perfusion of 30 μM telmisartan reduced the current density of L-type Ca^{2+} channel about 19% without affecting its time course. K^+ currents such as transient outward K^+ current (I_{to}) and delayed rectifier K^+ current (I_K) were not significantly affected by the same concentration of telmisartan. The most remarkable change was the slowing in the inactivation of voltage-gated Na^+ current (I_{Na}). The inactivation time constant (τ) of I_{Na} became more than 20-times bigger by perfusion of 30 μM telmisartan in a reversible manner. Sustained Na^+-influx by delayed inactivation of I_{Na} is supposed to increase the action potential duration (APD). Indeed, perfusion of telmisartan increased APD_{90} from 30 msec to 180 msec and even arrhythmias followed. The action of telmisartan was blocked by 10 μM tetrodotoxin (TTX) in a reversible manner. Recovery from the effect of telmisartan on inactivation time constant was very slow suggesting that it modulates the voltage-gated Na^+ channel after permeation into the cytosolic space of myocytes. The other parameters such as activation time constant and current-voltage relationship were not significantly affected. The voltage-dependence of steady-state activation and inactivation were not affected either. From above results, we conclude that high dose of telmisartan induces Na^+ overload by delaying the inactivation of I_{Na} and subsequent Ca^{2+} overload through reverse-mode operation of Na-Ca exchange, which could potentially cause cardiac cell death.

Key Words: Telmisartan, Voltage gated Na^+ channel, Inactivation time constant

IC-35

Inhibitory Mechanism of T-type Ca^{2+} Channel Inhibitor, Mibefradil on Voltage-dependent K^+ Channels in Coronary Arterial Smooth Muscle Cells

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We investigated the effects of mibefradil, a T-type Ca^{2+} channel inhibitor, on voltage-dependent K^+ channels in smooth muscle cells from rabbit coronary arteries. Mibefradil inhibited the Kv current in a dose-dependent fashion with an apparent K_d of 1.08 μM. It accelerated the decay rate of Kv channel inactivation without altering the kinetics of current activation. The rate constants of association and dissociation for mibefradil were 2.23 ± 0.07 $\mu M^{-1}s^{-1}$ and 2.40 ± 0.42 s^{-1}, respectively. Mibefradil did not have a significant effect on the steady-state activation and inactivation curves. The recovery time constant from inactivation was decreased in the presence of mibefradil, and application of train pulses (1 or 2 Hz) caused a progressive increase in the mibefradil blockade, indicating that mibefradil-induced inhibition of Kv current is use-dependent. The inhibitory effect of mibefradil was not affected by extracellular Ca^{2+} free condition. Moreover, the absence of intracellular ATP did not change the blocking effect of mibefradil. From these results, we suggest that mibefradil directly inhibited the Kv current, independently of Ca^{2+} channel inhibition.

Key Words: Mibefradil, Voltage-dependent K^+ channel, Coronary arterial smooth muscle cell

IC-36

The Study of TRPM7-mediated Ca^{2+} Signaling in Osteoclastogenesis

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A melastatin-related subfamily of transient receptor potential (TRPM7) channel is known to permeable to Ca^{2+} and Mg^{2+}, which is essential for cellular viabilities like cell growth, adhesion, and proliferation. TRPM7 channel activities are controlled by intracellular levels of Mg^{2+} ions and Mg-complexed nucleotides. Reducing cellular levels of these factors activates TRPM7-mediated currents that ex-

hibit a current-voltage relationship with inward and outward rectification. However, the roles of TRPM7 in RAW264.7, a mouse bone marrow derived monocytes and bone marrow macrophage (BMM) cells are unclear. We investigated the roles of TRPM7 in the cells using small interfering RNA (si-RNA) against TRPM7, RT-PCR, patch-clamp, and calcium imaging technique. TRPM7-mediated currents were recorded in HEK293, RAW264.7, and BMM cells. TRPM7-mediated increases of $[Ca^{2+}]_i$ were inhibited by transfection of si-TRPM7. Furthermore, Receptor activator of NF-kB ligand (RANKL)-induced Ca^{2+} oscillations were disappeared in si-TRPM7 RAW264.7 cells. These results suggest that TRPM7 channel plays role in Ca^{2+} signaling which is important for osteoclastogenesis.
Key Words: TRPM7, Mg^{2+}, RANKL, Osteoclastogenesis

IC-37

RASD1 Activates TRPC4 through $G\alpha_i$ Independently of GPCR

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Canonical transient receptor potential (TRPC) channels have six transmembrane (6-TM) domains and are Ca^{2+}-permeable and non-selective cation channels. It is generally speculated that TRPC channels are activated by stimulation of Gq-PLC-coupled receptors and oxidation. Activator of G-protein signaling1 (AGS1 or RASD1), the ras-related protein, interacts with Gi/Go and activates heterotrimeric G-protein signaling systems independent of G-protein-coupled receptor (GPCR). It is previously reported that AGS1 is related to GIRK channel and Ca^{2+} channel. However it is unknown whether AGS1 is associated with TRPC channels. We assumed that AGS1 might regulate TRPC4 channel, since AGS1 interacts with Gi/Go and TRPC4 is activated by Gi/o subunits. Here, we measured whole cell current of TRPC4/5 after the co-expression of TRPC4 or TRPC5 with constitutively active form of small GTPases in HEK293 cells. AGS1 (CA) mutant (Q to L) activated TRPC4 (38.8 ± 7.2 pA/pF) without GTPγS and independently of GPCR. Pertussis toxin (PTX), $G\alpha_i$ specific inhibitor, blocked RASD1-activated TRPC4 current (3.4± 1.6 pA/pF). When co-expressed with dominant negative $G\alpha_i$ protein subtype, TRPC4 activation by RASD1 was completely inhibited. With previous report that TRPC4 are activated primarily by selective $G\alpha_i$ subunits rather than $G\alpha_q$, these results suggest that AGS1 activates TRPC4 channel through modulating $G\alpha_i$ subunits and AGS1 is a new activator for TRPC4 channel.
Key Words: TRPC, GPCR, RASD1

IC-38

Regulation of TRPC6 Channels by Secreted Klotho

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Klotho is a mammalian senescence-suppression protein abundantly expressed in the kidney. The extracellular domain of Klotho is cleaved and secreted into blood and urine and may function as a humoral factor. The role of Klotho in the kidney, however, remains largely unknown. The canonical transient receptor potential type-6 channel, TRPC6, is a calcium-permeable cation channel expressed in many tissues including podocytes and mesangial cells of glomerulus. Gain-of-function mutations in *TRPC6* cause familial focal and segmental glomerulosclerosis. Increased expression and/or activity of TRPC6 may also be involved in the pathogenesis of secondary glomerular diseases. Transgenic overexpression of Klotho ameliorates glomerular injury in mice. Here, we investigated the potential role of Klotho in regulating TRPC6 thus contributing to the protection of glomerular injury. Carbachol (CCh)-induced TRPC6 channel activity was measured by whole-cell patch-clamp recording in HEK293 cell co-expressing TRPC6 and M3 muscarinic receptor. We found that incubation with purified Klotho protein inhibits CCh-stimulated TRPC6 channel activity. Klotho does not affect gating and activation kinetics of TRPC6 by CCh (i.e., activation time constant and current-voltage relationship is unchanged by Klotho). Moreover, Klotho attenuates TRPC6 channel activity activated by diacylglycerol. Klotho decreases surface abundance of TRPC6 measured by biotinylation assays. Klotho inhibits TRPC6 in cells cultured in serum media, but not in cells cultured in serum-free media. Addition of serum enhances surface expression of TRPC6 in cells cultured in serum-free media. We recently reported that Klotho has sialidase activity and regulates TRPV5 channel via this activity. We found that the regulation of TRPC6 is independent of the sialidase activity. Thus, Klotho decreases surface expression of TRPC6 probably by reducing serum stimulated export of the channel. Klotho may be protective for glomerular injury caused by upregulation of TRPC6 [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0024789)].
Key Words: Klotho, TRPC6, TRPV5, Glomerular injury, Exocytosis

IC-39

Expression of Cytokine with Asthma Related Allergens in Human Gingival Epithelial Cells

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Asthma is a chronic inflammatory disease of the airway characterized by variable airflow obstruction and bronchial hyper-responsiveness. In addition, patients with asthma have been reported that high prevalence of dental caries and gingivitis, which are induced by low salivation due to the medication for asthma. However, it is not known that asthma related allergens such as house dust mite (HDM) and German cockroach extract (GCE) have direct effects on the generation of gingival inflammation. In the present study, we investigated the level of interlukin-8 (IL-8) mRNA expression and the characteristics of Ca^{2+} signal by GCE and HDM in the human gingival epithelial cells. HDM and GCE induced increases in IL-8 mRNA expression level and intracellular concentration of Ca^{2+} $([Ca^{2+}])_i$, respectively. Endotoxin-free GCE activated protease-activated receptor (PAR) type2, but endotoxin-free HDM did not activate any PARs. In human gingival epithelial cells, lipopolisacaride, a Gram-negative endotoxin, did not induce Ca^{2+} signal. HDM and GCE containing endotoxins induced Ca^{2+} signal by releasing Ca^{2+} from thapsigargin (Tg)- sensitive Ca^{2+} stores via phospholipase C (PLC)/inositol 1, 4, 5-trisphosphate (IP_3) pathway. These results suggest that asthma related allergens induce Ca^{2+} signaling and cytokine release in human gingival epithelial cells.

Key Words: House dust mite, German cockroach, Gingival epithelial cells, Peptidoglycan, Toll-like receptor, Protease-activated receptor

IC-40

TRPM3 and TRPV4 Mediates Hypotonic Stress-induced RANKL Expression in Human Periodontal Ligament Cells

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The periodontal ligament (PDL) is specialized connective tissue fibers for attaching a tooth to the alveolar bone and for supporting the tooth to withstand the mechanical stress occurred during chewing and continuous orthodontic tooth movement. However, the mechanism underlying the hypotonic mechanical stress-induced cellular responses in PLD cells remains poorly understood. In this study, we investigated hypotonic stress-induced RANKL expression and determined which transient receptor potential (TRP) channels are affected by hypotonic stress in human primary cultured PDL cells. Hypotonic stress increased RANKL mRNA expression and the intracellular calcium concentration ($[Ca^{2+}]_i$). Treatment with gadolinium and lanthanum, non-specific plasma membrane Ca^{2+} channel blockers, completely inhibited these hypotonic stress-induced effects. Pregnenolone sulfate and 4α-Phorbol 12,13-Didecanoate, agonists of TRPM3 and TRPV4, also induced increases of $[Ca^{2+}]_i$. 2-Aminoethoxydiphenyl borate and ruthenium red, blockers of TRPM3 and TRPV4, significantly reduced hypotonic stress-induced increases of $[Ca^{2+}]_i$. Moreover, the channel activities of TRPM3 and TRPV4 were confirmed by a whole-cell patch-clamp technique. These results suggest that hypotonic stress induces increases of $[Ca^{2+}]_i$ through TRPM3 and TRPV4 to regulate RANKL in primary cultured human PDL cells.

Key Words: Human periodontal ligament, TRPM3, TRPV4, Mechanical stress, RANKL

IC-41

Dual Sensitivity of TREK-2 Channels to the Level of PIP_2 in Membrane

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Potassium ion (K^+) channels with 2-pore domain (KCNK) control diverse functions in several physiological processes. For example, these channels provide the electrical driving force that is required for calcium ion (Ca^{2+}) influx, cell volume regulation, and apoptotic volume decrease. Among the KCNK families, the TWIK-related K^+ channel-2 (TREK-2) is expressed in lymphocytes. Under physiological conditions, TREK-2 is inhibited by phosphatidylinositol 4, 5-bisphosphate (PIP_2), which is present in the inner leaflet membrane. Inside-out patch-clamp studies showed that the application of MgATP (1 mM) inhibited TREK-2 through the generation of PIP_2 by phosphoinositide kinases. Consistently, ATP-free conditions induced tonic activation of TREK-2. However, artificial scavenging of intrinsic PIP_2 by using poly-L-lysine restored the inhibition of TREK-2 activity. After confirming the total inhibition of TREK-2 by poly-L-lysine, PIP2 application resulted in dual effects: initial activation of TREK-2 and subsequent inhibition at higher concentrations. To elucidate the mechanism of this dual regulation effect of PIP2 on TREK-2 in physiological conditions, we performed a whole-cell patch-clamp study by using an HEK-293 cell line, in which TREK-2 was co-overexpressed with Dr-VSP. Dr-VSP is a voltage-sensitive phosphatase that is activated by membrane depolarization. Interestingly, when we applied a mild depolarizing pulse (+20 mV) on the HEK-293 cells to activate Dr-VSP, the TREK-2 currents showed initial activation and subsequent inhibition; this result is similar to those of the inside-out patch-clamp studies. This phenomenon might be the result of the depletion of membrane PIP_2. Thus, our results showed that TREK-2 was dually regulated by the level of PIP_2 in the inner leaflet membrane.

Key Words: TREK-2, VSP, PIP_2, Regulation

IC-42

Inactivations of JAK2/STAT3 Signaling Cause Impairments of Synaptic Plasticity in the aging *klotho* Gene Mutant Mice

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Klotho mutant mice, which harbor a defective *klotho* gene, have an extremely short. Further evidence suggests that the *klotho* gene is responsible for aging. Janus kinase (JAK) proteins and the signal transducer and activator of transcription (STAT) proteins are intracellular signaling pathway activated by soluble signaling molecules (i.e., cytokines and growth factors) and their receptors are expressed in both the developing and mature mammalian. In the present study, we employed McN-A-343 (McN) as M1 agonist, applied AG490 (AG) to inhibit the JAK2/STAT3 signaling. This study demonstrated: 1) p-JAK2 and p-STAT3 expression was significantly decreased in the hippocampus of *klotho* mutant mice, 2) M1 mAChR expression was significantly decreased in the hippocampus of *klotho* mutant mice, 3) AG antagonism against McN-induced effects on impaired NMDAR-dependent LTP in *klotho* mutant mice. Thus, it is possible that the *klotho* gene may be essential for maintaining cholinergic neuronal function in aging organisms, and that an M1 agonist may be useful in treating cognitive impairment associated with aging-related disorders.

Key Words: *klotho* mutant mice, JAK2/STAT3, M1 mAChR, Synaptic plasticity

IC-43

Inhibition of Cloned Kv4.3 Potassium Channels by the Antiestrogen Raloxifene

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Raloxifene, a second-generation of selective estrogen receptor modulation (SERMs), is widely used for the treatment of postmenopausal osteoporosis. The Kv4.3 current underlies the subthreshold-operating A-type Kv current in nervous systems. In the present study, we designed to characterize the effects of raloxifene on cloned Kv4.3 potassium channels. The interaction of raloxifene with cloned Kv4.3 channels stably expressed in Chinese hamster ovary cells was investigated using the whole cell patch-clamp technique. Raloxifene reduced the peak amplitude of Kv4.3 currents as well as accelerated the rate of decay of current inactivation on a concentration-dependent manner. Thus, the concentration-dependent decrease in Kv4.3 currents was assessed from the integral of the current during the depolarizing pulse. The IC_{50} value for the raloxifene block of Kv4.3 was 2.1 μM. We also studied the effects of tamoxifen and β-estrdiol, but inhibition of tamoxifen and β-estrdiol were not as strong as effect of raloxifene. The kinetics were significantly decreased in the rate of fast and slow inactivation by raloxifene at a concentration from 1 μM. The association (k_{+1}) and dissociation (k_{-1}) rate constants for the raloxifene block of Kv4.3 were 9.8 $\mu M^{-1}s^{-1}$ and 25.8 s^{-1}, respectively. The K_D (k_{-1}/k_{+1}) was 2.6 μM, similar to the IC_{50} value calculated from the concentration-response curve. The Kv4.3 inhibition by raloxifene was voltage-dependent, it was increased steeply in the full activation voltage range. Furthermore raloxifene shifted the voltage dependence of inactivation curves in the hyperpolarizing direction. Raloxifene caused a significant use-dependent block at frequencies of 1 and 2 Hz, and strongly delayed recovery from inactivation of Kv4.3 currents. The inhibitory effects of raloxifene on Kv4.3 was unaffected by the estrogen receptor antagonist ICI 182,780, indicating that these effects are independent of classical estrogen. Therefore, these results indicated that raloxifene directly blocks currents carried by Kv4.3 channels via open channel block and binding inactivation state.

Key Words: Serms, Open channel block

IC-44

Cyanidin-3-glucoside Inhibits ATP-Induced $[Ca^{2+}]_i$ Increase, ROS Formation and Mitochondrial Depolarization in PC12 Cells

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Cyanidin-3-glucoside (C3G) is a member of the anthocyanin family which is commonly present in pigmented fruits and vegetables. In addition to antioxidant effects, C3G has been reported to have neuroprotective effects. In this study, we determined whether C3G affects ATP-induced calcium signaling using digital imaging methods for Ca^{2+}, reactive oxygen species (ROS) and mitochondrial membrane potential. Treatment with 100 μM ATP for 90 s induced increases in intracellular free Ca^{2+} concentrations $[Ca^{2+}]_i$. Pretreatment with C3G (1 μg/ml to 100 μg/ml) for 30 min inhibited the ATP-induced $[Ca^{2+}]_i$ increases in a concentration-dependent manner (IC_{50}=15.45 μg/ml). Pretreatment with C3G (15μg/ml) for 30 min significantly inhibited the ATP-induced responses following removal of extracellular Ca^{2+} or depletion of intracellular $[Ca^{2+}]_i$ stores. Whereas treatment for 10 min with nimodipine (1 μM) significantly inhibited ATP-induced $[Ca^{2+}]_i$ increases, C3G further inhibited the subsequent ATP-induced responses. C3G also significantly inhibited KCl (50 mM)-induced $[Ca^{2+}]_i$ increases. Moreover, C3G significantly inhibited the mitochondrial depolarization induced by 100 μM ATP for 30 min. In addition, C3G blocked ATP-induced ROS formation. Therefore, these data suggest that cyanidin-3- glucoside inhibits ATP-induced $[Ca^{2+}]_i$ increases in PC12 cells by inhibiting both an influx of extracellular Ca^{2+} and a release of Ca^{2+} from intracellular stores. In addition, cyanidin-3-glucoside inhibits ATP-induced ROS formation and mitochondrial depolarization.

Key Words: Flavonoid, Mitochondrial membrane potential, PC12 cells, Reactive oxygen species

IC-45

Surface Expression of TTYH2 Channel is Suppressed by βCOP

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Protein tweety homolog 2 (TTYH2) was reported as Ca^{2+}-activated inwardly rectifying anion channels that is linked to renal cell carcinoma and colorectal cancer. Structurally, TTYH2 protein has five transmembrane domains with the extracellular N-terminus and the cytoplasmic C-terminus. However, it is not well known about regulatory mechanism of TTYH2 channel. In the present study, we identified a vesicle transport protein, βCOP, as a novel specific binding partner of TTYH2 by yeast two-hybrid screening using a human brain cDNA library with C-terminal region of TTYH2 (TTYH2-C) as a bait. We confirmed the protein-protein interactions of TTYH2 and βCOP in vitro. When βCOP was co-transfected with TTYH2 into COS-7 cells, both proteins were co-localized in the cytoplasm. In addition, the surface expression and activity of TTYH2 was decreased by βCOP expression. These data suggested that βCOP plays a critical role in the trafficking mechanism of TTYH2 channel.
Key Words: TTYH2, βCOP, Protein-protein interaction, Trafficking mechanism

IC-46

Block of hERG K^+ Channel by the Antipsychotic Drug Fluphenazine

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Fluphenazine is a potent antipsychotic drug that can increase action potential duration and induce QT prolongation in several animal models. Since block of cardiac human *ether-a-go-go-related* gene (*hERG*) channels is one of leading causes of acquired long QT syndrome, we investigated the acute effects of fluphenazine on hERG channels to determine the electrophysiological basis for its proarrhythmic potential. We examined the effects of fluphenazine on the hERG channels expressed in *Xenopus* oocytes using two-microelectrode voltage-clamp techniques. Fluphenazine induced a concentration-dependent decrease of the current amplitude at the end of the voltage steps and hERG tail currents. The IC_{50} of fluphenazine-dependent hERG block in *Xenopus* oocytes increased progressively relative to the degree of depolarization. Fluphenazine affected the channels in the activated and inactivated states but not in the closed states. The S6 domain mutation Y652A attenuated the hERG current block. The IC_{50} value of fluphenazine-dependent hERG block increased from 13.3 μM to 89.0 μM by the mutation from tyrosine to alanine at 652 amino acid of the channel. These results suggest that the antipsychotic drug, fluphenazine is a blocker of the hERG channels, providing a molecular mechanism for the drug-induced arrhythmogenic side effects.
Key Words: Antipsychotic drug, HERG channel, Fluphenazine, Rapidly-activating delayed rectifier K^+ channel

IC-47

Acute Alteration of Cardiac ECG, Action Potential, I_{Kr} and the hERG K^+ Channel by PCB 126 and PCB 77

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Polychlorinated biphenyls (PCBs) have been known as serious persistent organic pollutants (POPs), causing developmental delays, motor dysfunction. We have investigated the effects of two PCB congeners, 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on ECG, action potential, and the rapidly activating delayed rectifier K^+ current (I_{Kr}) of guinea pigs' hearts, and hERG K^+ current expressed in *Xenopus* oocytes. PCB 126 shortened the corrected QT interval (QTc) of ECG and decreased the action potential duration at 90% (APD_{90}), and 50% of repolarization (APD_{50}) ($P < 0.05$) without changing the action potential duration at 20% (APD_{20}). PCB 77 decreased APD_{20} ($P < 0.05$) without affecting QTc, APD_{90}, and APD_{50}. The PCB 126 increased the I_{Kr} in guinea-pig ventricular myocytes held at 36°C and hERG K^+ current amplitude at the end of the voltage steps in voltage-dependent mode ($P < 0.05$). However, PCB 77 did not change the hERG K^+ current amplitude. The PCB 77 increased the diastolic Ca^{2+} and decreased Ca^{2+} transient amplitude ($P < 0.05$), while PCB 126 was without effect. The results suggest that PCB 126 shortened the QTc and decreased the APD_{90} possibly by increasing I_{Kr}, while PCB 77 decreased the APD_{20} possibly by other modulation related with intracellular Ca^{2+}. The present data indicate that the environmental toxicants, PCBs, can acutely affect cardiac electrophysiology including ECG, action potential, intracellular Ca^{2+}, and channel activity, resulting in toxic effects on the cardiac function in view of the possible accumulation of the PCBs in human body.
Key Words: Action potential, ECG, hERG channel, PCB 126, PCB 77, Short QT syndrome

IC-48

Effects of Hydrogen Sulfide (H_2S) on Neuronal Excitability in the Afferent and Efferent Bladder Neurons of Rat

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Hydrogen sulfide (H_2S) is a gasomediator that is endogenously synthesized from cysteine by cystathionine γ lyase (CSE) and cystathionine β synthetase (CBS), and exerts its biological actions mainly through modulating different types of ion channels in the nervous system. Several studies have suggested that H_2S contributes to modulation of the urogenital functions. Recently, we have observed that bladder outlet obstruction (BOO) increases excitability of the afferent and efferent bladder neurons of rats through regulation of T-type Ca^{2+} and SK channels. Importantly, real-time PCR (RT-PCR) analysis revealed that BOO significantly up-regulates expression of CSE and CBS in dorsal root ganglion (DRG) and pelvic ganglion (PG) from rats. Thus, we examined potential roles of H_2S in modulating excitability of DiI-labeled DRG and PG neurons projecting to the bladder using the whole-cell patch-clamp technique. Repeated application of NaHS, a H_2S donor for 24 hr significantly increased excitability of the afferent and efferent bladder neurons by decreasing the rheobase and AHP duration. Thelong-term treatment of NaHS was found to increase expression of the T-type a1H Ca^{2+}, but decrease that of SK channels in DRG and PG neurons. Acute bath application of NaHS also increases T-type Ca^{2+} currents, suggesting non-genomic effects of H_2S. In addition, we found that H_2S selectively regulates the a1 isoform of T-type Ca^{2+} channel stably expressed in HEK 293 cells. Taken together, these data suggest that H_2S is a potential molecular candidate for mediating BOO-induced increase in excitability of the afferent and efferent bladder neurons.

Keywords: Hydrogen sulfide (H_2S), $Ca_V3.2$ T-type Ca^{2+} channel, Bladder outlet obstruction (BOO), Pelvic ganglion (PG), Dorsal root ganglion (DRG)

IC-49

Functional Plasticity of the Efferent and Afferent Bladder Neurons in Rats with Benign Prostatic Hyperplasia

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Benign prostatic hyperplasia (BPH), a common problem affecting middle-aged and elderly men, is characterized by overgrowth of the prostatic tissue surrounding the urethra which causes lower urinary tract symptoms (LUTS) such as urgency, frequency, nocturia, incomplete bladder emptying, and weak urine flow. Overactive bladder (OAB) is a syndrome characterized by urinary urgency with frequency and nocturia. To date, neurogenic mechanisms underlying OAB remain incompletely understood. The hypothesis of the present study is that OAB is associated with functional changes in major pelvic ganglion (MPG) and dorsal root ganglion (DRG) neurons projecting to the bladder. To prove the hypothesis we examined whether BPH alters expression and activity of the nicotinic acetylcholine receptors (nAChRs) involved in ganglionic transmission, and excitability of the bladder MPG and DRG neurons. Toward this end, experimental BPH were induced by sc injection of testosterone/17-β-estradiol in rats. The bladder muscles were hypertrophied in BPH rats. Cystometry revealed that BPH increased bladder capacity, threshold pressure for voiding, micturition volume, residual volume after voiding, and intermicturition oscillatory frequency, indicating development of OAB. Real-time PCR analysis showed that the nAChRs α3 and β4 subunits were up-regulated in the MPG neurons of BPH rats. Consistent with the molecular data, the nAChR currents were significantly increased in DiI-labeled parasympathetic MPG neurons projecting to the bladder detrusor muscles. Under the current-clamp mode of the patch-clamp technique, action potentials were recorded in the bladder DRG and MPG neurons of control and BPH rats. BPH produced hyperexcitability of the bladder DRG and MPG neurons via reducing rheobase and AHP duration. The ionic mechanisms underlying the hyperexcitability include up-regulation of T-type Ca^{2+} and SK channels expressed in the bladder DRG and MPG neurons. In conclusion, BPH-induced OAB is associated with enhanced ganglionic transmission and/or hyperexcitability of the afferent and efferent neurons projecting to the bladder.

Keywords: Nicotinic Acetylcholine Receptors (nAChRs), $Ca_V3.2$ T-type Ca^{2+} Channel, Overactive Bladder (OAB), Pelvic Ganglion (PG), Dorsal root ganglion (DRG)

IC-50

Factors Altered TREK Channels Expression in Rat Dorsal Root Ganglia

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TREK channels (TREK-1 and TREK-2), members of two-pore domain K^+ (K2P) channels, are responsible for setting the resting membrane potential in many kinds of cells including dorsal root ganglia (DRG) neurons. However, their expression levels in DRG neurons are controversial among researchers. The aim of this study is to find factors that may regulate expression of TREK channels in rat DRG neurons. TREK-1 mRNA and protein expression levels were down-regulated in DRG isolated from neonatal rat compared with those in adult. TREK-2 expression levels were vice versa. TREK-1 expression levels were decreased in the presence of nerve growth factor (NGF), whereas TREK-2 expression levels were increased by NGF treatment. The cloned heterodimers of TREK-1 constructed with TREK-2 (TREK-1/TREK-2) showed single channel conductance similar to that of TREK-2 in HEK293A cells. These results show that different stages of culture con-

ditions, dimerization, and expression pattern give rise to changes in TREK expression in the DRG neurons.
Key Words: Dimerization, Dorsal root ganglia, Nerve growth factor, TREK channel

IC-51

Biophysical Properties of *KCNQ1* Mutation Associated with Atrial Fibrillation and Bradycardia

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Background: Loss-of-function mutations in KCNQ1 gene have been associated with long QT syndrome, whereas gain-of-function mutations have been implicated in atrial fibrillation (AF) and short QT syndrome. However, the presence of both AF and bradycardia in the same family is poorly characterized. **Objective:** The purpose of this study was to identify a mutation in KCNQ1 gene and to characterize a novel KCNQ1 variant in a Korean family with both AF and bradycardia. **Methods:** Mutation analysis of the KCNQ1 gene was performed by Sanger sequencing. In order to characterize the physiological consequences of a novel KCNQ1 variant, HEK293 cells were transfected with wild-type (WT) or mutant KCNQ1 subunits, with KCNE1. **Results:** A novel variant (c.721G>T; V241F) in the KCNQ1 gene was identified in a woman with AF. Interestingly, her two sons with the same variant had sinus bradycardia and AF, respectively. Functional analysis of the novel variant revealed a gain-of-function effect on the IKs. Under conventional voltage clamp the V241F- KCNQ1 shifted voltage-dependent activation by ~-86 mV, with a marked instantaneous current component evident on membrane depolarization. Thus, the V241F is likely to lead to AF by reducing action potential duration and effective refractory period in atrial myocytes, or slow pacemaker activity in the sinoatrial node. **Conclusion:** We speculate that V241F could be a pleiotropic missense mutation capable of inducing different clinical manifestations including AF and bradycardia.
Key Words: *KCNQ1*, Atrial fibrillation, Bradycardia

IC-52

Identification of Possible Molecular Entity for Fluid Pressure-Gated Cl^- Current in Rat Atrial Myocytes Using Pharmacological Interventions

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We have previously reported that fluid pressure (FP, ≈16 dyne/cm^2) activates DIDS/9-AC sensitive Cl^- current in rat atrial myocytes (Kor J Physiol Pharmacol 2011;13:S86a). This current has been proposed to be partly responsible for the depolarization of diastolic membrane potential during FP stimulation. To know the molecular entity for the FP-gated Cl^- current we examined the effects of known Cl^- channel inhibitors on this current using whole-cell patch clamp in isolated rat atrial myocytes. Pressurized fluid flow (~16 dyne/cm^2) was rapidly applied onto the entire surface of single myocytes. To minimize nonselective cation current, external Na^+ and K^+ were replaced by equimolar $NMDG^+$, and external Ca^{2+} was removed. Low Cl^- K-rich internal solutions containing 4 mM EGTA were dialyzed into the cells, where ramp pulse currents were studied. Under these conditions, FP-gated current was shown as a large outwardly rectifying current with an inward rectifying component. Tamoxifen (0.01 mM) that blocks the volume-regulated outward rectifying Cl^- channel inhibited the outward I_{FP} by ~92% and slightly reduced the inward I_{FP}. The tamoxifen-resistant inward I_{FP} was eliminated by 1 mM external Zn^{2+}, the inhibitor of volume-regulated inward rectifying Cl^- channel. The I_{FP} was not altered by the exposure to *N*-(p-amylcinnamoyl)anthranilic acid (ACA), the blocker of Ca^{2+}-activated Cl^- channels. The blocking effects of DIDS and 9-AC on I_{FP} were similar. These results suggest that the FP-gated Cl^- currents may be mediated by two pharmacologically distinct volume-regulated Cl^- channels.
Key Words: Fluid pressure, Atrial myocytes, Cl^- current, Volume-regulated outward rectifying Cl^- channel, Volume-regulated inward rectifying Cl^- channel

MP-1

Exendin-4 Inhibits iNOS Expression at the Protein Level in LPS-Stimulated Raw264.7 Macrophage by the Activation of cAMP/PKA Pathway

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Glucagon-like peptide-1 (GLP-1) and its potent agonists have been widely studied in pancreatic islet β-cells. However, GLP-1 receptors are present in many extrapancreatic tissues including macrophages, and thus GLP-1 may have diverse actions in these tissues and cells. Therefore, we examined the mechanism by which exendin-4 (EX-4), a potent GLP-1 receptor agonist, inhibits lipopolysaccharide (LPS)-induced iNOS expression in Raw264.7 macrophage cells. EX-4 significantly inhibited LPS-induced iNOS protein expression and nitrite production. However, Northern blot and promoter analyses demonstrated that EX-4 did not inhibit LPS-induced iNOS mRNA expression and iNOS promoter activity. Electrophoretic mobility shift assay (EMSA) showed that EX-4 did not alter the binding activity of NF-κB to the iNOS promoter. Consistent with the result of EMSA, LPS-induced IκBα phosphorylation and nuclear translocation of p65 were not inhibited by EX-4. Also, actinomycin D chase study and the promoter assay using the construct containing 3'-untranslated region of iNOS showed that EX-4 did not affect iNOS mRNA stability. Meanwhile, cycloheximide chase study demonstrated that EX-4 significantly accelerated iNOS protein degradation. The EX-4 inhibition of LPS-induced iNOS protein was significantly reversed by adenylate cyclase inhibitors (MDL-12330A and SQ 22536), a PKA inhibitor (H-89) and PKAα gene silencing. These findings suggest that EX-4 inhibited LPS-induced iNOS expression at protein level, but not at transcriptional mechanism of iNOS gene and this inhibitory effect of EX-4 was mainly dependent on cAMP/PKA system.

Key Words: Exendin-4 (EX-4), iNOS, NF-κB, cAMP/PKA, Raw264.7 macrophage

MP-2

Docosahexaenoic Acid Up-Regulates eNOS Activation through Promoting Sirt1 Expression in Endothelial Cell

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Docosahexaenoic acid (DHA) and Sirt1 is well known to prevent vascular endothelial dysfunction and associated cardiovascular disorders. Also, DHA and Sirt1 play essential roles in promoting production of nitric oxide (NO) and activation of endothelial nitric oxide (eNOS) in the vascular system. However, the relationship between DHA and Sirt1 in the context of endothelial function is unknown. In this study, we report the a novel DHA mediated mechanism that regulates Sirt1 expression and eNOS activation in endothelial cell. Human umblical vein endothelial cells (HUVECs) were cultured in the presence of DHA or eicosapentaenoic acid (EPA) for 24 h. We first confirmed DHA and EPA did not have the cytotoxicity in concentration of 100 nM - 30 uM of both in HUVECs. DHA significantly increased the Sirt1 protein and RNA expression dose-dependently in HUVECs. In contrast, EPA did not affect the Sirt1 protein and RNA expression in HUVECs. DHA up-regulated Sirt1 promoter activity in HUVECs after transfection of Sirt1 promoter construct but EPA did not. Furthermore, DHA deacetylated eNOS stimulating eNOS activity and NO production. DHA-induced increase in endothelial NO is mediated through Sirt1 activation and eNOS deacetylation. However, inhibition of Sirt1 in the endothelial cell inhibits eNOS activation and NO production. Our results demonstrated that DHA plays a fundamental role in regulating eNOS activation and endothelial NO by up-regulation of Sirt1.

Key Words: DHA, EPA, eNOS, Sirt1, NO

MP-3

Detection of Plasma APE1/Ref-1 in Lipopolysaccharide-treated Rats

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Apurinic/apyrimidinic endonuclease1/Redox factor-1 (APE1/Ref-1) is a multifunctional protein involved in base excision DNA repair and in transcriptional regulation of gene expression. APE1/Ref-1 is known to be regulated at both the transcriptional and translational levels, and then be controlled subcellular localization by several signaling. In this present study, we investigated the possibility of extracellular release of APE1/Ref-1. Especially, we tried to show the detection of plasma APE1/Ref-1 in the endotoxemic rats. Endotoxemic animal models were generated with intraperitoneal injection of lipopolysaccharide (LPS) in Sprague Dawley rats, which was confirmed with up-regulation of iNOS mRNA, protein, and increased nitric oxide production. An immune-reactive band of 37 kDa with anti-APE1/Ref-1 antibody was detected in cell-free plasma of LPS-treated rats. The plasma APE1/Ref-1 was initially detected at 3 h, reached the maximum at 6-12h and tends to return to the basal level at 18 h. To quantify plasma APE1/Ref-1, recombinant human APE1/Ref-1 was use as standard in immunoblot analysis, which was showed the linearity with amount of rhAPE1/Ref-1 at the range of 0.2-1.0ng. The highest plasma APE1/Ref-1 showed in LPS-treated rats was about 117ng/ml. Molecular weight of hepatic APE1/Ref-1 showed in immunoblot is not changed by LPS exposure, suggesting that secreted APE1/Ref-1 might be decomposes in the plasma after secretion. In LC-MS/MS, an immune-reactive band of 37 kDa was identified as rat APE1/Ref-1. Interestingly, the recombinant APE1/Ref-1 protein inhibited TNF-a-induced VCAM-1,

ICAM-1 and COX-2 expression in HUVEC. Collectively, we demonstrated for the first time that APE1/Ref-1 is clearly detected in the plasma of LPS-treated rat, implying the role of regulation in inflammatory signals. Therefore, APE1/Ref-1 might be used as serological biomarker such as systemic inflammatory disease.
Key Words: APE1/Ref-1, LPS, Secretion, HUVEC, Inflammatory disease

MP-4(PO-7)

SHP-2 Binds to Caveolin-1 and Regulates Src Activity via Competitive Inhibition of Csk in Response to H_2O_2 in Astrocytes

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Reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals, are well known as signal regulatory molecules in the brain and can lead to the development of diverse neuropathological conditions. Recently, we have reported that SHP-2 is translocated and activated in response to H_2O_2, and that caveolin-1 is involved in the ROS-induced SHP-2 activation. However, the function of SHP-2 is poorly understood at present. In the present study, we showed that caveolin-1 interacted with SHP-2 in response to H_2O_2. Using co-immunoprecipitation assays, we found that SHP-2, like Csk, bound only to the wild-type caveolin-1 with phosphorylated tyrosine and not to the phosphorylation-deficient mutant of caveolin-1 (Y14A). Next, we conducted an in situ proximity ligation assay (PLA) to examine the interaction among caveolin-1, SHP-2, and Csk in response to H_2O_2. Using peptide arrays, we found that both SHP-2 and Csk bound specifically to the phosphorylated caveolin-1 but not the unphosphorylated caveolin-1. In the presence of Csk siRNA, compared to GFP siRNA, the binding between caveolin-1 and SHP-2 was enhanced by H_2O_2 treatment, leading to reduced Src phosphorylation at tyrosine 527 (Tyr 527) and enhanced Src phosphorylation at tyrosine 416 (Tyr 416). In contrast, siRNA targeting SHP-2 facilitated H_2O_2-mediated binding between caveolin-1 and Csk, resulting in an inverse effect on Src phosphorylation at Tyr 527 and Tyr 416. Collectively, our results indicate that in the presence of H_2O_2, SHP-2 bound to caveolin-1 and regulated Src activation through the competitive inhibition of Csk in astrocytes.
Key Words: Astrocyte, SHP-2, Caveolin-1, Oxidative Stress

MP-5

PX-12 Inhibits the Growth of Lung Cancer Cells via Cell Cycle Arrest and Apoptosis

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1-methylpropyl 2-imidaxolyl disulfide, PX-12 as a thioredoxin (Trx) inhibitor has an antitumor effect in various cancer cells, which is currently in clinical development. In this study, we investigated the anti-growth effects of PX-12 on A549 and Calu-6 lung cancer cells in relation to reactive oxygen species (ROS) and glutathione (GSH) levels. PX-12 inhibited the growth of A549 and Calu-6 cells with an IC_{50} of approximately 30 μM and 5 μM at 72 hours, respectively. DNA flow cytometric analysis indicated that PX-12 induced a G2/M phase arrest in the cell cycle in A549 and Calu-6 cells. This agent also induced apoptosis, which was accompanied by caspase-3 activation and the loss of mitochondrial membrane potential (MMP; $\Delta\Psi_m$). The susceptibility of Calu-6 cells to PX-12 was higher than that of A549 cells. In relation to ROS and GSH levels, PX-12 increased ROS levels including $O_2^{\cdot-}$ and induced GSH depletion in A549 and Calu-6 cells. N-acetyl cysteine (NAC; a well-known antioxidant) attenuated apoptotic cell death in PX-12-treated lung cancer cells. Unexpectedly, L-buthionine sulfoximine (BSO; an inhibitor of GSH synthesis) did not affect lung cancer cell death induced by PX-12. In conclusion, PX-12 inhibited the growth of A549 and Calu-6 lung cancer cells via a cell cycle arrest as well as apoptosis, which was related to ROS. This work was supported by a grant from the Ministry of Science & Technology (MoST)/Korea Science & Engineering Foundation (KOSEF) through the Diabetes Research Center at Chonbuk National University (2010-0029497) and the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (2010-0021808).
Key Words: PX-12, Cell cycle, Apoptosis, Thioedoxin

MP-6

8-Bromo cAMP Regulates Migration of Mouse Embryonic Stem Cells by Actin Reorganization or Stabilization through cdc42/Rac1 Signaling Pathways

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Aim: To investigate the effect of 8-Br AMP on Cx43 regulation and its related signal cascade in embryonic stem (ES) cells. **Matrials and Methods:** Mouse ES cells were obtained by American Type Culture Collection (ES-E14TG2a). Mouse ES cells were cultured in DMEM without feeder layer plus LIF. Immunofluorescence staining. Scrape Loading/Dye Transfer assay. Confocal microscope image. Western blotting analysis. **Results and Conclusion:** 8-Bromo cAMP increased phospho-Cx43 and decreased Cx43 protein level in plasma membrane compartment. Moreover, 8-Bromo

cAMP inhibited transfer of Lucifer yellow to neighboring cells. Thus, we further examined the molecular mechanisms responsible for the phosphor-Cx43 by 8-Bromo cAMP. 8-Bromo cAMP stimulated Rac1/Cdc42 signaling pathways through PKA and Epac. In addition, 8-Bromo cAMP induced increase of MLC phosphorylation and Arp3, TOCA, PAK, and N-WASP protein expression, which were inhibited by cdc42 siRNA. Furthermore, 8-Bromo cAMP-induced Cx43 phosphorylation and Cx43 protein endocytosis disrupted interaction of Cx43 with tight junctional proteins or adherent junctional proteins in plasma membrane compartment, which were mediated by Rac1/Cdc42 signaling pathways. This suggests that Cx43 structural changes are contributed to ES cell junctional formation. Furthermore, 8-Bromo cAMP-induced Cx43 phosphorylation and Cx43 protein endocytosis disrupted interaction of Cx43 with tight junctional proteins or adherent junctional proteins in plasma membrane compartment, which were mediated by Rac1/Cdc42 signaling pathways. This suggests that Cx43 structural changes are contributed to ES cell junctional formation.

Key Words: cAMP, Migration, Cx43, Gap Junction

MP-7

Sildenafil Alleviates Bronchopulmonary Dysplasia in Neonatal Rats by Activating the Hypoxia-Inducible Factor Signaling Pathway

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Bronchopulmonary dysplasia (BPD) is a major cause of morbidity in premature babies given oxygen therapy. Currently, sildenafil is being examined clinically to improve pulmonary function in BPD patients. Based on the pharmacological action of sildenafil, cGMP elevation in lung tissue is considered to underlie its beneficial effects, but this mechanism is not understood at the molecular level. Here, we examined the possibility that sildenafil helps the pulmonary system adapt to hyperoxic stress. To induce BPD, fetal rats were delivered earlier using an intra-amniotic injection of LPS, and premature neonates were exposed to hyperoxia, followed by intra-peritoneal injections of sildenafil. Alveolarization was impaired in rats exposed to hyperoxia, and this was significantly recovered by sildenafil. An immunohistochemical examination revealed that sildenafil effectively increased vascular distribution in lungs. Furthermore, the oxygen sensor HIF-1/2α and the angiogenic factor VEGF were highly expressed in the lungs of sildenafil-treated rats. In human small airway epithelial cells, HIF-1/2α and its downstream genes, including VEGF, were confirmed to be induced by sildenafil at both the protein and mRNA levels. Mechanistically, cGMP in airway cells was accumulated after sildenafil treatment due to interfering phosphodiesterase type 5 (PDE5), and subsequently cGMP activated HIF-mediated hypoxic signaling by stimulating the PI3K-Akt-mTOR pathway. This study provides a better understanding about the mode-of-action of sildenafil, and suggests that HIF can be a potential target for treating BPD patients.

Key Words: BPD, Sildenafil, AKT/mTOR pathway, HIF-1α, VEGF

MP-8(PO-8)

G-Protein Regulatory (GPR) Motif Modulates SDF1α-Induced *MUC*1 Gene Expression and Regulates Airway Inflammation

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Mucus overproduction and airway obstruction are common features in airway mucosal inflammation. The mechanism by which SDF1α induces *MUC1* overexpression, however, has not been fully explored. The aims of this study were two-fold; firstly, to examine the Activator of G-protein signaling (AGS) 3-dependent mechanism by which SDF1α reduces *MUC1* gene expression, and secondly, to identify specific molecules which could suppress SDF1α-induced MUC5AC expression at a G-protein coupled receptor level. Here, we suggest that SDF1α induces *MUC1* gene expression via CXCR4 receptor. In addition, we showed that AGS3 plays as a suppressor for SDF1α-induced *MUC1* and *TNF*α gene expressions by regulating with Gαi. More interestingly, G-protein Regulatory (GPR) motif in AGS3 bound to Gαi and decreased MUC1 expression, whereas increased TNFα expression. In addition, GPR mutation (DDQR→DDAR) increased MUC1 expression, but decreased TNFα, IL-6, and IL-8 expressions. We suggest that GPR mutation peptide play as suppressive compound to decrease airway inflammation. These results give additional insights into the molecular mechanism of negative regulation of mucin overproduction and enhance our understanding of mucus hypersecretion during airway mucosal inflammation.

Key Words: Inflammation, SDF1, AGS, GPR motif, Mucin 1

MP-9

Paclitaxel Plus Doxorubicin Suppress Growth of Human Esophageal Squamous Cancer Cells by G2 Cell Cycle Arrest

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Despite of paclitaxel and doxorubicin are widely used as chemotherapy agents against to variety of cancers, their

combination effects on esophageal squamous cell carcinoma (ESCC) has not been clearly elucidated. Thus the present study was set out to investigate biological effects of paclitaxel and doxorubicin in ESCC cells (TE-8 and TE-12). Paclitaxel plus doxorubicin significantly inhibited the proliferation of TE-8 and TE-12 cells in a dose-and time-dependent manner compared to when paclitaxel or doxorubicin is treated alone. FACS analysis showed that the percentages of cells in the G2 phase were significantly increased 12 h after being treated with paclitaxel plus doxorubicin. And paclitaxel plus doxorubicine increased phosphorylated cdc2 and phosphorylated Wee1 protein levels and decreased p21 levels. In addition, paclitaxel plus doxorubicin significantly induced cleaved poly (ADP-ribose) polymerase and cleaved caspase 7 and 9 levels. Therefore, these results suggest that the combination of paclitaxel and doxorubicin induces G2/M cell cycle arrest and apoptosis by activating caspase-7 in ESCC cells.
Key Words: Paclitaxel, Doxorubicin, Esophageal squamous cell carcinoma, G2 arrest

MP-10

OXPHOS Complex Dysfunction in Endothelial Cell Causes ROS Production and Endothelial Cell Dysfunction

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Endothelial dysfunction is well known to play an essential role vascular disease and decrease NO production. Oxidative stress has emerged as the major contributing factor in endothelial dysfunction. Mitochondria dysfunction also induces oxidative stress and endothelial dysfunction. However, the key mechanism of mitochondria dysfunction induced endothelial dysfunction is not clear. To determine the role of mitochondria of endothelial cell, we performed down-regulation of CRIF1 which was done with transient transfection of human umbilical vein endothelial cells (HUVECs) with short interfering CRIF1 (siCRIF1), because CRIF1 plays a critical role in integration of OxPhos ploypeptides into the mitochondrial membrane in mammals. Down-regulation of CRIF1 decreased the mitochondrial membrane potential (MMP) and increased the mitochondrial ROS, measured by staining the cells with TMRE and MitoSox dye respectively and measuring the relative fluorescence intensity. In addition to, down-regulation of CRIF1 increase in phosphorylation of P66Shc causing a further rise in ROS. Importantly, eNOS activation as well as the expression of SIRT1 was found to be decreased in CRIF1 siRNA trasfected cell indicative of endothelial dysfunction. Taken together, these results suggest that OxPhos complex plays an important role in the maintenance of mitochondrial function and indirectly endothelial function.
Key Words: OxPhos complex, CRIF1, P66Shc, eNOS, Mitochondrial dysfunction

MP-11

rhBMP2 Inhibits Growth of Esophageal Cancer Cells through Hippo Signaling Pathway

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Despite of recombinant human bone morphogenetic protein-2 (rhBMP-2) has been reported as a stimulatory effecter on cancer cell growth because of its characteristics like morphogen, the biological functions of rhBMP-2 in human esophageal cancer cells are unknown. To investigate whether rhBMP-2 has the inhibitory effect to the growth of human esophageal squamous carcinoma cells (ESCC), three human ESCC cell lines (TE-8, TE-12 and TT) were used to test the response to rhBMP-2. rhBMP-2 significantly inhibited the proliferation of ESCC cells in a dose-dependent manner (0, 10 nM, 250 nM, 500 nM and 1 ?M) by MTT assay. Cell cycle arrest at the G1 phase was induced 24 h after being treated with rhBMP2. rhBMP-2 also reduced cyclin D_1, cyclin-dependent kinase (CDK) 4 and CDK 6 activities, and stimulated p-Smad1/5/8, p53, and p21 levels at 12h. Meanwhile, rhBMP-2 diminished PARP protein expression levels and activated cleaved PARP, cleaved casepase-7, cleaved-casepase 9 levels in ESCC cells. In addition, rhBMP-2 increased MST1, MOB1, p-LAS1 protein levels and decreased p-YAP protein level. Therefore, these results suggest that rhBMP-2 may function as a tumor suppressor in human esophageal cancer mediated through activating of hippo signaling pathway.
Key Words: Human esophageal cancer cell, BMP-2, Cell cycle, Hippo signaling pathway

MP-12(PO-9)

Enhanced Formation of Vascular Neointima in DJ-1 Knockout Mice is Involved in Hydrogen Peroxide-Stimulated S1P1 Receptor Activation

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Sphingosine-1-phosphate (S1P), an active lipid mediator, exerts biological effects such as cell proliferation and migration in a variety of cell types including vascular smooth muscle cells (VSMCs). DJ-1/Park7 is known as multifunctional protein involved in several signal transduction pathways that plays essential roles in biological functions in

mammalian cells. However, interaction between S1P and DJ-1/Park7 protein in atherosclerosis has not been reported. Here, we investigated the involvement of S1P and DJ-1/Park7 in pathogenesis of atherosclerosis by using DJ-1 knockout (KO) mice. VSMCs from DJ-1 KO mice showed the high levels of H2O2 generation compared with those from normal control. VSMCs deficient in DJ-1 expressed higher levels of S1PR1 mRNA than normal control. In functional test, VSMC migration and proliferation in response to S1P increased in DJ-1 KO compared with normal control mice. Moreover, vascular neointimal formation by the ligation of carotid artery also showed predominant increment in DJ-1 KO mice. Our data demonstrate a possibility that S1P may be linked to enhanced vascular neointimal formation in DJ-1/Park7 KO mice.
Key Words: DJ-1/Park7, Sphingosine 1-phosphate, Sphingosine 1-phosphate receptor 1, Proliferation, Migration

MP-13

Physiology of Taste Receptors in Salivary and Other Exocrine Glands

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Taste is the one of the most important sense for the survival of an organism. We will search in salivary and other exocrine glands and will elucidate the physiological roles of those probable receptors. Using microarray analysis, the presence of taste receptors and relating signal proteins such as phospholipase $C\beta_2$ (PLCβ2) in submandibular glands, sublingual glands, lacrimal glands, and pancreas of DBA2 mouse were examined. The RT-PCRs was performed in PLCβ2, 3 of type I taste receptors (T1Rs) as well as 35 of type II taste receptors (T2Rs). Gene microarrays revealed that the expression of PLCβ2 as well as T1Rs and T2Rs. The expression patterns of T2Rs in exocrine glands, examined by RT-PCR were not similar to each other. These results suggest that taste receptors and related-signalling proteins were expressed in the exocrine glands and they may play physiological roles.
Key Words: RT-PCR, Exocrine glands, Taste receptor

MP-14

Gas6/Mer Complex Leads Transcriptional Production of HGF through the RhoA-Dependent Pathway and Epithelial Wound Repair

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Recent studies demonstrated Gas6 signaling modulates cytokine secretion and helps to regulate the immune response nad tissue repair. The present study was designed to confirm Gas6/Mer complex-mediated signaling pathways leading to HGF expression. Additionally, the functional consequences of activation of Mer and induction of HGF in macrophages upon exposure to Gas6 were evaluated by examining Gas6-mediated epithelial wound repair. Gas6 or apoptotic cells enhanced phosphorylation of Mertk with a peak at 5 min after exposure. Gas6 increased HGF mRNA and protein expression in a dose-dependent manner, and HGF expression peaked at 2 h after exposure. Gas6 activated RhoA, Akt, and specific MAP kinases, including p38 MAP kinase, ERK, and JNK with the peak at 15 min after exposure. Down-regulation of the RhoA/Rho kinase pathway by pharmacological inhibitors or RhoA-specific siRNA suppressed Gas6-induced phosphorylation of Akt and the MAP kinases and HGF mRNA expression. Inhibition of PI3K by decreased phosphorylation of the MAP kinases. The pharmacological inhibitors of PI3K and the MAP kinases blocked Gas6-induced HGF mRNA. A Mertk-blocking antibody inhibited Gas6-induced HGF mRNA and protein expression, RhoA activity, and phosphorylation of Akt and MAP kinases. A HGF receptor-blocking antibody and c-Met inhibitor into macrophages conditioned medium from RAW 264.7 cells had been exposed to Gas6 or apoptotic cells caused an enhancement of wound closure. Anti-Mer antibody, anti-HGF anti-body or c-Met inhibitor blocked epithelial wound repair induced by conditioned medium derived from the RAW 264.7 cells after stimulation with Gas6 or apoptotic cells. Our data provide evidence that Gas6/Mertk complex triggers activation of RhoA/PI3K/MAP kinase pathway lead to transcriptional HGF production, that promotes growth of epithelial cells.
Key Words: Gas6/Mertk complex, HGF, RhoA/PI3K/MAP kinase pathway, Epithelial wound repair

MP-15

Regulation of CREB Phosphorylation by IP3 Receptor-Mediated Nuclear Signaling in Ventricular Myocytes

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The calcium sensitive transcription factors are implicated in cardiac development and cellular remodeling associated with cardiac disease. Although nuclear Ca^{2+} increase has been suggested to control the cAMP response element binding protein (CREB)-mediated gene expression neither the source of Ca^{2+}, nor the role of nuclear channels is clear. We tested possible role of inositol 1,4,5-triphosphate receptors (IP_3Rs) on the regulation of CREB activity in isolated adult rat ventricular myocytes, and examined signal transduction for the activation of CREB during the exposures to IP_3 generating hormones, endotheline-1 (ET-1)

and phenylephrine (PE). ET-1 (10 nM) and PE (100 μM) largely enhanced CREB phosphorylation via ET_A receptor and α_1-adrenergic receptor, respectively, which was dependent on extracellular and intracellular Ca^{2+} concentrations. Inhibitions of IP_3Rs, ERK1/2, CaMKII, p38, protein kinase C (PKC) or phospholipase C (PLC) using their specific blockers inhibited ET-1- or PE-induced CREB phosphorylation. ET-1 and PE both enhanced CaMKII and ERK1/2 phosphorylation through the IP_3Rs. The ERK phosphorylation was independent of CaMKII or intracellular global Ca^{2+} increase, but sensitive to PKC inhibitors. Exposures to a protein kinase A (PKA) activator and to the inhibitor of PI3 kinase and PKA showed no changes on both ET-1- and PE-induced CREB activation. Confocal calcium imaging revealed that the ET-1 increased nuclear Ca^{2+} concentration which was inhibited by 2-APB, the IP3R blocker. These results suggest that sequential signaling, mediated by PLC, IP_3R, Ca^{2+}, and CaMKII, causes CREB phosphorylation during ET_A receptor and α_1-adrenergic receptor stimulation in rat ventricular myocytes, and that a different pathway involving PKC and ERK also affects the CREB activation.

Key Words: CREB phosphorylation, Ventricular myocytes, IP3R, CaMKII, Nuclear Ca^{2+}

MP-16(PO-10)

Type 1 Angiotensin II Receptor-NADPH Oxidase-Type 2 Angiotensin II Receptor Axis Mediates Angiotensin II Stimulation of Neuronal Nitric Oxide Synthase in Murine Left Ventricular Myocyte

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Accummulating evidence indicate that increased protein expression and activity of neuronal nitric oxide synthase (nNOS) in hypertrophic or failing myocardium are important protective mechanisms in reversing oxidative stress and contractile dysfunction. Recently, we have shown that a potent pathogenic stimulator, angiotensin II (Ang II) stimulates cardiac nNOS, which in turn, suppresses the activity of NADPH oxidase, reduces intracellular reactive oxygen species (ROS) and facilitates myocyte relaxation. Here, we investigated the mechanisms mediating Ang II regulation of nNOS in rat left ventricular (LV) myocytes. Our results showed that Ang II (1 μM, 3 hrs) significantly increased the protein expression and the activity of nNOS in LV myocyte homogenates. Both type 1 and type 2 Ang II receptor (AT1R, AT2R) antagonists, losartan and PD123319 blocked Ang II stimulation of nNOS, whereas AT2R agonist, CGP 42112A increased nNOS, suggesting a role of AT2R. Furthermore, inhibition of NADPH oxidase (apocynin) or reducing intracellular ROS (4,5- dihydroxy-1,3-benzenedisulfonic acid, tiron) prevented Ang II-stimulation of nNOS mRNA, protein and activity, indicating that AT1R-NADPH oxidase-ROS axis is the upstream regulator of nNOS. Ang II induced the translocation of AT2R to the plasma membrane (surface membrane biotinylation) within 20 min. Interestingly, losartan, apocynin or tiron pre-treatment blocked Ang II-induced AT2R translocation. Importantly, Ang II facilitated LV myocyte relaxation. Selective nNOS inhibitor, S-methyl-L-thiocitrulline (SMTC), losartan, PD123319, apocynin or tiron abolished nNOS-mediated faster relaxation by Ang II. These results reveal, *for the first time*, that Ang II promotes nNOS protein expression and activity *via* AT1R-NADPH oxidase-AT2R axis in murine left ventricular myocytes.

Key Words: Angiotensin II, NADPH oxidase, ROS, nNOS, NO

MP-17

Secretion of Acetylated APE1 in Trichostatin A treated HEK293A

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Apurininc/apyrimidic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multi-functional protein which is regulated in subcellular localization via several localization signals. We hypothesized that APE1/Ref-1 could be secreted by the post-translational modification, such as intracellular acetylation. HEK293 cells were incubated with trichostatin A (TSA), an inhibitor of histone deacetylase, for various concentration and periods. In some experiments, the cells were transfected with flag-APE1/Ref-1, EGFP-tagged APE1/Ref-1. APE1/Ref-1 in cell culture supernatants was detected by immunoblot after immunoprecipitation or acetone precipitation. The translocation of nuclear EGFP-APE1/Ref-1 into cytoplasm was visualized under fluorescent microscopy. The secreted EGFP-APE1/Ref-1 was also quantified with fluorometer. TSA treatment sustained intracellular acetylation, which was assessed with anti-acetylated lysine antibody. Although undetectable at basal state, APE1/Ref-1 was present in supernatant of p300-transfected cells, and TSA treatment induced an increase of the amount of secreted APE1/Ref-1. By using acetone precipitation, we confirmed that APE1/Ref-1 in supernatants was increased by the exposure with TSA (1 mM) within 30 min and persisted its level in 12 h. The amount of APE1/Ref-1 in supernatants was increased in a dose-dependent manner without any change of cell viability. Acetylation at the N-terminal K6/K7 residues of APE1/Ref-1 is one of a post-translational modification. Furthermore, double lysine mutants of APE1/Ref-1 (K6R/K7R) were not affected by TSA, suggesting APE1/Ref-1 secretion is required the acetylation at K6/K7 residues of APE1/Ref-1. Taken together, these data demonstrate that APE1/Ref-1 can be secreted into the supernatant in response to intracellular acetylation in HEK293 cells. The detection of APE1/Ref-1 in supernatants or extracellular medium would be very helpful to understand the cellular signals or pathophysiology of diseases.

Key Words: APE1/Ref-1, Secretion, Acetylation, Trichostatin A

MP-18

GAS6 Attenuates TLR Induced Inflammation through LXR Activation in Macrophages

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Mer receptor tyrosine kinase (Mer) is both an important mediator of apoptotic cell clearance (efferocytosis) and a regulator of macrophage cytokine production. Liver X receptor (LXR) is a key regulator of inflammation and cholesterol homeostasis. LXR activation dampens induction of inflammatory genes in response to various stimuli and positively regulates expression of anti-inflammatory genes. We examined whether Mer plays a role in reducing TLR-induced inflammation through increased liver X receptor (LXR) activation *in vitro.* Treatment with Gas6, a Mer ligand, enhanced mRNA and protein expression of LXRα/β and its target molecules dose dependently in murine peritoneal macrophages and RAW 264.7 cells. Neutralizing antibody or siRNA of Mer inhibited Gas6-induced LXRα/β activation in RAW 264.7 cells. Furthermore, Gas6 inhibited TLR-induced mRNA expression of proinflammatory mediators, including TNF-α, MIP-2, and IL-1β in RAW 264.7 cells. Specific siRNA of LXRα increased both zymosan-triggered production of TNF-α, MIP-2, and IL-1β and failed to reduce zymosan-induced cytokine releases in presence of Gas6 in RAW 264.7 cells. These data suggest that Gas6-induced Mer activation may dampen TLR-induced inflammation through LXRα/β activation in murine macrophages. This work was supported by the National Research Foundation (NRF) Grant funded by the Korean government (MEST) (2010-029353).
Key Words: Mer receptor tyrosine kinase, Liver X receptor, Inflammation, Gas6, Macrophages

MP-19

Mer Receptor Tyrosine Kinase Upregulates Liver X Receptor Signaling and Modulates Inflammatory Cytokines during Acute Systemic Inflammation

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Mer signal plays the central roles in the intrinsic inhibition of the inflammatory response to pathogens by macrophages and dendritic cells. Liver X receptor (LXRα and LXRβ) are oxysterol-activated transcription factors that have been shown to transrepress inflammatory gene expression. In the present study, we investigated the role of Mer signaling in LXR activation and consequent modulation of pro or anti-inflammatory mediators after i.p. injection of zymosan, using anti-Mer neutralizing antibody. Treatment with anti-Mer antibody significantly reduced phosphorylation of Mer and Akt, a Mer downstream molecule in spleen and lung, at hours 6, 24, and 72 after zymosan treatment. After zymosan injection, LXRα expression in spleen and lung reduced at 6 hours and reached the control level at 24hours and increased at 72 hours. The level of LXRβ expression dropped at 6 hours and reached the control level at hours 24, 72. The levels of LXRα/β at these time points after zymosan treatment were reduced by anti-Mer antibody. The levels of mRNA and proteins of LXRα/β target molecules, such as ABCA1 and Mer, were in parallel reduced by treatment with anti-Mer Ab. Treatment with anti-Mer antibody enhanced zymosan-induced pro-inflammatory mediators at each time point. The zymosan-induced anti-inflammatory mediators, such as TGF-β and HGF, were inhibited by treatment with anti-Mer Ab in peritoneal fluid. Similarly, treatment with anti-Mer antibody enhanced zymosan-induced mRNA and protein expression of TNF-α and MIP-2, but suppressed TGF-β and HGF in spleen and lung tissue. Importantly, treatment with the LXR agonist, T0901317, inhibited zymosan-induced increases in TNF-α, MIP2 and IL-1β, but enhanced TGF-β and HGF in peritoneal fluid. Co-administration of T0901317 with anti-Mer antibody reversed the increases in these pro-inflammatory mediators but further enhanced the induction of anti-inflammatory mediators at hours 6, 24 in peritoneal fluid. These results indicate that the Mer signaling leads LXRα/β activation and consequently down-regulates inflammatory mediators, contributing for the resolution of acute systemic inflammation.
Key Words: Mer receptor tyrosine kinase, Liver X receptor, Zymosan, T0901317, Inflammatory responses

MP-20(PO-11)

A Redox Switch Regulated by APE1/Ref-1 Governs Endothelial SIRT1 Activity

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The DNA repair enzyme/reducing protein Apurinic/Apyrmidinic Endonuclease / Redox Factor-1 (APE/Ref-1) and the SIRT1 protein lysine deacetylase both play important roles in vascular endothelial homeostasis. APE1/Ref-1 maintains many redox sensitive transcription factors in their reduced active form. SIRT1 is subject to redox modifications but whether APE1/Ref-1 regulates the redox state and activity of SIRT1 is not known. To determine if APE1/Ref-1 governs the redox state and activity of SIRT1, and whether SIRT1 mediates the effect of APE1/Ref-1 on endothelium-dependent vascular function. APE1/Ref-1 maintains cysteine (thiol) residues in SIRT1 in the reduced (free)

form and is obligatory for endothelial SIRT1 activity. APE1/Ref-1 increases the free thiol content of SIRT1 in vitro and in endothelial cells, targeting functionally critical cysteine residues in the catalytic domain of SIRT1 for reduction, thus activating SIRT1. Cysteine residues in the N-terminal redox domain of APE1/Ref-1 are essential for stimulating SIRT1 activity and for increasing free thiol content of SIRT1. In addition, APE1/Ref-1 protects endothelial SIRT1 from hydrogen peroxide-induced oxidation and inactivation. Activation of SIRT1 by APE1/Ref-1 translates into deacetylation of the SIRT1 target endothelial nitric oxide synthase (eNOS) by APE1/Ref-1. SIRT1 mutated at cysteine residues targeted by APE1/Ref-1 which renders it non-reducible by APE1/Ref-1 functions in a dominant inhibitory fashion and prevents lysine deacetylation of eNOS induced by APE1/Ref-1. When compared to wild-type mice, APE1/Ref-1+/- mice have lower free thiol content of SIRT1, and diminished SIRT1 activity in tissues. Overexpression of SIRT1 in aortas of APE1/Ref-1+/- mice restores endothelium-dependent vasorelaxation and vascular bioavailable NO to levels similar to those observed in wild-type mice. APE1/Ref-1, by maintaining endothelial SIRT1 in the reduced form, is required for SIRT1 activity. Reductive activation of endothelial SIRT1 by APE1/Ref-1 mediates the salutary effect of APE1/Ref-1 on endothelium-dependent vasorelaxation.

Key Words: APE/Ref-1, SIRT1, eNOS, Acetylation

MP-21

Ca^{2+} Response to Tastant in Mouse Submandibular Salivary Glands

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Background: Taste gives an appetite and it protects us from poisons. Taste sense is very crucial factor for quality of life and survival. Taste cells utilize G protein-coupled receptors to detect sweet, bitter and umami taste whereas salty and sour taste are detected with ion channels. However, research on distribution of taste receptor and taste signal transmission was rarely investigated. Recently, some of these taste receptors were identified in non-taste cells. Salivary glands and other exocrine glands are related to the roles in sensing of taste and transduction of taste. Therefore, we examined whether submadibular acinar cells respond to tastant and analyzed relative difference between several taste stimuli. **Methods:** To investigate the response to taste stimuli in mouse salivary glands, we isolated submadibular acinar cells by sequential trypsin/collagenase treatment. Acinar cells were loaded with fura-2 and measured Ca^{2+} activity while applying several different tastants. Treated compounds were quinine, cycloheximide, denatonium, phenylthiourea and sucrose octaacetate (bitter); saccharine (sweet); glutamate (umami); and carbachol (positive control). **Results:** Ca^{2+} responses elicited by each chemical stimulus showed distinct peak amplitude. Quinine, cycloheximide, denatonium, phenylthiourea and sucrose octaacetate elicited increase of Ca^{2+} activity with different patterns, depending on each compound and cells. However, saccharine and glutamate did not show change in Ca^{2+} activity. **Conclusions:** Our study show that murine salivary glands respond to bitter taste with different range of chemical sensitivities. This suggests that individual cells distinguish between bitter taste and the possibility of other exocrine glands also directly respond to taste.

Key Words: Taste receptor, Exocrine gland, Ca^{2+} imaging

MP-22

Pathogenic Role of HIF-1α in Prostate Hyperplasia in the Presence of Chronic Inflammation

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Benign prostatic hyperplasia (BPH) commonly occurs in older men with chronic prostatitis. Although BPH is frequently accompanied by inflammation, it is unclear whether inflammation underlies prostate enlargement. Recently, we reported that HIF-1α which is known to be induced by proinflammatory cytokines, is involved in testosterone-induced prostate hyperplasia. Therefore, we hypothesized that cytokines secreted from infiltrated macrophages under inflammatory conditions stimulate prostate enlargement by upregulating HIF-1α. In the present study, we injected LPS into rat prostates to mimic prostatitis and evaluated prostate hyperplasia 14 days later. Epithelial cells of LPS-treated prostates were found to be highly proliferative and HIF-1α levels in prostate tissues to be elevated. When prostate epithelial cells were incubated in conditioned medium from macrophages activated with LPS, they robustly expressed HIF-1α, and under these conditions IL-1β, IL-6, and TNF-α cytokines were found to mediate HIF-1α induction. In addition, HIF-1α was found to enhance the expression of Twist, which initiates epithelial-mesenchymal transition (EMT). Furthermore, profound EMT features were observed in LPS-treated rat prostates, and the natural HIF-1α inhibitors ascorbate and curcumin were found to attenuate EMT and prostate hyperplasia both *in vivo* and *in vitro.* Based on these results, we propose that HIF-1α mediates prostate enlargement under inflammatory conditions, and we suggest that HIF-1α be viewed as a promising target for blocking the transition from prostatitis to BPH.

Key Words: Prostatic hyperplasia, LPS, Inflammation, HIF-1α, EMT

MP-23

The AP Enodnuclease/Redox Factor APE1/ref-1 Translocalizes to Mitochondria after Phorbol 12-Myristate 13-Acetate (PMA)-Induced Oxidative Stress and Regulates Mitochondrial Function

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The AP enodnuclease/redox factor APE1/ref-1 is a multifunction protein that plays an important role in both the repair of baseless sites that arise in DNA and in transcriptional responses to oxidative stress. Oxidative stress induces endogenous reactive oxygen species (ROS) by electron transportation chain (ETC), which makes the mitochondrion the main redox-sensitive subcellular organelle. An important mitochondrial regulatory role for APE1 has recently been reported, so therefore we explored the role of APE1 in regulation of mitochondrial function after PMA-induced oxidative stress. Treatment with PMA in MS-1 cells induced mitochondrial hyperpolarization and bax activation and increased mitochondrial superoxide production and MnSOD expression. We observed that mitochondrial translocation of APE1 is induced following PMA treatment. To investigate the functional meaning of the APE1 mitochondrial translocation, we examined that overexpression or downregulation of APE1 regulates PMA-induced mitochondrial hyperpolarization and MnSOD expression. APE1 overexpression decreased PMA-induced mitochondrial hyperpolarization and MnSOD expression. In APE1-deficient cells, alteration of mitochondrial membrane potential was higher compare to APE1- proficient cells. These results suggest that mitochondrial translocated-APE1 regulates mitochondrial function after PMA-induced oxidative stress.

Key Words: APE1/ref-1, Mitochondrial function

MP-24(PO-12)

Mitochondrial Dysfunction and ER Stress by Palmitate in Mouse Podocyte

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Podocytes play a major role in glomerular filtration barrier, but also participate in pathogenic process of diabetic nephropathy. Diabetic patients have elevated plasma levels of saturated free fatty acid (FFA) that induces ER stress and apoptosis in different cell types. In this study, we investigated the cytotoxic mechanism of FFA in immortalized mouse podocyte. Incubation with palmitate, a saturated FFA, increased cytosolic or mitochondrial reactive oxygen species (ROS) production and apoptotic cell death in a dose dependent manner. Palmitate depolarized the mitochondrial membrane potential and caused a morphodynamic change to mitochondrial fragmentation. Palmitate strongly upregulated ER stress proteins such as GRP78/Bip, spliced Xbp1 and CHOP. However, a monounsaturated FFA oleate itself or combination with palmitate increased neither ROS level nor ER stress protein expression. Palmitate markedly depleted the luminal Ca^{2+} level in ER and abolished the cyclopiazonic acid (CPA)-induced cytosolic Ca^{2+} increase, that were confirmed by D1ER, a ER Ca^{2+} indicator, and Fura-2, a cytosolic Ca^{2+} indicator, respectively. Interestingly, the palmitate-induced ROS production, ER Ca^{2+} depletion and apoptosis were blocked by sulfo-N-succinimidyl oleate (SSO), a selective inhibitor of FAT/CD36 known as fatty acid transporter. In addition, palmitate-induced ER Ca^{2+} depletion and cytotoxicity were attenuated by mitochondrial antioxidant, mitoTEMPO. Taken together, these data suggest that palmitate as the predominant circulating saturated FFA leads to mitochondrial dysfunction and ER stress via mitochondrial ROS generation and ER Ca^{2+} depletion through FAT/CD36 in mouse podocyte.

Key Words: Podocyte, Free fatty acid, ER calcium depletion, Mitochondrial dysfunction, Reactive oxygen species

MP-25

Exosome from Keratinocyte Stimulates Proliferation and Migration in Keratinocytes

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Intercellular communication in most cells is important for maintaining their homeostasis. Therefore, cells secrete not only growth factors and cytokines but also extracellular membrane vesicles. Extracellular membrane vesicles distinguish membrane vesicle from exosome. Especially, exosome shows diameters of 30～100 nm and is secreted from endosomal membrane compartment through plasma membrane by various cell stimulators. It composes of many proteins, lipids and RNAs, transfer membrane receptors, proteins and mRNAs between different cells, and is also known to be an immune response regulator and induce angiogenesis. However, keratinocyte exosome function has not been known. This study determined the role and structural feature of exosome isolated from HaCaT (kerationocyte cell line) in biological mechanism in keratinocytes. Isolated HaCaT exosome was confirmed to have diameters of 30～100 nm by atomic force microscopy analysis. HaCaT proliferation was induced in re-

sponse to both non-boiling-exsome and boiling exosome. However, HaCaT migration and sprout out growth were stimulated by only non-boiling-exsome. Taken together, these results suggest that keratinocyte exosomes may induce proliferation by lipid or heat-resistant components, while sprout out growth and migration by protein or heat-weak components in keratinocytes.
Key Words: Exosome, Keratinocyte, Proliferation, Migration, Sprouting assay

MP-26

Silibinin Induces Cell Death through Different Mechanisms in Human Breast Cancer Cells MCF7 and MDA-MB-231

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In the present study, we examined the efficacy of silibinin as an antitumorigenic agent in the breast cancer by evaluating silibinin-induced cell death in the human breast cancer cells MCF7 which is known to be deficient of caspase-3 and MDA-MB-231 which normally expresses caspase-3. Silibinin suppressed cell viability in a time- and dose- dependent manner in the both cells. Silibinin-induced cell death was attenuated by antioxidants, *N*-acetylcysteine (NAC) and Trolox, suggesting that the effect of silibinin was dependent on generation of reactive oxygen species (ROS). Western blot analysis showed that silibinin induced ROS-dependent dephosphorylation of ERK and Akt. When cells were transiently transfected with constitutive active MEK (caMEK) and Akt (caAKt), they showed resistance to silibinin-induced cell death. In MDA-MB-231 cells, silibinin-induced apoptosis was accompanied by increased cleavage of caspase-3 and was prevented by caspase inhibitors. In contrast, in MCF7 cells, silibinin-induced cell death was accompanied by translocation of apoptosis inducing factor (AIF). Silibinin-stimulated nuclear translocation of AIF was blocked by NAC, caMEK, and caAKt. Furthermore, silibinin-induced cell death was prevented by silencing of AIF expression using small interfering AIF (siAIF) technique in MCF7 cells, but not in MDA-MB-231 cells. These findings suggest that silibinin induces death of breast cancer cells through an AIF-dependent mechanism in MCF7 cells and through a caspase-3-dependent mechanism in MDA-MB-231 cells.
Key Words: Silbinin, Breast cancer, caspase-3, AIF

MP-27

Protective Effect of Cilostazol against Oxysterol-induced Disruption of Tight Junction Integrity and Apoptosis in Human Colonic Epithelial Cells

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The present study was performed to examine whether cilostazol has a beneficial effect on intestinal epithelial cells to preserve the tight junction integrity and cell viability under oxysterol-induced oxidative insult. CaCo-2 cells were grown as monolayers on polycabonate membrane filters and were treated with 7β-hydroxycholesterol (7β-HC) in the presence and absence of cilostazol and the changes in the tight junction integrity and cell viability were examined. In 7β-HC-treated cells, there was a significant decrease in transepithelial resistance (TER), increase in permeability to FITC-dextran, and increase in apoptotic cell death. In these cells, activation of caspases, depolarization of mitochondrial membrane potential, and induction of MPT were also observed. Immunocytochemical studies demonstrated that redistribution of tight junction proteins ZO-1 and occludin occurred in 7β-HC-treated cell monolayer. Cilostazol effectively prevented these 7β-HC-induced events and suppressed generation of reactive oxygen species (ROS). The antioxidant NAC and the NADPH oxidase inhibitor DPI also effectively suppressed the 7β-HC-induced ROS generation, TER drop and apoptotic cell death. Taken together, these results indicated that cilostazol protected the CaCo-2 cell monolayer against 7β-HC-induced disruption of the tight junction integrity and apoptotic cell death, and suppression of NADPH oxidase-dependent ROS generation might be the key events responsible for the protection mechanism.
Key Words: Cilostazol, Oxysterol, Tight junction, Colonic epithelial integrity

MP-28

Apoptotic Cell Instillation Promotes Antifibrotic Feedback Loop through Coordinate Induction of COX-2/PGE2 Signaling, and HGF in Mice

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Previous our study reported that instillation of apoptotic cells after bleomycin induced persistent enhancement of HGF mRNA and protein expression, resulting in attenuation of lung injury and fibrosis. In the present study, we investigated effects of apoptotic cell instillation on the other potent antifibrotic molecules, such as COX-2/PGE2. Furthermore, we characterized the functional interaction between these molecules and HGF upon *in vivo* exposure to apoptotic cells in the injured lung processing extensive fibrosis, using selective inhibitors of these molecules. The levels of COX-2 mRNA and protein expression increased up to 14 days and slightly declined at 21 days after bleomycin treatment. The instillation of apoptotic cells further enhanced the levels of COX-2 mRNA and protein each day after bleomycin treatment. Similarly, the COX-2 enzymatic product, PGE2 in BAL fluid increased up to 14 days and slightly declined at 21 days after bleomycin treatment.

The levels of PGE2 in BAL fluid were further enhanced following apoptotic cell instillation. Coadministration of the COX-2 selective inhibitor, NS-398, with apoptotic cells reversed induction of PGE2 as well as HGF mRNA and protein following apoptotic cell instillation. Importantly, NS398 reversed reduced apoptotic activity of caspase-3 and -9 and the levels of hydroxyproline content, indicating collagen content in lung tissue. In addition, apoptotic cell-induced COX-2 expression and PGE2 production were reversed by coadministration of the c-Met antagonist PHA665752. These findings indicate that enhanced HGF/c-Met signaling and COX-2/PGE2 signaling following apoptotic cell instillation into the bleomycin-stimulated lungs are interrelated through positive cross-talk between these two important pathways, providing a mechanism for the antifibrotic effects mediated by apoptotic cell clearance.
Key Words: Apoptotic cells, Bleomycin, Lung injury, Cyclooxygenase-2, Hepatocyte growth factor

MP-29

Changes in Reactive Oxygen Species and Thioredoxin by Suberoyl Bishydroxamic Acid Affect A549 Cell Death

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Suberoyl bishydroxamic acid (SBHA) as a histone deacetylase (HDAC) inhibitor can induce apoptosis through the formation of reactive oxygen species (ROS). In this study, we investigated the effects of SBHA on A549 cell death in relation to ROS, glutathione (GSH) and the regulation of thioredoxin (Trx). SBHA inhibited the growth of A549 cells in time- and dose-dependent manners. This agent decreased ROS level at the early times of 30 mins. However, SBHA significantly increased ROS levels including $O_2^{\cdot -}$ level at 24 and 72 hours. SBHA also induced GSH depletion at 24 and 72 hours. N-acetyl cysteine (NAC; a well-known antioxidant) decreased apoptotic cell death, ROS levels and GSH depletion in SBHA-treated A549 cells. In addition, SBHA changed the levels of antioxidant-related proteins, especially Trx1. The expression and activity of Trx1 were reduced by SBHA in A549 cells. While the down-regulation of Trx1 intensified cell death, ROS level and GSH depletion in SBHA-treated A549 cells, the over-expression of Trx1 decreased ROS level regardless of cell death. In conclusion, changes in ROS and Trx1 levels by SBHA were related to A549 cell death. This work was supported by a grant from the Ministry of Science & Technology (MoST)/Korea Science & Engineering Foundation (KOSEF) through the Diabetes Research Center at Chonbuk National University (2010-0029497) and the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (2010-0021808).
Key Words: Suberoyl bishydroxamic acid, Reactive oxygen species, Cell death, Thioredoxin

MP-30

NecroX-5 Prevents Hypoxia/Reoxygenation Injury by Inhibiting the Mitochondrial Calcium Uniporter

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Preservation of mitochondrial function is essential to limit myocardial damage in ischaemic heart disease. We examined the protective effects and mechanism of a new compound, NecroX-5, on rat heart mitochondria in a hypoxia/reoxygenation (HR) model. NecroX-5 reduced mitochondrial oxidative stress, prevented the collapse in mitochondrial membrane potential, improved mitochondrial oxygen consumption, and suppressed mitochondrial Ca^{2+} overload during reoxygenation in an in vitro rat heart HR model. Furthermore, NecroX-5 reduced the ouabain- or histamine-induced increase in mitochondrial Ca^{2+}. These findings suggest that NecroX-5 may act as a mitochondrial Ca^{2+} uniporter inhibitor to protect cardiac mitochondria against HR damage.
Key Words: NecroX-5, Mitochondria, Hypoxia/reoxygenation, Calcium uniporter

MP-31

HS-1793, Resveratrol Analogue Reduces Ischemia Reperfusion Injury via Attenuating Mitochondrial Calcium Overload in Rat Hearts

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Resveratrol, a plant derived phytoalexin, has cardioprotective effect against ischemia/reperfusion (IR) injury. HS-1793 (C16H12O3), a novel analogue of resveratrol, has more potent than resveratrol in physiological effect. However its cardioprotective effect was not clear. The present study aimed to test the cardioprotective effect of HS-1793 against IR injury and investigate mitochondria mediated the underlying mechanism. To address cardioprotective potential of HS-1793, we analyzed myocardial infarct region, heart rate and left ventricular pressure in the IR treated and HS-1793 pre-treated rat heart. Cytosolic, mitochondrial calcium and mitochondrial reactive oxygen spices (ROS) production were assessed in the IR treated or HS-1793 pretreated single cardiomyocytes using con-

focal microscopy system. Mitochondrial function was evaluated by mitochondrial oxygen consumption ratio with isolated mitochondria from IR and HS-1793 treated heart. HS-1793 treated rat heart has more resistance to IR injury as evidence by reduced infarct size. Moreover HS-1793 ameliorated IR induced mitochondrial dysfunction by reducing mitochondrial ROS production, improving mitochondrial oxygen consumption and suppressing mitochondrial Ca^{2+} overload during reperfusion. However, HS-1793 did not directly affect whole-cell current or action potential duration of single cardiomyocyte. Our data suggest that HS-1793 has potential to protect cardiac mitochondrial against IR injury by modulating mitochondrial calcium ion flux.
Key Words: Resveatratrol analogue, Mitochondrial calcium, Ischemia reperfusion injury

MP-32

Mitochondrial Peroxiredoxin III Protects Colon Cancer Stem Cells from Cell Death

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Cancer Stem Cell (CSC) is a subset of the bulk tumor responsible for initiating and maintaining the disease. In soft agar assay and sphere culture, the $CD133^{high}$ sorted cells were dramatically increased to form spheroid colonies compared to $CD133^{low}$ cells. The $CD133^{high}$ cell subpopulation also showed the resistance to 5-FU induced-cell death. Although there is increasing evidence that a rare population of undifferentiated cells is responsible for tumor formation and maintenance in colon cancer, this has not been explored for mitochondrial functions. We found that the mitochondrial calcium, ROS, and membrane potential was enhanced in $CD133^{high}$ compare to $CD133^{low}$ cells. Using a microarray analysis and real timee RT-PCR, we confirmed to increase the expression of several genes in $CD133^{high}$ cells. From gene expression profile analysis, we identified peroxiredoxin (Prx) 3, one of mitochondrial gene that utilize to protect CSC from cell death. We conclude that treatment of colorectal cancer, which is created and propagated by a small number of undifferentiated tumorigenic $CD133^{high}$, should be the combination of mitochondrial Prx3-targeted drug via functional studies of Prx3.
Key Words: Colon, Cancer stem cells, CD133, Mitochondria, Peroxiredoxin III, Apoptosis

MP-33

The Effect of Phytoncides on the Inflamed Synovial Fibroblast Cells

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Phytoncides are reported as antimicrobial volatile organic compounds from plants and prevent them from rotting or being eaten by some insects and animals. They were used as the medicine in Korea, Russia, Japan and other many countries. They also have been used for treatment of inflammatory disease. Because they have the anti-inflammatory activity. We already saw the effect of phytoncides on the inflammation-induced by carrageenan in the rat knee joint model as *in vivo* model. In addition, we need to evaluate the effect of phytoncides with *in vitro* system again. One percentage carrageenan/0.2 kg into the knee joint to induce the acute inflammation in male Sprague-Dawley rats under enflurane anesthesia. After 4 hours we watched the swollen knee joint, sacrificed rats and isolated the synovial membrane to make inflamed synovial fibroblast cells. In order to see the effect phytoncides, we cultured the inflamed synovial fibroblast (InSF) with and without phytoncides for 12 hours. We harvested the cells, did perform the western blotting to see the expression of pro-(TNFα, COX-2, IL6, IL1β) and anti-(IL10) inflammatory molecules. The effect of phytoncides on the InSF cells as dose-dependent manner. Especially, in 1ppm of phytoncide treated group, the pro-inflammatory molecules, TNFα; COX-2; IL6; IL1β, were decreased. In contrast, the anti-inflammatory molecule, IL10 were increased dramatically. In conclusion, it is possible that phytoncides may play a role in inflamed synovial fibroblast cells to reduce inflammatory molecules.
Key Words: Phytoncides, Carrageenan, Inflamed synovial fibroblast, Pro-inflammatory molecule and anti-inflammatory molecule

MP-34

Functional Network Analysis of Gene Expression for Identifying Informative Genes Involved In Differentiation of Neural Progenitors

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Time series gene expression profiles can provide us the first window to observe activities of genes to process information interacting with many others for a well-organized behavior such as stem cell development. In this study, we investigated coordinately expressed genes on a functional association network mined from the scientific literature to find out key members of neuronal differentiation. We have generated and analyzed nine points time series of transcriptomic data of differentiating neural progenitor cells derived from HSF6 human embryonic stem cells. Applying modularity calculation and identifying overrepresented ontologies with unbiased methods, we obtain nine sub-networks, each of which reveals biologically meaningful function. We identify fifty genes of high betweenness as

candidates that have a potency to regulate phenotypical changes of through perturbing information flow of many pathways as bottlenecks. TP53 with the highest betweenness is a hub of the sub-network that is strongly interconnected with two clusters of sub-networks that are relatively weakly connected each other and involved in cell cycle progression and developmental process respectively. It suggests that TP53 may be a master regulator that interplays between self-renewal and cell differentiation of neural progenitor cells. This analysis is more powerful than the classical profiling method in that it describes activated functional sub-networks and identifies significant genes by evaluating centrality of those biological systems.
Key Words: Genome wide expression, Neural progenitor, Differentiation, Interaction network, Betweenness centrality

MP-35

DJ-1/Park7 Deficiency Contributes to (pro)renin Receptor-Mediated Hypertension

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DJ-1/Park7 protein is a transcriptional regulatory protein and its deficiency might be associated with the expression of hypertension-related genes. Therefore, we determined whether DJ-1/Park7 protein contributes to the regulation of renal (pro)renin receptor (PRR) expression related with hypertension pathogenesis. The levels of PRR, angiotensinogen (AGT), renin, angiotensin I (Ag I), the activity of extracellular signal-regulated kinase (ERK) 1/2, pro-fibrotic genes and systolic blood pressure were compared between wild type (WT) and DJ-1 knockout (DJKO) mice. The levels of PRR mRNA and protein in the kidney were significantly increased in DJKO compared with WT, whereas levels of AGT, renin and Ag I were not different between WT and DJKO. The levels of ERK1/2 activity and pro-fibrotic genes were much greated in DJKO than in WT. Moreover, renin increased the ERK1/2 activity and profibrotic gene expression in glomerular cells, which were inhibited by treatment with handle region peptide (HRP), PRR inhibitory peptide. Systolic blood pressure was increased in DJKO compared with WT. We conclude that DJ-1/Park7 protein contributes to the regulation of renal PRR expression related with hypertension pathogenesis. Thus, we suggest that DJ-1/Park7 protein may act as an important regulator of renal hypertension.
Key Words: DJ-1, (pro)renin receptor, Hypertension, ERK1/2

MP-36

Role of IRAK1 on TNF-induced Proliferation and NF-κB Activation in Human Bone Marrow Mesenchymal Stem Cells

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In this study, we determined the effect of TNF-α on hBMSCs proliferation as well as the role of IL-1 receptor-associated kinase 1 (IRAK1) on TNF-α signaling. Western blot analysis revealed that TNF-α treatment increased the phosphorylation of IRAK1 in hBMSCs. The downregulation of IRAK1 inhibited TNF-α-induced NF-?B activation and COX-2 expression. TNF-α treatment increased hBMSCs proliferation in a dose-dependent manner and increased ERK, JNK, and NF-κB activity. U0126, an ERK inhibitor, decreased hBMSCs proliferation and significantly blocked TNF-α-induced hBMSCs proliferation. In cells with IRAK1 or TRADD downregulation, the U0126 treatment inhibited hBMSCs proliferation and significantly suppressed TNF-α-induced hBMSCs proliferation. The downregulation of IRAK1 or TRADD inhibited TNF-α-induced ERK and JNK activation, and hBMSCs proliferation. Inhibition of NF-κB by decoy oligonucleotides reduced the TNF-α-induced hBMSCs proliferation. Immunoprecipitation analysis showed that IRAK1 does not physically interact with TNF receptor 1 (TNFR1) even in the presence of TNF-α. Suppression of IRAK1 binding protein (IRAK1BP1) inhibited TNF-α-induced increase of theproliferation and ERK1 phosphorylation of hBMSCs in the presence of TNF-α. Our data indicate that TNF-α modulates hBMSCs proliferation through ERK signaling pathways, and that IRAK1 plays an important role in TNF-α-induced NF-κB activation in hBMSCs.
Key Words: hBMSCs, TNF-α, IRAK1, ERK signaling, NF-κB

MP-37

miR-146a Regulates Tumor Outgrowth Induced by Human Adipose Tissue-Derived Mesenchymal Stem Cells *in vivo*

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Mesenchymal stem cells (MSCs) have therapeutic potential, including the ability to self renew and differentiate into cells multiple lineages, but transplanted MSCs can be accelerate tumor growth *in vivo*. In this study, we determined

whether microRNAs (miRNAs) can regulate MSC-induced tumor outgrowth in mice. Overexpression of miR-146a in Human adipose tissue-derived mesenchymal stem cells (hADSCs) inhibited hADSCs-induced tumor growth, cell proliferation and osteogenic differentiation and induced the inhibition of NF-κB promoter activity by TNF-α. Transplantation of miR-146a-transfected hADSCs decreased both tumor vascularity compared with transplantiation of control olio-transfected hADSCs and mRNA expression of angiogenic factors. Similar with effect of miR-146a, Downregulation of Transforming growth factor-β-activated kinase 1 (TAK1) inhibited tumor growth *in vivo* and NF-κB promoter activity. Transplantiation of TAK1 siRNA-transfected hADSCs also decreased tumor vascular density and angiogenesis. Our data indicate that miR-146a inhibited tumor growth-induced hADSCs via downregulation of NF-κB and angiogenesis.

Key Words: hADSCs, TNF-α, miR-146a, TAK1, NF-κB

MP-38

A Serum Protein Fetuin B is a Possible Biomarker for Identifying Acute Myocardial Infarction

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The rupture of an atherosclerotic plaque is one of the main causes of coronary artery thrombotic occlusion and subsequent myocardial infarction, but research into the causes and treatment of plaque rupture is hampered by the lack of a suitable biomarker. In the current study, to identify the new biomarker for atherosclerotic plaque rupture, we analyzed the protein expression of profiles of serum of acute myocardial infarction (AMI) or stable angina (SA), using proteomic analysis. The expression of three proteins altered in AMI compared with SA. In particularly, the expression level of fetuin B showed higher increases in patients with AMI than SA. Furthermore, fetuin B significantly increased T cell migration in a concentration-dependent manner, and also enhances the expression of MMP-2 in TNF-α- or LPS-stimulated U937 cells and HUVECs. However, fetuin A did not change T cell migration and the expression of MMP-2. Therefore, fetuin B may be a potential biomarker to guide the reliable prevention of patients from increased risk of acute myocardial infarction.

Key Words: Fetuin B, TNF-α, U937 cells, HUVECs, MMP-2, Proteomics, Acute myocardial infarction

MP-39

Role of NFAT5 on Osteogenic Differentiation from Human Adipose Tissue-Derived Mesenchymal Stem Cells

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Human adipose tissue-derived mesenchymal stem cells (hADSCs) have therapeutic potential, including the ability to self-renew and differentiate toward multiple lineages. The hADSCs before in clinical application, in order to raise efficiency of the cell treatment which uses the stem cell from is more important what to seek the elements which participate to a therapeutic effect. In this study, we determined the role of NFAT5 on the hADSCs osteogenic differentiation. Down regulation of NFAT5 significantly inhibit osteogenic differentiation and decrease the activity of NF-κB promoter. But, down regulation of NFAT5 is not effective in proliferation and adipogenic differentiation of hADSCs. Our study showed that hADSCs osteogenic differentiaton by regulation of native NFAT5 expression. Collectively, these findings indicate that NFAT5 functional role in hADSCs osteogenic differentiation.

Key Words: hADSCs, TNF-α, NFAT5, NF-κB

MP-40

Exogenous H_2O_2 and Pyrogallol Induce Growth Inhibition and Death in Human Pulmonary Artery Smooth Muscle Cells via GSH Depletion

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Reactive oxygen species (ROS) are involved in many of pathophysiological processes of vascular smooth muscle cells (VSMCs) such as cell growth and death. Pyrogallol (PG) as a polyphenol compound induces the $O_2^{\cdot -}$-mediated death of several types of cell. In the present study, we investigated the effects of exogenous H_2O_2 and PG on cell growth and death in human pulmonary artery smooth muscle (HPASM) cells in relation to changes in intracellular ROS and glutathione (GSH) levels. H_2O_2 decreased HPASM cell growth with an IC_{50} of approximately 250～500 μM at 24 hours and induced apoptosis, as evidenced by annexin V-staining cells and Z-VAD treatment. However, PG did not strongly induce growth inhibition and death in HPASM cells. H_2O_2 increased ROS levels including mitochondrial $O_2^{\cdot -}$ and induced GSH depletion in HPASM cells. N-acetyl cysteine (NAC; a well-known antioxidant) attenuated apoptotic cell death and ROS levels in H_2O_2-treated HPASM cells, and this agent also prevented GSH depletion. Interestingly, PG did not increase ROS levels in-

cluding mitochondrial $O_2^{\cdot -}$. Moreover, unexpectedly NAC induced a strong increase in mitochondrial $O_2^{\cdot -}$ level in PG-treated HPASM cells and significantly increased cell death and GSH depletion. In summary, H_2O_2 and PG induced growth inhibition, death and GSH depletion in HPASM cells. However, HPASM cells were resistant to PG compared with other cells. NAC attenuated cell death in H_2O_2-treated HPASM cells whereas it augmented cell death in PG-treated HPASM cells. This work was supported by a grant from the Ministry of Science & Technology (MoST)/Korea Science & Engineering Foundation (KOSEF) through the Diabetes Research Center at Chonbuk National University (2010-0029497) and the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (2010-0021808).
Key Words: Smooth muscle cell, H_2O_2, Pyrogallol, Cell death, ROS

MP-41

Role of Formyl Peptide Receptor-Like 1 for Homing of Endothelial Progenitor Cells and Ischemic Neovasculogenesis

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Mobilization of endothelial progenitor cells (EPCs) from bone marrow and homing of EPCs to ischemic tissues are required for neovascularization. N-formyl peptides produced by Gram negative bacteria have been implicated in host defense by stimulating chemotaxis of leukocytes through mechanisms involving N-formyl peptide receptors. In the present study, we investigated the ability of the G protein-coupled receptor formyl peptide receptor-like 1 (FPRL1) in homing ability of EPCs and vascular regeneration of ischemic tissues. WKYMVm, an FPRL1 agonist, stimulated chemotactic migration and angiogenesis of human EPCs in vitro. Intramuscular injection of WKYMVm attenuated severe hindlimb ischemia and promoted vascular regeneration. When EPCs were transplanted via tail vein into nude mice, they incorporated into capillary vessels in ischemic hindlimb, augmented neovascularization, and improved ischemic limb salvage. Intramuscular injection of WKYMVm promoted the homing of transplanted EPCs to ischemic limb and vascular regeneration. siRNA-mediated silencing of FPRL1 expression abrogated WKYMVm-induced in vitro chemotactic migration and in vivo homing of EPCs to ischemic limb. These results suggest that WKYMVm promotes neovascularization and regeneration of injured tissues by stimulating homing of EPCs via FPRL1-dependent mechanism.
Key Words: N-formyl peptide receptor, WKYMVm, Endothelial progenitor cells, Ischemia, Neovasculogenesis

MP-42

Role of Periostin in the Migration and Tube Formation of Human Endothelial Progenitor Cells

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Periostin, which belong to fasciclin family, is an extracellular matrix protein and is implicated in a various biological processes, including differentiation, adhesion, migration, invasion, and survival. It has been shown that periostin is expressed in injured tissues, such as in heart with myocardial infarction. Periostin has been reported to promote angiogenesis and tissue regeneration. However, the molecular mechanism associated with periostin-stimulated tissue regeneration is still unclear. In the present study, we demonstrated that recombinant periostin protein stimulated migration and endothelial tube formation of human endothelial progenitor cells. To identify the functional domains of periostin implicated in the angiogenesis, five fragments of periostin, including four FAS I domain repeats and carboxyl terminal domain, were overexpressed in Escherichia coli and purified the recombinant protein using Ni-NTA affinity chromatography. We found that the first FAS I domain is responsible for the periostin-stimulated migration and endothelial tube formation of human endothelial progenitor cells. These results suggest that the recombinant protein of the FAS I domain of periostin will be useful for therapeutic angiogenesis and tissue regeneration of ischemic injured tissues.
Key Words: Periostin, Migration, Tube formation, Angiogenesis, Tissue regeneration

MP-43

Restoration of Tyrosine Hydroxylase by a Novel Fusion Protein of Human Metallothionein1A (Zn-TMhM) with TAT and Artificial Mitochondrial Targeting Sequence in MPP^+-Damaged Neuronal Cells

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Oxidative stress, an excess production of reactive oxygen species (ROS) relative to antioxidant defense, plays a major role in developing various degenerative diseases. Since mitochondria are the major sites of generating ROS as well as damaging by ROS, the immediate delivery of antioxidant molecules to mitochondria may be beneficial to

prevent or alleviate the diseases. We designed a novel mitochondrial targeting sequence (MTS) to transduce human antioxidant metallothionein 1A (hMT1A) into mitochondria *in silico*. The designed MTS contains both cell-penetrating TAT (YGRKKRRQRRR) and a short artificial MTS peptide (LLRAALRKAAL, aMTS) for mitochondrial import. Secondary structure of TAT-aMTS peptide was predicted as α-helix showing amphiphilicity. We cloned the gene coding for TAT-aMTS-hMT1A (TMhM) for mammalian or bacterial expressions. TMhM was present in mitochondria when it was overexpressed. Zn-TMhM protein was purified from *E. coli* and applied in SH-SY5Y neuronal cells. TAT-aMTS was cleaved out from Zn-TMhM and mature form of hMT1A was detected in mitochondria after transduction. Successful mitochondrial delivery of Zn-TMhM protein ameliorated the damages on mitochondria and tyrosine hydroxylase in MPP^+-induced Parkinson's disease cell model. TAT-aMTS-mediated protein transduction and MT1A would be extremely useful for treating patients with mitochondrial deficits.

Key Words: IProtein transduction domain, Human metallothionein1A fusion protein, Antioxidant agent, Artificial mitochondrial targeting sequence, MPP^+

MP-44

Tumor Necrosis Factor-Alpha Conditioned Medium from Human Mesenchymal Stem Cells Stimulate Angiogenesis in a Murine Model of Hindlimb Ischemia

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Ischemia causes a restriction in blood supply, as a result dysfunction of tissue. Mesenchymal stem cells (MSCs) stimulate injured, inflamed tissue repair and regeneration through cell differentiation, migration and secretion of various cytokines and chemokines. However, cell-based stem cells therapy is increasing evidence that the contribution of MSC differentiation is limited due to poor engraftment and survival of MSCs at the injured tissue. Here, this study show to stimulate angiogenesis in hind limb ischemia model through MSCs paracrine function. Using proteomic technology, we found that tumor necrosis factor-α (TNF-α) induces secretion of various angiogenic chemokines, including IL-6, IL-8 from MSCs. To explore the paracrine function of MSCs on regeneration of ischemic tissues, the effects of TNF-α-conditioned medium on regeneration of ischemic tissues were investigated using a hind limb ischemia model. In the mouse ischemic model, femoral muscle injection of TNF-α-conditioned medium (TNF-α CM) inhibited limb loss and stimulated regeneration of blood vessels into the ischemic limb via IL-6 and IL-8 dependent mechanism *in vivo.* Also TNF-α-conditioned medium enhanced TNF-α CM enhances recruitment of macrophages and cell proliferation in ischemic tissue. Furthermore, TNF-α CM potentiated the migration of endothelial progenitor cells (EPCs) *in vitro* and EPCs homing *in vivo.* These results suggest that TNF-α CM will be useful for regeneration of ischemic injured tissues due to their prevented tissue necrosis and increased angiogenic potential.

Key Words: Ischemia, TNF-α CM, Angiogenesis, IL-6, IL-8

MP-45

Role of Phosphatidylinositol-(3,4,5)-Triphosphate (PIP_3) in Mechanical Hyperalgesia Following Spinal Nerve Ligation in the Rat

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It is known that reactive oxygen species (ROS) play a crucial role in the generation and the maintenance of hyperalgesia that develops following peripheral nerve injury. However, mechanisms by which ROS generates nerve injury-induced hyperalgesia are not yet clear. Phosphatidylinositol-(3,4,5)-triphosphate (PIP_3) is observed to be upregulated by ROS under the condition of fat accumulation or steatosis. Increased level of PIP_3 is found in hippocampal neurons to promote AMPA receptor generation, which results in a long-lasting enhancement of synaptic transmission. In the present study, we investigate whether PIP_3 makes contribution to ROS-induced mechanical hyperalgesia seen in rats. Mechanical hyperalgesia, measured with von Frey hairs, was induced using naive rats either by intrathecal (i.t.) injection of ROS donor tertiary-butyl hydroperoxide (t-BOOH) at the lumbar spinal cord level or by L5 spinal nerve ligation. In these two animal pain models, the effects of blocking PIP_3 production on the development of mechanical hyperalgesia were examined. Spinal cord of t-BOOH injected group and spinal nerve ligation model group also examined histologically in the level of L4,5 root origin. An i.t. injection of t-BOOH in naive rats induced mechanical hyperalgesia that was attenuated by about 60% when pretreated with wortmannin a selective inhibitor of PIP_3 producing-enzyme phosphoinositide 3-kinase. Mechanical hyperalgesia induced by L5 spinal nerve ligation was completely abolished by pretreatment with ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN), and was partly attenuated by pretreatment with wortmannin. t-BOOH injected group showed clear enhancement of PIP_3 immunoreactivity in Lamina I-III of dorsal horn. In case of spinal nerve ligation model, immunorecativity appeaed partially. There was no prominent immunoreactivity in the superficial spinal dorsal horn of both models treated with wortmannin. The results indicate that PIP_3 contributes to ROS-induced mechanical hyperalgesia. It is also suggested that PIP3 production via the action of ROS is partly involved in the generation of neuropathic pain following peripheral nerve injury. The research was supported by a grant from Stem Cell Research Center (SC-4140).

Key Words: Reactive oxygen species; Phosphatidylinositol-(3,4,5)-triphosphate, Peripheral nerve injury, Mechanical

hyperalgesia, Peripheral, Neuropathic pain

MP-46

Tribbles Homolog 3 (TRB3) Downregulates Endotoxin-Induced NO Production and NF-κB Activation in Murine Macrophages

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Tribbles homolog 3 (TRB3), a human homolog of Drosophila tribble, has been found to interact with a variety of signaling molecules to regulate cellular functions. Recently, it was shown that TRB3 inhibited MCP-1 expression in podocytes, suggesting its role in the regulation of inflammation. Moreover, the expression of TRB3 is reported to be induced by lack of nutrients. Previously, we have shown that endotoxin-induced iNOS expression was enhanced under high glucose condition. In this study, we investigated the effects of TRB3 on LPS-induced iNOS expression in Raw264.7 cells to explore the possible role of TRB3 in glucose enhancing effect. The expression level of TRB3 significantly increased in Raw264.7 cells exposed to low glucose (5 mM). On the other hand, high glucose (25 mM) enhanced LPS-induced iNOS expression and NO production and upregulated NF-κB activity in a reporter assay. Ectopic expression of TRB3 attenuated LPS-induced iNOS expression, NO production and transcriptional activity of NF-κB, whereas the knock-down of endogenous TRB3 using siRNA upregulated these responses. These results suggest that TRB3 might downregulate endotoxin-induced iNOS expression and NO production through attenuation of NF-κB activity, providing insight into glucose-enhancing effect on LPS-induced iNOS expression in murine macrophages.

Key Words: NO, iNOS, NF-kB, TRB3

MP-47

Prostaglandin E_2 Induces ICAM-1 Expression through EP-4 associated Signaling Pathways in bEnd.3 Brain Endothelial Cells

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Background and Purpose: Prostaglandin E_2 (PGE_2) has been implicated in the regulation of adhesion molecules, leukocyte adhesion and infiltration into inflamed site. However, the underlying mechanism therein involved remains ill-defined. In this study, we explored its cellular mechanism of action in the regulation of the intercellular adhesion molecule-1 (ICAM-1) expression in the brain endothelial cells. **Key Results:** PGE_2 upregulates the expression of ICAM-1 in bEnd.3 brain endothelial cells and the leukocyte adhesion to endothelial layer. Pharmacological study using selective agonists and antagonists for E prostanoid receptors suggests EP4 mediates PGE_2-induced ICAM-1 expression. PGE_2 increased the cellular cAMP level and forskolin and dbcAMP induced ICAM-1 expression. The downstream effector molecule responsible for cAMP-induced ICAM-1 expression was exchange protein directly activated by cAMP (Epac) but not protein kinase A. PGE_2-induced expression of ICAM-1 was blocked by the PI3K/Akt specific inhibitor (LY294002, Akti) and the transfection of dominant negative construct of Akt. PGE_2 induced the phosphorylation of IκBβ kinase and IκBα and the translocation of p65 and increased NF-κB dependent reporter gene activity. NF-κB inhibitors (Bay-11-7082, MG-132) attenuated PGE_2-induced ICAM-1 expression. Akti diminished NF-κB reporter gene activity induced by PGE_2, dbcAMP and 8-Cpt-cAMP. Taken together, our findings demonstrate that PGE_2 induces ICAM-1 expression by cAMP-dependent pathways via EP4 receptors and the cAMP/Epac-mediated signaling proceeds through PI3K, Akt and NF-κB in bEnd.3 cerebrovascular endothelial cells. This study was supported by grants from Ajou University School of Medicine and Gyunggi-do through CCRB-GRRC.

Key Words: Prostaglandin E_2, Exchange protein activated by cAMP, Intercellular adhesion molecule-1, PI3K/Akt

MP-48

Low-Intensity Ultrasound Decreases the Erythrocyte Swelling induced by Gramicidin D

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The edema of a cell is most common cellular pathologic phenomenon. So it is important for some clinicians to manage the cellular edema. The most powerful way reducing cellular edema ever developed is diuretics, which can increase urine output. Other way to make cells to excrete their fluids is to increase plasma osmotic pressure. It is not effective to local edema and doesn't have immediate therapeutic responses. By the way, ultrasound devices are the most non-invasive modalities in medical fields. It is distinguished with radiation in no harm to body. There are also many reports that ultrasound modalities can affect erythrocytes used as an experimental model for cellular changes. On the basis of these facts, we investigated the effect of low-intensity ultrasound (LIUS) on the cellular edema using rat red blood cells (RBCs). To induce the RBCs swelling, RBCs were treated with gramicidin D (40 ng/ml) for 20 min. The gramicidin D-induced RBCs swelling was evaluated with the hematocrit asaay. We found that the hematocrit value was significantly decreased in

LIUS-stimulated group compared with non-stimulated group in the RBCs swelling. We also examined whether the LIUS effect mechanism involves water channel, aquaporin 1 (AQP1), activity. When $HgCl_2$ (an AQP1 inhibitor) was treated, $HgCl_2$ could not completely countervail the effect of LIUS. In conclusion, LIUS has an effect on reduction of RBCs swelling induced by gramicidin D and AQP1 may be related in this process somehow. These results suggest that LIUS may be utilized to an alternative treatment to regulate edema or related disorders. However, the exact mechanism by that mechanical stimulation of LIUS reduces RBCs swelling is not elucidated and the further study is needed.

Key Words: Low-Intensity Ultrasound (LIUS), Edema, Water transport, Gramicidin D, Aquaporin

MP-49

Effects of Low Intensity Ultrasound on the Cell Viability and Mitochondrial Activity of Retinal Pigment Epithelium

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Purpose: The retinal pigment epithelium (RPE) is compose of monolayer of tightly connected pigmented cells and supports the function of the normal vision function. Death of RPE by oxidative stress is tightly involved in many cases of macular degeneration. The aim of this study was to investigate the effects of low-intensity ultrasound (LIUS) on the cell viability and mitochondrial activity in RPE cells under various pathologic stresses *in vitro*. **Methods:** ARPE-19 cells were grown on a plastic dish and treated with various damaging agents such as SNP, BSO and H_2O_2. Then, cells were untreated or treated with LIUS of 100 mW/cm^2 once for 20 min a day. Viability of cells was examined with Wst-1 and TUNEL assays. Cytoplasmic and mitochondrial ROS levels were measured using flow cytometry. JC-1 staining was also performed to measure mitochondrial membrane potential in cells. **Results:** Cell damaging agents increased cell death, cytoplasmic and mitochondrial ROS levels, and mitochondrial membrane potential in a dose-dependent manner. LIUS treatment significantly reduced cell death and mitochondrial ROS levels. Changes in the mitochondrial membrane potential were also decreased by LIUS. **Conclusion:** This study determined that LIUS affect changes in mitochondrial activities and cell death of ARPE-19 by various pathologic stresses, thereby might be a promising treatment for macular degeneration.

Key Words: Low intensity ultrasound, Retinal pigment epithelium, Oxidative stress, Mitochondria, Macular degeneration

MP-50

Glucosamine Protects Long-Term Hypoxia-Induced Mouse Embryonic Stem Cell Apoptosis Through Attenuation of ER Stress: Involvement of SP1 Glycosylation and HSP70 Expression

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Aims: To investigate the effect of Glucosamine (GlcN) in hypoxic condition and its related signal pathways in mouse embryonic cells (mESCs). **Materials and Methods:** To investigate the effect of GlcN on protection of mESCs, [3H]-Thymidine incorporation, LDH release and MTT assay were carried out. In addition, western blotting, immunoprecipitation, immunofluorescence microscopy assay, siRNA transfection were used for determination of GlcN related signaling pathways. **Results:** Incubation of mESCs at hypoxic condition increased CHOP (C/EBP-homologous protein) expression, but decreased GRP78 (glucose-regulated protein 78) expression in a time-dependent manner, which were blocked by pretreatment of GlcN. In addition, pretreatment of GlcN increased OGT (O-GlcNAc transferase) expression and decreased OGA (O-GlcNAcase) expression, subsequently increased level of specificity protein 1 (SP1) glycosylation in hypoxic condition, which was blocked by pretreatment of O-GlcNAc trasferase inhibitor ST 045849 but not by O-GlcNAcase inhibitor PUGNAc. GlcN-induced glycosylation of SP1 increased expression of heat shock protein 70 (HSP70), which was attenuated by pretreatment of OGT- or SP1-siRNA. Furthermore, GlcN-induced protection of ER stress in hypoxia was reversed by HSP70-siRNA or HSP70 inhibitor VER155008. These results suggest that increase of HSP70 by O-GlcNAc-SP1 exerts protective effects on hypoxia-induced ER stress. In hypoxic condition, GlcN decreased cleaved caspase-3 and increased bcl-2 and cellular inhibitor of apoptosis proteins (c-IAPs), which were attenuated by pretreatment of HSP70-siRNA. Finally, SP1-, HSP70-siRNA, mithramycin A, or VER155008 blocked hypoxia-induced increase in LDH release level and decrease in [^{3}H]-thymidine incorporation and MTT reduction. **Conclusion:** GlcN protected hypoxia-induced apoptosis of mESCs through attenuation of ER stress via SP1 glycosylation and HSP70 expression.

Key Words: Mouse embryonic stem cells, Hypoxia, Glucosamine, Specificity protein 1, Heat shock protein

MP-51

Effects of TRPC3,6 Blocker, 503A, on the Gene Expressions of Hypertrophic Markers and TRPCs in Sham and TAC Mouse Ventricular Myocytes; Comparison with TRPC3,6DKO

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We examined the new TRPC3,6 blocker, 503A on the expression of hypertrophic marker genes by using qPCR in cultured WT and TRPC3,6 DKO mouse ventricular myocytes after treatment of ET-1 to induce hypertrophic condition. The drug didn't affect the mRNA level of ANP, BNP, RCAN, TRPC3, and TRPC6 in WT and TRPC3,6DKO. The lack of the effect with the 503A in control mouse ventricular myocytes could be due to low level of TRPC expression. Therefore, we used TAC (transverse aortic constriction) model to induce hypertrophy, which can increase the expression of TRPC. Indeed, TAC-model of WT has up to ~9 folds increased level of TRPC3,6 expression compared to sham-WT mouse ventricular myocytes. In TAC-WT ventricular myocytes, 503A effectively reduced the increased expression of ANP, BNP, RCAN, and TRPC6 induced by ET-1. However, 503A failed to change the increased expression of ANP, BNP, and TRPC6 induced by ET-1 in TAC-TRPC3,6DKO. We can use this primary culture system of mouse cardiomyocytes to examine the long-term (> 1 day) cellular responses such as gene expressions and protein modifications by various stimulants in our KO mouse. Also, the results can open the possibility that the drug can be used as a new treatment for cardiac hypertrophy.

Key Words: Hypertrophy, Mouse cardiac cell culture, TAC model, TRPC, TRPC3,6-DKO

MP-52

Exogenous Hydrogen Peroxide Induces Lipid Raft-Mediated STAT-6 Activation in T Cells

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Reactive oxygen species (ROS) perform various functions in infection, inflammation, and cancer progression. Recently, hydrogen peroxide (H_2O_2) was reported to trigger the phosphorylation of signal transducer and activator of transcription STAT-6 in a time- and dose-dependent manner in the lipid rafts of astrocytes. STAT-6 is a transcription factor activated by interleukin IL-4 and IL-13, and is known to play a central role in T_H2 polarization of the immune system as well as leukemia and lymphoma progression. In the present study, we showed that H_2O_2 induced rapid and strong phosphorylation of STAT-6 in Jurkat and EL4 cells, similar to that observed in astrocytes. Interestingly, the extent of STAT-6 phosphorylation was further enhanced by co-treatment with anti-CD3 antibody. We disrupted the lipid rafts by using the cholesterol-depleting agent methyl-beta-cyclodextrin (MCD) and found a significant reduction in the H_2O_2-induced STAT-6 phosphorylation in the Jurkat cells. Further, incubation with cholesterol restored the H_2O_2-induced STAT-6 phosphorylation in these cells. Taken together, these results indicate that STAT-6, which is involved in T cell polarization and activation, was phosphorylated or activated by exogenous ROS, and that further activation of STAT-6 was induced through the raft-mediated signaling pathway by co-stimulation with anti-CD3 antibody and H_2O_2.

Key Words: ROS, STAT-6, T cell, Lipid raft

MP-53

Effects of PDE9A Knock-Out on the Gene Expressions of Hypertrophic Markers in Cultured Mouse Cardiomyocytes of Transverse Aortic Constriction (TAC) Model

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We examined the effect of PDE9A knock-out on the mRNA level of hypertrophic marker genes, such as ANP, BNP, and RCAN, by using qPCR in cultured WT and PDE9A-KO mouse cardiomyocytes. We induced hypertrophic condition by treatment of ET-1 for >1 day and we used PDE9A inhibitor to examine the subtype-specificity of PDE9A. In WT mouse cardiomyocytes, PDE9A inhibitor at 5 μM effectively reduced the increase of gene expression of ANP and BNP induced by ET-1 although the drug at 1 μM didn't affect. However, the drug's negating effect on the gene expression was lack in the PDE9A-KO mouse cardiomyocytes. PDE9A-KO itself decreased BNP expression in sham mouse cardiomyocytes when compared with WT. Next, we tested the effect of TAC model on the hypertrophic marker gene expression in PDE9A-KO cardiomyocytes. PDE9KO itself still reduced the expression of ANP, BNP, and RCAN in TAC mouse cardiomyocytes when compared with WT-TAC. Also, in WT-TAC, PDE9A inhibitor at 5 μM effectively reduced the increase of gene expression of BNP (not ANP and RCAN) induced by ET-1, however, the drug's negating effect on the gene expression of BNP was lack in the PDE9A-KO-TAC mouse cardiomyocytes. We think that further experiments such as protein expression and biochemical modification by knock-out of PDE9A and TAC modeling should be needed to clarify the function of PDE9A in hypertrophy of cardiomyocytes. For this purpose, we can use this primary culture system of mouse cardiomyocytes to examine the long-term (>1 day) cellular responses by various stimulants in our KO mouse. Also, the results show the possibility that the new target for the treatment of cardiac hypertrophy could be PDE9A in the future.

Key Words: Hypertrophy, Mouse cardiac cell culture, PDE9A, PDE9A-KO, TAC model

MP-54

3, 3-Diindolylmethane induces Apoptosis via Activating Hippo Signaling Pathway in Gastric Cancer Cells

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Recent studies revealed that 3, 3-diindolylmethane (DIM) has antitumor effects both in vivo and in vitro models in various cancer cells. However, the biological functions of DIM in human gastric cancer cells are unknown. And genetic and biological studies have confirmed the importance of the novel hippo tumor suppressor pathway in regulating cell proliferation, apoptosis, organ size, and tumorigenesis in mammals. Thus, the purpose of this study was to investigate the cytotoxic effects of DIM in human gastric cancer cells and to elucidate whether DIM induce cell death by activating hippo signaling pathway. Four human gastric cancer cell lines (SNU1, SNU16, SNU484, and SNU638) were used to test the response of DIM. MTT, cell cycle and western blot analyses were conducted. DIM significantly inhibited the proliferation of human gastric cancer cells in a dose-dependent manner. The percentage of G1 phase cells increased 24h after being treated by DIM. DIM reduced CDK2, CDK4, CDK6, CyclinD1 protein levels and increased p53 protein levels. DIM also induced cleaved poly (ADP-ribose) polymerase, cleaved-caspase-9 levels, and diminished pro-caspase-3 protein expression levels. Additionally, DIM increased P-LATS1, Mob1, P-Mob1, P-Yap protein levels and reduced Yap protein levels. These results indicate that DIM leads to G1 cell cycle arrest and induces apoptosis by activating hippo signaling pathway in human gastric cancer cells.

Key Words: DIM, Human gastric cancer, Apoptosis, Hippo signaling pathway

NC-1

Neuroprotective Mechanisms of Dieckol against both Neuronal Mitochondrial Dysfunction and Microglia-Mediated Neurotoxicity

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In this study, we investigated the neuroprotective effect of anti-oxidant dieckol isolated from marine brown alga *Ecklonia cava* on glutamate-induced neuronal cell death and lipopolysaccharide (LPS)-stimulated microglial activation. Dieckol significantly attenuated reactive oxygen species (ROS) production, mitochondrial Ca^{2+} overload, ATP depletion, loss of mitochondrial membrane potential ($\Delta\Psi_m$), and the subsequent cell death induced by glutamate toxicity both in primary cortical neurons and HT22 neurons. These events are mediated by a lipid-oxidizing enzyme, 12/15-lipoxygenase (12/15-LOX), as evidenced by LOX inhibitors baicalein and AA-861. Dieckol also reduced translocation of the pro-apoptotic bcl-2 family member Bid to mitochondria, followed by mitochondrial apoptosis inducing factor (AIF) translocation to the nucleus. Furthermore, diekcol itself induced heme oxygenase-1 (HO-1) expression via the Nrf2 (NF-E2 related factor 2) nuclear translocation. In addition, dieckol potently suppressed LPS-stimulated pro-inflammatory mediators including nitric oxide (NO), inducible nitric oxide synthase (iNOS), ROS, and cytokines in BV-2 cells. Dieckol pretreatment markedly induced down-regulation of LPS-induced nuclear translocation of nuclear factor κB (NF-κB) p65 through preventing the degradation of I-kappa B and phosphorylation of Akt and mitogen-activated protein kinases (MAPKs). In addition, LPS-induced NADPH oxidase protein levels were also suppressed by dieckol, suggesting that dieckol played an inhibitory role via downregulation of NADPH oxidase as well as the known scavenging activity of ROS in microglial activation. Concomitantly, dieckol protected neurons against microglia-mediated neurotoxicity in both microglial conditioned media and neuron-microglia co-culture system. Altogether, these results suggest that dieckol possesses neuroprotective potential via inhibition of oxidative mitochondrial dysfunction and neuroinflammation implicated in neurodegeneration.

Key Words: Neuroinflammation, Neuroprotection, Glutamate oxidative stress, Mitochondria dysfunction, Dieckol

NC-2

Benefits of Beta-lapachone in Brain Metabolic Insult

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Interruption of a smooth blood flow in cerebral artery causes brain ischemia and induces a striking metabolic change in the corresponding area. Therefore, it is still required to discover a valuable neuroprotection agent against metabolic distress. In this study, we tested the hypothesis that beta-lapachone, which is not defined any effect in brain insult yet, may contribute to delay development of injury. When focal cerebral ischemia/reperfusion insult was exerted, beta-lapachone markedly rescued the brain cells from metabolic distress. In addition, beta-lapachone ameliorated neuronal death from challenge of energy supplement in the blockage of glycolysis, not oxidative phosphorylation. The striking phenomenon of beta-lapachone was the improvement of intracellular ATP and preservation of mitochondrial integrity, regardless of restoration of glycolytic activity. The possibility of this mitochondrial protection was that beta-lapachone provoked the oxidation of intracellular excitatory amino acid, glutamine and glutamate in mitochondria through the truncated tricarboxylic acid cycle. The oxidation of these amino acids was accompanied by activation of phosphate-activated glutaminase and glutamate dehydrogenase. Beta-lapachone can be one of energetically preventive neuroprotection agent due to utilizing the alternative metabolites when cellular energy metabolism was damaged by shortage of major energy supplement, such as stroke.

Key Words: Beta-lapachone, Energy metabolism, Neuronal death

NC-3

The Effect of Riboflavin in the Neuropathic Pain Model

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Vitamin B has been widely used to relieve various pain, including carpal tunnel syndrome, migraine, and chronic pain that results from vitamin B deficiency. Previous study has demonstrated that vitamin B produce antinociceptive effect against chemical- and heat-induced pain. Moreover, riboflavin (vitamin B2), among the vitamin B, alleviated the formaldehyde-induced pain response and inhibited action of the inflammatory mediator. Although there are some studies showing the effect of riboflavin on inflammatory pain model, the fewer studies have been reported in neuropathic pain model. This study was designed to determine if riboflavin reduces the neuropathic pain induced by transection of spinal nerve (L5& L6). Male Sprague-Dawley rats were anesthetized and then introduced a spinal nerve transection (SNT). Riboflavin was injected intraperitoneally (100, 300 mg/kg) or intrathecally (0.1 mmol in 10 μl) into rats more than 10days after spinal nerve injury. To evaluate pain, paw withdrawl threshold (PWT) and paw withdrawl latency (PWL) were measured for 24 hours (0.5, 1, 2, 4, 24). As a result of riboflavin treatment, no PWT

were significantly decreased. PWL also was not decreased after the riboflavin injection. These results indicate that riboflavin might not alleviate pain response induced by SNT.
Key Words: Vitamin, Riboflavin, Neuropathic pain

NC-4

Role of JAK2/STAT3 on Injury-Induced Astrogliosis

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Background: Astrocytes undergo reactive responses to various CNS insults. This process is well-known as astrogliosis, which is characterized by extensive hypertrophy, increased glial filament acidic protein and proliferation. Although astrogliosis is beneficial for injured central nervous system, excessive astrogliosis can form a local biochemical and physical barrier that hampers axonal regeneration. In this study, we investigated effect of JAK2 inhibition on astrogliosis *in vitro* and *in vivo* model. **Observations:** In *in vitro* astrocyte cultures, scratch wound with sterile 10 μl pipette tip increased the number of proliferative cell markers, BrdU and Ki-67 positive cells. Cell bodies and cytoplasmic processes of astrocytes showed hypertrophy and extended to the denuded area. However, treatment of JAK2 inhibitor AG490 significantly decreased the number of proliferative cells in wound edge, and also prevented repopulation of denuded area. Moreover, JAK2 and the downstream molecule of JAK2, STAT3 were also activated by scratch wound injury. Furthermore, scratch injury-induced cyclin D1 expression and activation of JAK2/STAT3 signaling pathway was reduced by AG490. In addition, cortical stab wound injury to mouse cerebral cortex led to significant increase of Ki-67 positive cells level and expression of GFAP in the vicinity of lesion site, which were remarkably reduced by AG490 injection. **Conclusions:** Taken together, our study showed that the stab wound in the brain or scratch wound in astrocyte culture induced astrocyte proliferation and astrogliosis through JAK2/STAT3 signaling. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No.2012011417).
Key Words: Scratch, JAK2, STAT3, Proliferation

NC-5

Determination of Spontaneous Firing Rate by the Area Ratio of Proximal Dendritic Compartments to Soma in the Dopamine Neurons

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Dopamine neurons in the midbrain generate action potentials spontaneously at a regular rhythm and the rate of spontaneous firing determines tonic dopamine levels in the brain. Spontaneous firing activity of the multipolar midbrain dopamine neurons can be determined and/or modulated by many factors including distribution and density of ion channels at specific compartments of the neuron. Since dopamine neurons consist of the large soma and multiple excitable dendrites, the morphological characteristics of the dopamine neurons, such as the size of the soma, the number of primary dendrites, and relative excitable areas of proximal dendritic compartments, may affect excitability of the neurons. However, the relationship between the intrinsic excitability and the morphological features has never been investigated in the midbrain dopamine neurons. To find the best factors determining the intrinsic firing rate in the dopamine neurons, we analyzed the morphological parameters of the tyrosine hydroxylase-positive dopamine neurons in the rat midbrain. Isolated dopamine neurons showed various sizes of the soma, 3-6 proximal dendrites, and different ratios of proximal dendritic compartments to soma areas. The number of primary dendrites had no correlation with spontaneous firing rate, while the soma size showed a very little correlation with the rates of spontaneous firing. However, the ratios of proximal dendritic area to somatic area are strongly proportional to the spontaneous firing rate in the dopamine neurons. These data indicate that the proximal dendritic compartment is the most important unit for generation of spontaneous firing and that the spontaneous firing rate could be determined by the ratio of excitable areas of the proximal dendrites to the somatic areas in the midbrain dopamine neurons.
Key Words: Dopamine neuron, Dendrite, Area ratio, Spontaneous firing

NC-6

Role of NKCC1 and KCC2 in the Modulation of Supraoptic Nucleus Neuronal Activity during Sleep-Wake Cycle

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Magnocelluar neurosecretory cells of the hypothalamic supraoptic nucleus (SON) release vasopressin (antidiuretic hormone) and oxytocin (natriuretic hormone). Vasopressin increases water reabsorption from the kidney and oxytocin causes natriuresis. The SON controls body fluid volume and osmolarity through the action of vasopressin and oxytocin. It has been reported that the neurosecretory function of the SON is modulated during sleep-wake cycle. However, the mechanism of circadian modulation is not clear. SON neurons receive GABAergic synaptic inputs and GABAergic synaptic action may be inhibitory or excitatory according to the intracellular chloride concentration. Cation-chloride co-transporters are important in the control of chloride concentration and SON neurons express abun-

dant amount of chloride-importer NKCC1 and chloride-exporter KCC2. In this study, we examined the role and mechanism of NKCC1 and KCC2 in the circadian modulation of SON activity during sleep-wake cycle. The expression of NKCC1 was slightly increased during day-time and decreased during night-time. The expression of KCC2 was increased at late day-time and early night-time. The change in KCC2 expression was more prominently than that in NKCC1. The results indicate that SON neuronal activity might be increased through an altered GABAergic synaptic influence at early sleep period and decrease at late sleep and early wake state. In sleep deprivation experiments as a prolonged wake condition, the expression of NKCC1 was increased and that of KCC2 was decreased. The results indicate that SON neuronal activity might be decreased through an altered GABAergic synaptic influence in sleep deprived condition. This study suggests that NKCC1 and KCC2 might play an important role in the circadian modulation of supraoptic nucleus neuronal activity. in sleep deprived condition or prolonged wake state.
Key Words: Supraoptic nucleus, Sleep-wake cycle, NKCC1, KCC2

NC-7

Partial Mitochondrial Membrane Depolarization via Mitochondrial K^+ Channels is Involved in the Neuroprotective Mechanism of Indomethacin against Glutamate-Induced Excitotoxicity

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Partial mitochondrial depolarization has been recently evaluated as a novel mechanism of neuroprotection via inhibiting neurotoxic mitochondrial calcium overload during neuronal insults. In this study we investigated the neuroprotective effect of indomethacin-induced mitochondrial membrane potential in glutamate-induced excitotoxicity model and clarified the underlying mechanism of action. We demonstrated here that indomethacin significantly attenuated glutamate-induced mitochondrial calcium overload, reactive oxygen species (ROS) overproduction, and the subsequent cell death in the primary cortical neurons. Indomethacin substantially evoked mitochondrial membrane depolarization but did not show any cytotoxicity. Furthermore, the blockade of mitochondrial K^+ channels such as ATP-sensitive potassium channels (K_{ATP}) and large-conductance calcium-activated potassium channels (BKca) significantly attenuated indomethacin-induced mitochondrial depolarization. These results suggest that indomethacin-induced mitochondrial depolarization may be a novel mechanism of neuroprotection via inhibiting neurotoxic mitochondrial calcium overload during neuronal insults and mitochondrial K^+ channels may be play a role in indomethacin-induced mitochondrial depolarization.
Key Words: Indomethacin, Mitochondria, Mitochondrial calcium, Mitochondrial membrane potential, Cell death, Glutamate

NC-8

Role of Oxidative Stress and Nitric Oxide in the Wake-Promoting Action of Serotonergic Dorsal Raphe Nucleus Neurons

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Reactive oxygen species (ROS) are produced as a normal product of cellular metabolism and induce oxidative stress and endoplasmic reticulum (ER) stress. Reactive nitrogen species including nitric oxide (NO) is also produced by oxidative metabolism of the cells and NO is well known as a biological messenger. Sleep is as an important mechanism that recovers the cell function to the normal state by decreasing ROS and oxidative stress developed during wakefulness. The dorsal raphe nucleus (DRN) is known as a wake-promoting area in sleep-wake cycle and most DRN neurons are serotonergic which projects to various regions of the brain. It has been reported that sleep-related neurons of the brain including DRN show a specific expression pattern of ER stress markers and neuronal nitric oxide synthase (nNOS) during sleep-wake cycle and a sleep-deprivation condition. In this study, we examined the role of ROS and NO in the wake-promoting action of serotonergic DRN neurons during sleep-wake cycle. The expression of GRP78, a master regulator of ER stress, was decreased during the day-time. The nNOS expression was increased during the day-time and decreased during the night-time. The expression of c-Fos was increased during the day-time. The results indicate that DRN neuronal activity might be increased during the day-time through unknown mechanisms activated by NO or inhibited by ROS. In addition, nNOS expression was significantly increased during sleep-deprivation. The result indicates that the DRN neuronal activity might be activated by NO increased during sleep-deprivation or a prolonged wake state.
Key Words: Dorsal raphe nucleus, Sleep-wake cycle, Nitric oxide, Oxidative stress, Serotonin

NC-9

Reduction in Synaptic Nitric Oxide Function Contributes to Neuronal Excitation of presympathetic PVN Neurons in Rats with Myocardial Infarction

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Recent *in vivo* studies indicate that the reduction of Nitric Oxide (NO) contributes to the elevation of neuronal activity in the hypothalamic paraventricular nucleus (PVN) and sympathetic overactivity in the rats with heart failure. How-

ever, the synaptic mechanism underlying such neuronal plasticity is not well understood. To determine whether the reduced synaptic NO signaling system mediates the plasticity of presympathetic PVN neurons, we analyzed spontaneous firing activity and IPSCs in the PVN neurons projecting to the rostral ventrolateral medulla (PVN-RVLM) in the rats with the heart failure by using slice patch clamp methods. Myocardial Infarction (MI) was induced by coronary artery ligation in rats, and the PVN-RVLM neurons were labeled by a retrograde dye, and electrical activity of PVN-RVLM neurons were recorded at 2, 4, 6, and 8 weeks post MI. The firing rate of PVN-RVLM neurons was higher in MI than in Sham rats. Neuronal nitric oxide synthase (nNOS) immunoreactivity was lower in MI rats than in Sham rats. L-arginine reduced the firing activity of PVN-RVLM neurons, and the inhibitory effect of L-arginine was more pronounced in MI than in Sham rats. L-arginine increased the frequency of miniature IPSCs and its effect was more pronounced in MI than in Sham rats. All these MI-induced changes occurred at 2 weeks and lasted for up to 8 weeks post MI. Collectively, our findings indicate that the basal NO signaling system in the PVN is reduced in MI rats. It is likely that the elevated firing activity in the presympathetic PVN neurons in MI rats is due to the reduced synaptic NO signaling system, and resulting decrease in the GABAergic inhibitory inputs to the PVN neurons. This plasticity occurred as early as 2 weeks and lasted for up to 8 weeks post MI. The results provide a synaptic mechanism for the sympathetic hyperactivity commonly seen in heart failure.

Key Words: Sympathetic overactivity, Heart failure, Nitric oxide, Rostral ventrolateral medulla, Slice patch clamp

NC-10

Monitoring Neural Signals with a Newly Developed Closed-loop Neuromodulation System

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Neuromodulation (NM) system is a minimally invasive device which delivers mild electrical pulses to specific sites in the nervous system to treat various nervous malfunctions, such as movement disorders (including Parkinson's disease, essential tremor and dystonia), chronic pain, spasticity, overactive bladder, urinary retention, fecal incontinence, benign prostatic hyperplasia and gastroparesis. However, currently available NM systems simply deliver electrical stimulation to a target tissue without monitoring neural activities during pre or post-stimulation periods. Therefore, it is impossible to unveil the neural circuit mechanism of the treatment by the NM systems used in clinics at this time. We have developed a wirelessly powered neural prosthesis (WPNP) capable of 1) recording of neural signals and 2) delivering electrical stimulation to neural tissue. The system consists of a MCU (MSP430F2616, TI, USA) for acquisition of neural signals and stimulation of neural tissue, a Bluetooth-based wireless module (FB155BC, FirmTech, Korea) for transmission of neural signals to external receiver, and 2 Litz coils for wireless energy transfer. The recording part includes neural signal detection, amplification using the character of TLV2374 rail-to-rail OP amplifier and FIR (finite impulse response) band-pass filter (300-1,200 Hz). The part of wireless energy transfer is a system that supplies power by induced electromotive force between parallel coils. The efficiency of the system was verified by anesthetized rats. Each animal is anesthetized with isoflurane. A laminectomy was performed between L1 and L2 vertebra corresponding to L6 and S1 spinal cord segments. A 2 channel tungsten electrode (50 μm in diameter, A-M systems, USA), connected to the developed WPNP, was implanted for the neural activities recording from the L6 to S1 dorsal horn. The neural activities were displayed on the LabView-based monitoring program in real time. We believe that our WPNP system will open a new era for the closed-loop NM devices in world markets. Supported by Brain Research Center (BRC, 2012K001127), Ministry of Knowledge Economy (MKE, 10033634-2012) and National Research Foundation of Korea (NRF, 2012-0005787).

Key Words: Neuromodulation system, Neural prosthesis, Spinal cord, Rat, Neural signal

NC-11

mGluR1 Associates with Lipid-Rafts for Receptor Activity and Calcium Signaling by Interacting with Caveolin

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Group I metabotropic glutamate receptors (mGluR1/5) have important roles in the synaptic activity of the central nervous system. They modulate neuronal excitability by mobilizing intracellular Ca^{2+} following receptor activation. Until recently, accumulating evidence has indicated the association of Ca^{2+} signaling with lipid rafts. A specialized subset of lipid rafts contains caveolin which promotes rafts-localization of other membrane proteins. In the present study, we investigated the role of lipid rafts on the mGluR1-mediated Ca^{2+} transients with hippocampal primary culture neurons. We show that the disruption of lipid rafts using methyl-β-cyclodextrin markedly decreased mGluR1-mediated Ca^{2+} transients and rafts-localization of the receptor. Furthermore, transfection of mGluR1α mutant disrupted with caveolin-binding domain reduced rafts-localization of the receptor as well. Also, application of peptide blocker of binding between mGluR1α and caveolin reduced mGluR1-mediated Ca^{2+} transients and raft-localization of the receptor as well. Taken together, these results suggest that the

binding of mGluR1 to caveolin is crucial for mGluR1 localization to lipid rafts and modulation of mGluR1-mediated Ca^{2+} signaling.
Key Words: mGluR, Lipid-rafts, Caveolin, Calcium signaling, Hippocampal primary neuron

NC-12

Cytidine 5'-Diphosphocholine (CDP-Choline) Reduced Hypoglycemia-Induced Neuron Death

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Diabetic patients who attempt strict management of blood glucose levels frequently experience hypoglycemia. Severe and prolonged hypoglycemia causes neuronal death and cognitive impairment. There is no effective tool for prevention of these unwanted clinical sequelae. Citicoline (CDP-choline; cytidine 5'-diphosphocholine) is an important intermediate in the biosynthesis of cell membranes phospholipids. Citicoline serves as a choline donor in the metabolic pathways for biosynthesis of acetylcholine and neuronal membrane phospholipids, mainly phosphatidylcholine. The ability of citicoline to reverse the neuronal injury has been tested in animal models of cerebral ischemia and also has been performed clinical trial in stroke patients. However, no previous report has examined the effect of citicoline on hypoglycemia-induced neuron death. To clarify the therapeutic potency of citicoline on hypoglycemia-induced neuron death, we used an animal model of insulin-induced hypoglycemia. Acute hypoglycemia was induced by intraperitoneal injection of human insulin (10 U/kg), and then iso-electricity was maintained for 30 minutes. Citicoline injection was started immediately after hypoglycemia (500 mg/kg, i.p.). Neuronal injury and microglia activation was evaluated at 1 week after hypoglycemia. Here we found that post-treatment of citicoline showed significant less neuron death and microglia activation in the hippocampus compared to vehicle treated group. Taken together, these results suggest that neuronal membrane stabilization by citicoline can rescue neurons after severe hypoglycemia as seen in several ischemia studies. The present study suggests that citicoline may have a high therapeutic potential to reduce hypoglycemia -induced neuronal death.
Key Words: Citicoline, Hypoglycemia, Diabetic patient, Neuron death

NC-13

Decreased Cysteine Uptake by EAAC1 Gene Deletion Exacerbates Neuronal Oxidative Stress and Neuronal Death after Traumatic Brain Injury

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EAAC1 (excitatory amino acid carrier type 1 or EAAT3) is a high affinity glutamate transporter that actively moves glutamate from the extracellular space into neurons. EAAC1 has a negligible effect on glutamate clearance from the extracellular space because this function is performed primarily by the astrocyte glutamate transporters, GLT1 and GLAST. However, EAAC1 uptake of cysteine into neurons contributes to neuronal antioxidant function by providing cysteine substrate for glutathione synthesis. We have previously shown that mice lacking EAAC1 exhibited increased susceptibility to neuronal oxidative stress after ischemia, and developed brain atrophy and cognitive decline with aging. Here we evaluated the role of EAAC1 in neuronal resistance to traumatic brain injury (TBI). Young adult C57BL/6 wild-type or *EAAC1*$^{-/-}$ mice were subjected to a weight drop model. The outcome measures were neuronal death, superoxide production, oxidative injury and microglia activation. Neuronal death evaluated by Fluoro-Jade B staining 24 hours after TBI showed more than twice as many degenerating neurons in *EAAC1*$^{-/-}$ mice as in wild-type mice. Superoxide production measured by the dihydroethidium method 3 hours after TBI similarly showed a marked increase of ROS in the *EAAC1*$^{-/-}$ mice compared to wild-type mice. Oxidative injury into hippocampal neurons detected by 4HNE immunofluorescence staining and microglia activation evaluated by F4/80 immunoreactivity was also higher in the *EAAC1*$^{-/-}$ mice. These findings suggest that cysteine uptake by EAAC1 is important for neuronal antioxidant function and survival following TBI.
Key Words: Traumatic brain injury, EAAC1, Microglia, Neuron death, Reactive oxygen species, Lipid peroxidation

NC-14

Muscarine-Induced Parallel Fiber-Purkinje Cell Synaptic Transmission Alters Spontaneous Firing Activity of Purkinje Cells in the Vestibulo-Cerebellum

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Cholinergic neuro-modulation is whole-brain-wide phenomena such as muscarine-induced theta oscillation of hippocampal neurons. It was validated that the vestibulo-cerebellum (cerebellar flocculus and lobule IX~X of vermis) receives much more cholinergic input than other regions of cerebellum. A previous study showed that muscarine application evoked membrane depolarization of granule cells in the vestibulo-cerebellar cortex, and therefore spontaneous excitatory post-synaptic current (sEPSC) of Purkinje cell was increased. However, this study did not show how

enhanced parallel fiber-Purkinje cell (PF-PC) synaptic transmission by muscarine affects output of Purkinje cells, the sole output neurons of cerebellum. In our study, we have examined this relationship between enhanced PF-PC synaptic transmission by muscraine and output of Purkinje cells. We recorded spontaneous firing activities of Purkinje cells in the vermis lobule X, the vestibulo-cerebellum, and found out that muscarine application induced potentiation of the firing rate. Moreover, this potentiation is maintained even for a long time after muscarine application for 10 minutes, which implies that prominently increased PF-PC synaptic transmission induced intrinsic plasticity of Purkinje cells. Unlike other regions of cerebellum, Purkinje cells in the vermis lobule X have very heterogeneous firing rate, ranging from less than 1 Hz to about 10 Hz. In our results, we observed that the degree of this potentiation was inversely proportional to the basal firing rate, which indicates that Purkinje cells with low-frequency firing rate were more potentiated, whereas cells with high-frequency firing rate were less potentiated. In conclusion, our results strongly demonstrate that muscarine-induced PF-PC synaptic transmission potentiated the spontaneous activity of Purkinje cells in the vestibulo-cerebellum for a long time, and the degree of this potentiation was highly dependent on the basal firing rate of these Purkinje cells.

Key Words: Cerebellum, Vestibulo-cerebellum, Purkinje neuron, Muscarine, Intrinsic plasticity

NC-15

Neuroporetctive Effects of Lipoic Acid on Kainic Acid-Induced Neurotoxicity in Organotypic Hippocampal Slice Culture

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Kainic acid (KA) has been used to study the mechanisms of status epilepticus-induced neuronal damage and epileptogenesis since KA treatment of organotypic hippocampal slice culture (OHSC) induces region-specific neuronal death and reorganization of hippocampal circuitry. The present study investigated the neuroprotective effects of lipoic acid, an antioxidant, against oxidative stress induced by KA in OHSC of rats. Cultured slices were injured by exposure to 5 μM KA for 18 hr and then treated with different concentrations of lipoic acid. Neuronal cell death measured as propidium iodide uptake was reduced at 24 hr after lipoic acid treatment. We also observed an increased number of surviving CA3 neurons in the lipoic acid-treated groups using cresyl violet staining. Lipoic acid treatment significantly decreased the expression of NQO1 in the lipoic acid-treated groups was significantly lower than that in the KA only group. These results suggest that lipoic acid may protect hippocampal neurons against oxidative stress. This study was supported by the Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (20090076605).

Key Words: Lipoic acid, Kainic acid, Organotypic hippocampal slice culture, Antioxidant, Oxidative stress

NC-16

Comparative Study of Electrophysiological Properties at Spinocerebellum and Vestibulocerebellum

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Cerebellum is a brain region important in motor control. Vestibular Ocular Reflex (VOR) learning is usually used to test cerebellar functions. Cerebellum can be subdivided into cerebrocerebellum, spinocerebellum and vestibulocerebellum. Spinocerebellum receives inputs from spinal cord and auditory system, while vestibulocerebellum receives vestibular inputs from semicircular canals and vestibular nuclei. Even though vestibulecerebellum and spinocerebellum receive different inputs and vestibulocerebellum is the place for regulating balance and eye movements, recordings are usually taken at spinocerebellum. Flocculus is supposed to be the initial place of VOR learning and long-term depression is the mechanism for it. Here whole-cell recordings are made at lobules 3 to 5 of cerebellar vermis and flocculus, which are known to be the part of spinocerebellum and vestibulocerebellum, resepectively; and analyzed them for long-term depression and other electrophysiological properties. First, fast EPSC was measured and showed no difference in Input-Output curve and Paired Pulse Ratio, meaning they have similar presynaptic properties. Second, slow EPSC was measured in presence of NBQX. It is known that lobule X, a part of vestibulocerebellum, does not show slow current. Flocculus showed slow current, but input-output relationship curve of slow EPSC showed that for same input current, flocculus showed smaller size of slow current. Third, it is possible to record long-term depression at flocculus and it shows similar tendency. Further study will be tested on knockout mice with defects in VOR learning.

Key Words: Flocculus, Cerebellum, Synaptic plasticity, Long-term depression, Slow EPSC

NC-17

Involvement of 5-HT_1 and 5-HT_2 Receptors in Serotonin-Mediated Inhibition and Followed Excitation by Exogenous 5-HT on GnRH Neurons

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GnRH neurons are the central regulator of hypothalamic gonadal axis in human and non human primates. Serotonin (5-Hydroxy-tryptamine, 5-HT) is well known as a biogenic amine neurotransmitter in the CNS. It has been reported that 5-HT containing axons are juxtaposed to GnRH neurons. Although there are evidence for interaction between GnRH neuronal system and serotoninergic innervations, there is little report for the functional expressions of 5-HT receptors on GnRH neurons. In here, we examined the direct effects of 5-HT on GnRH neurons by using brain slice patch clamp technique. In the majority of GnRH neurons tested, 5-HT induced membrane hyperpolarization in a dose dependent manner with an EC_{50} of 11.87 μM followed by tonic excitation. 5-HT-induced hyperpolarization remain persisted in the presence of amino acid receptor blocking cocktail (AARBC) containing; AP-5, an NMDA receptor antagonist (20 μM), picrotoxin a, GABA receptor antagonist (50 μM), CNQX, a non-NMDA glutamate receptor antagonist (10 μM) and strychnine, a glycine receptor antagonist (2 μM) with TTX, a Na^+ channel blocker (0.5 μM) and in the presence of AARBC with 7-nitroindazole (7-NI), an NO synthase inhibitor manifesting the existence of 5-HT receptors on postsynaptic GnRH neurons. Further, 5-HT-induced hyperpolarization was blocked by $5HT_{1A}$ antagonist WAY-100635 and $5HT_{1A}$ mRNA was detected via single cell RT-PCR. In addition, the 5-HT-induced hyperpolarization was blocked by Ba^{2+}, a K^+ channel blocker (200 μM). In addition, this hyperpolarization was partly blocked by adenylyl cyclase activated by forskolin, and was almost completely blocked by forskolin followed by Ba^{2+}. The tonic excitation induced by 5-HT was abolished by $5\text{-}HT_2$ antagonist ketanserin. Further, Increase in endogenous 5-HT by selective serotonin reuptake inhibitor fluoxetine hydrochloride induced hyperpolarization suggesting the major effect of endogenous 5-HT on GnRH neurons as inhibitory. These results suggest that 5-HT mediates inhibitory actions on the GnRH neurons through $5HT_{1A}$ receptor activation and K^+ channel opening, partly via adenylyl cyclase inhibition and tonic excitation via $5\text{-}HT_2$ receptors on GnRH neurons.

Key Words: GnRH neurons, Serotonin. Gramicidin perforated patch clamp

NC-18

Role of Genistein on Gonadotropin-Releasing Hormone Neurons in Juvenile Female Mice

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We investigated the effects of the phytoestrogen genistein on gonadotropin-releasing hormone (GnRH) neurons using single cell electrophysiology on GnRH-green fluorescent protein (GnRH-GFP) transgenic juvenile female mice. Perforated patch-clamp recordings from GnRH-GFP neurons showed that approximately 83% of GnRH neurons responded to 30 μM of genistein with a markedly prolonged membrane depolarization. This effect not only persisted in the presence of tetrodotoxin, but also in the presence of amino acid receptor antagonists. Using a voltage clamp technique, we found that 30 μM of genistein increased the synaptic current of GnRH neurons voltage clamped at −60 mV in the presence of glutamate receptor blocker but not GABA receptor blockers. Pre-incubation of GnRH neurons with 30 μM of genistein enhanced kisspeptin-induced membrane depolarization and firing. GnRH neurons of juvenile mice injected with genistein *in vivo* showed enhanced response kisspeptin compared to vehicle injected controls s. The TRPC-5 blocker 2-aminoethoxydiphenyl borate (2-APB, 75 μM) blocked the genistein mediated responses on GnRH neurons. These results demonstrate that in juvenile female mice, genistein acts directly on GnRH neurons to induce excitation via GABA neurotransmission and TRCP5 channels and enhance kisspeptin-induced activation.

Key Words: GnRH neurons, Patch clamp, Genistein, Kisspeptin, Juvenile

NC-19

Developmental Upregulation of Presynaptic NCKX Underlies the Decrease of Mitochondria-Dependent Post-Tetanic Potentiation at Calyx of Held Synapses

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The sensitivity of post-tetanic potentiation (PTP) to high frequency stimulation (HFS) steeply decays during the first two postnatal weeks. We investigated the underlying mechanisms for the developmental change of PTP induced by HFS (100 Hz, 2 sec) at postnatal days 4-6 and 9-11 at the calyx of Held synapse. Low concentration tetraphenylphosphonium (2 μM), an inhibitor of mitochondrial calcium Na/Ca exchanger, suppressed the amount of post-tetanic residual calcium and PTP to a larger extent at the immature calyx synapse, indicating a developmental reduction of mitochondrial contribution to PTP. The higher amount of mitochondrial Ca^{2+} uptake during HFS and consequent post-tetanic residual Ca^{2+} at the immature calyx of Held was associated with higher peak of HFS-induced Ca^{2+} transients, most likely because the mitochondrial Ca^{2+} uptake during HFS was supralinearily dependent on the presynaptic $[Ca^{2+}]$ level. Probing into the contribution of Na^+/Ca^{2+} exchangers to calcium clearance, we found a specific upregulation of the K^+-dependent Na^+/Ca^{2+} exchanger (NCKX) activity in the maturer calyx of Held. We conclude that the upregulation of NCKX limits Ca^{2+} buildup during HFS and inhibits mitochondrial Ca^{2+} uptake during HFS, which in turn results in the reduction of post-tetanic residual Ca^{2+} and PTP at the mature calyx of Held.

Key Words: Presynaptic, Calcium clearance, Post-tetanic potentiation, Calyx of held

NC-20(PO-13)

Vulnerable Seizure Activity in Neonatal Brain through Little COX Activity

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Seizures occurs with abnormal excessive electrical activity in the brain. The seizure activity is more common in young children than adults. Most neonatal seizures are extremely difficult to control with current anti-epileptic drugs (AEDs). In the previous our reports, COX-2 inhibitors aggravate KA-induced seizures and PGF2α might act as endogenous anticonvulsant in the adult mice. Therefore, we assumed whether management of COX activity regulates KA-induced neonatal seizure. Neonatal (post-natal day 9) mice are far more prone to KA-induced seizures than the adult (P35). The seizure activities in the adult, which was aggravated by COX inhibition, showed less than those in the neonate. However, the neonate seizure activities were not affected by COX inhibition. Interestingly, in the brain, COXs mRNA and protein expression increased during development. The maturation of COXs activity was correlated with glycosylation. However, in the neonate brain COX-1/2 were rarely expressed and could not be activated by excitable stimulus. By western blot analysis, sqRT-PCR and EIA assay, the neonate hippocampus expressed little COX-1/2 and little PGF2α with/without KA stimulation, whilst the adult hippocampus responded the increased COX-2 and PGF2α by KA. Also, the seizure activity in the neonate was alleviated by intracisternal PGF2α administration. Taken together, the expression and activity of COXs were developed and activated with brain maturation during development. Our findings suggested that neonatal vulnerability to seizure is closely associated with little COXs activity and following little PGF2α release. **Acknowledgement:** This study was supported by the Korea Research Foundation (KRF) grant funded by the Korea government (MEST) (20110002759), the Chronic Inflammatory Disease Research Center (KOSEF R-13-2003-019-01005-0) and a grant of the Korean Health Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Korea (A091120).
Key Words: Cyclooyxgenase, PGF2a, Seizure, Neonate

NC-21

Souble CCL5 from BM-MSCS in the Brains of AD Mice with Ab Deposition

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Microglia have the ability to eliminate amyloid b (Ab) by a cell-specific phagocytic mechanism, and bone marrow (BM) stem cells have shown a beneficial effect through endogenous microglia activation in the brains of AD mice. However, the mechanisms underlying BM-induced activation of microglia have not been resolved. We show that BM-derived mesenchymal stem cells (MSCs) induced the migration of microglia when exposed to Ab in vitro. Cytokine array analysis of the BM-MSC media obtained after stimulation by Ab further revealed elevated release of the chemoattractive factor, CCL5. The CCL5 was increased when BM-MSCs were transplanted into the brains of Ab-deposited AD mice, but not normal mice. Interestingly, alternative activation of microglia was associated with elevated CCL5 expression. Furthermore, by generating a chimeric mouse, we ascertained that the activated microglia resulted from endogenous BM cells that were recruited into the brain by CCL5. Additionally, we observed that neprilysin (NEP) and IL-4 derived from the alternative microglia were associated with a reduction in Ab deposition and memory impairment in AD mice. These results suggest that the recruitment of the alternative microglia into the brain is driven by CCL5 secretion from the transplanted BM-MSCs, which itself is induced by Ab deposition in the AD brain. This work was supported by the grants for the Bio & Medical Technology Development Program (2010-0020234) and Basic Science Research Program (2010-0003949, 2010-0009421) funded by the National Research Foundation (NRF) of the Ministry of Education, Science and Technology, Republic of Korea (Stem Cells, 2012, in press, *Co-corresponding author).
Key Words: Alzheimer's disease model, Bone marrow-derived mesenchymal stem cells, CCL5, Recruitment, Alternatively activated microglia

NC-22

Potentiation of Scolopendra Subspinipes Mutilans on NGF-Induced Neurite Outgrowth Mediated with MAPK Kinase Pathway in PC12 Cells

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The Scolopendra subspinipes mutilans (SSM) have been used on neurological disorders in the traditional oriental medicine. However, the molecular mechanism of SSM with regarding to the physiological effects has poorly been understood on cellular levels. The neurite growth of PC12 cells under presence of nerve growth factor (NGF) have been used as a model for the neural growth or regeneration *in vitro* experiment. Present study examined whether the neurite outgrowth of PC12 cells were affected by the treatment with SSM on their behavioral pattern and whether the MAPK kinase intracellular signaling involve in SSM-mediated outgrowth of PC12 cells. Treatment of SSM resulted in statistically significant the neurite outgrowth of PC12 cells under low concentration of NGF (5 ng/ml) with a dose-dependent manner. The expression of beta III Tubulin and GAP 43, a neuronal-specific marker in PC12 cells also was significantly up-regulated by SSM co- treatment with NGF, indicating that the processes of the neuronal dif-

ferentiation are potentiated by SSM. In addition, the study revealed that the the phosphorylation of ERK 1/2 protein in transitions of PC12 cells was upregulated in presence of SSM with a dose-dependent manner. On the other hand, application of ERK1/2 inhibitor on culture medium caused a significant suppression of SSM-mediated neurite outgrowth, which suggested biological effect of SSM treatment on PC12 cells is closely associated with induction of MEK/Erk1/2-mediated signaling pathway. In summary, this study suggested that the treatment with SSM under the presence of NGF may expect the synergistic effects for neuronal regeneration or neural plasticity. This study was supported by a grant from the Korea basic science institute (KBSI).

Key Words: Scolopendra subspinipes mutilans, PC12, NGF, MAPK kinase pathway

NC-23

The Role of Phorbol 12-Myristate 13-Acetate in the Induction of Long-Term Potentiation

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Phorbol-12-myristate-13-acetate (PMA) has been reported as tumor promoter and co-mitogen materials with other factors. PMA is known to potentiate exocytosis and modulate vesicle fusion kinetics in neurons and endocrine cells. We focus on the fact that PMA act as selective PKC agonist and increase formation of new spine of hippcampus. The purpose of this study was to examine which PKC isoforms are responsible for PMA-induced augmentation of long term potentiation (LTP) and if PMA modulates LTP through NMDARs in the CA1 stratum radiatum of hippocampus *in vitro*. We found that PMA enhanced the induction of LTP by one-episode of theta burst stimulation in dose-dependent manner without affecting to magnitude of baseline field excitatory postsynaptic potential. LTP facilitated by PMA (200 nM) were blocked in presence of a nonspecific PKC inhibitor Ro 31-8220 (10 μM); a selective PKCδ inhibitor rottlerin (1 μM) and a PKCε inhibitor TAT-εV1-2 peptide (500 nM). However, PMA did not induce LTP in the presence of DL-APV (50 μM, NMDA receptor blocker). Our results suggest that PMA is involved in synaptic plasticity in the nervous system via activation of PKCδ and/or PKCε.

Key Words: Phorbol-12-myristate-13-acetate, Long term potentiation, Protein Kinase C, Hippocampus

NC-24

Cholinergic Modulation of Synaptic Transmission in Layer 2/3 Pyramidal Neurons of Rat Visual Cortex

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Acetylcholine controls synaptic transmission and plasticity, which is involved in learning, memory, and adaptive sensory processing in humans and in experimental animals. However, the mechanistic interaction of cholinergic inputs with signaling pathways underlying acute and long-term synaptic plasticity remains unclear. We investigated cholinergic regulation of synaptic transmission in layer 2/3 pyramidal neurons of the rat visual cortex, where vertical and horizontal inputs are integrated, using the whole-cell patch-clamp technique in acute slices. Synaptic responses were evoked by bipolar tungsten electrodes located in layer 4 and layer 1. Bath application of the non-specific cholinergic agonist carbachol decreased the amplitude of evoked excitatory and inhibitory postsynaptic currents (eEPSCs and eIPSCs, respectively) to a similar extent. These effects were mimicked by the muscarinic cholinergic agonist muscarine in concentration-dependent manners, and this was abolished by the muscarinic receptor antagonist atropine. The muscarine showed no effect on the amplitude of the postsynaptic current elicited by pressure-applied glutamate. Moreover, muscarine increased the paired-pulse ratio of evoked excitatory postsynaptic potentials (eEPSPs) while it concomitantly decreased the amplitude of eEPSP, suggesting that its action was mediated by presynaptic mechanisms. Consistent with this observation, muscarine decreased the frequency of miniature EPSCs and miniature IPSCs, but had no effect on their amplitude. The muscarine-induced decrease in mEPSC frequency was partially blocked in the presence of the M_2 receptor antagonist methoctramine, the M_4 receptor antagonist PD 102807 or the M_1 receptor antagonist pirenzepine. We also found that muscarine-induced long-term depression is prevented by the endocannabinoid CB1 receptor antagonist AM-251 in inputs activated by stimulation of layer 4. Taken together, these results suggest that multiple types of muscarinic receptors are involved in synaptic transmission in layer 2/3 pyramidal neurons of the rat visual cortex. Moreover, long-term presynaptic muscarinic modulation might be mediated indirectly by the endocannabinoid signaling. Supported by the Basic Science Research Program through the NRF (2012-046621).

Key Words: Acetylcholine, Synaptic transmission, Visual cortex, Presynaptic

NC-25(PO-14)

Altered Property of Endogenous Analgesic System Following Chronic Neuropathic Pain

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It is now well established that descending pain modulation mechanisms of the central nervous system are involved in the maintenance of the chronic neuropathic pain. The endogenous analgesic effect as well as existent pain is thought to affect various brain regions and change their mode of actions. Thus, understanding the altered brain connectivity of chronic pain state and analgesic state may help us to understand possible underlying mechanism of maintenance of chronic pain. Among the related brain regions, midbrain periaqueductal gray (PAG) is thought to be one of the most important pain-related area in terms of integrating descending pain control from various brain regions. In this study, we investigated the change of the brain following chronic neuropathic pain and activation of PAG analgesic effect. Spinal nerve ligation surgery was operated to adult rats to establish chronic neuropathic pain. Twenty days after surgery, group I metabotropic glutamate receptor (mGluR) agonist was administered into ventrolateral PAG (VL-PAG). Activation of the mGluR within VL-PAG successfully evoked intrinsic analgesic effect sufficient to completely reverse neuropathic mechanical allodynia. Brain glucose metabolisms were measured with a pair of 18F-Flurodeoxyglucose-PET scans during chronic pain state and analgesic state respectively. Sham animals were also measured with same manner as control. Brain areas which showed metabolic change were selected as region of interest (ROI). Connectivity between each ROI was determined based on inter-subject covariation and brain networks of each state were constructed. Analysis of brain images revealed that activation of the VL-PAG altered resting-state metabolism within various brain areas. Nevertheless, metabolic connectivity of these areas was preserved in pain animals despite robust pain alleviation, suggesting reflection of secondary alterations related to the endogenous pain modulating system rather than pain perception or behavioral phenotype. Interestingly, this phenomenon was not prevalent for sham animals in spite of similar metabolic increase and decrease variation following VL-PAG activation. This preservation of connectivity specific to chronic pain brain indicates that there occurred alteration of mode of action within the brain areas related to endogenous analgesic system, which in turn reflect plastic change of the brain network involving chronic pain.

Key Words: Neuropathic pain, Brain network, Periaqueductal gray

NC-26

Low-Intensity Ultrasound Attenuates Ischemia-Induced Edema Formation in Rat Hippocampal Slices

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Brain edema, the net increase in water content of the brain parenchyma which results in an increase in brain tissue volume, is a major contributing factor to morbidity and mortality of a wide variety of nervous system disorders including head trauma, tumors, stroke, infections, and metabolic disorders. A consequence of this volume increase is the development of increased intracranial pressure leading to brain herniation, irreversible brain damage, and ultimately, death. Classically, two major types of brain edema have been shown to exist, namely, cytotoxic and vasogenic edema, however, a considerable overlap exists between these two types. In this study, we examined whether low intensity ultrasound (LIUS) stimulation can reduce brain edema formation. We used *in vitro* hippocampal slices as a model for studying cytotoxic brain edema. Cytotoxic edema can be induced in hippocampal slices *in vitro* by incubation in oxygen glucose deprived (OGD) artificial cerebrospinal fluid (ACSF) solution. Hippocampal slices were prepared from male Sprague-Dawley rats and divided into various groups and are incubated with/without LIUS stimulation of various intensities in normoxic and/or OGD conditions. Right after the incubation, the hippocampal slices were weighed for the wet weights, and are dried at 80^{o}C overnight in dry oven. The hippocampal slices were weighed again for their dry wet. The water content was calculated as (wet weight - dry weight) × 100/wet weight. Additionally, immunohistochemical (IHC) staining assays for aquaporin 4 (AQP4) was performed to study the localization pattern of AQP4 in normoxic, OGD, and LIUS stimulated groups of the hippocampal slices. We found that OGD conditions induced edema, and LIUS stimulation attenuates edema formation in intensity-dependent manner in the hippocampal slices. The water content of the LIUS stimulated hippocampal slices were significantly lower than those incubated in the OGD condition. As water channel protein AQP4 has been implicated in brain edema formation, we asked whether the lowering of edema formation by LIUS stimulation is related with AQP4 in one way or other. We found that the membrane localization of AQP4 in the astrocytic end-feet in blood vessels was increased in the edematous hippocampal slices, while the LIUS stimulated hippocampal slices showed decreased membrane localization of AQP4. These data suggest that LIUS stimulation attenuates brain edema formation possibly by decreasing AQP4 membrane localization in *in vitro* hippocampal slices.

Key Words: Low-Intensity Ultrasound (LIUS), Brain edema, Oxygen glucose deprivation (OGD), Water content, Aquaporin 4 (AQP4)

NC-27

Serotonergic Regulation of Long-Term Synaptic Depression is Mediated by Layer-Specific Modulation of Inhibitory Synaptic Transmission in the Rat Visual Cortex

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Serotonin [5-hydroxytryptamine (5-HT)] is one of the major neuromodulators involved in the regulation of long-term synaptic plasticity in the visual cortex. It is known that 5-HT suppresses the induction of long-term synaptic plasticity in rat visual cortex at the end of critical period. In our previous study, 5-HT inhibited the induction of N-methyl-D-aspartate receptor (NMDAR)-dependent long-term depression (LTD) in the pathway from layer 4 to layer 2/3 in the primary visual cortex prepared from 5-week-old rats. We also found that 5-HT selectively enhances GABAergic inhibitory synaptic transmission in perisomatic area. To identify the causal relationship between the enhancement of inhibitory transmission and the suppression of LTD by 5-HT, we studied the role of inhibitory modulation on the serotonergic regulation of induction of LTD. Excitatory postsynaptic potential (EPSP), evoked by extracellular current pulse (0.1 ms), was recorded in layer 2/3 pyramidal neurons in slices of visual cortex from 5-week-old rats. Stimulation was applied at either layer 1 or layer 4 to recruit different fibers to different cellular region. LTD was evoked by 1-Hz application of 900 stimuli. 5-HT selectively inhibited the LTD induced by layer 4 stimulation. 5-HT also selectively enhanced GABAergic inhibitory transmission evoked by layer 4 stimulation. The enhancement of inhibition by 5-HT was about 20% of the control. When we applied a $GABA_A$ receptor blocker at a concentration to suppress 20% of inhibition (bicuculline, 300 nM), the LTD-suppressing effect of 5-HT was completely abolished. These results suggest that 5-HT inhibition of LTD is mediated by modulation of GABAergic inhibitory transmission in a layer-specific manner. *Supported by the NRF* (No. 2012-046621).

Key Words: Visual cortex, 5-HT, LTD, Inhibition

NC-28

Inhibitory Effects of Cyanidin-3-Glucoside on Glutamate-Induced $[Zn^{2+}]_i$ Increase in Primary Cultures of Rat Hippocampal Neurons

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Cyanidin-3-glucoside (C3G) is a member of the anthocyanin family which belongs to flavo-noids. In addition to antioxidant effects, flavo-noids have been reported to affect functions of various ion channels including Ca^{2+} channels. Glutamate has been reported to induce an increase in intracellular free Zn^{2+} concentration ($[Zn^{2+}]_i$) in neurons through Ca^{2+} signaling, oxidative stress, and mitochondrial dysfunction. We investigated whether C3G affects glutamate-induced $[Zn^{2+}]_i$ increase *in* cultured rat hippocampal neurons from embryonic day 18 maternal Sprague-Dawley rats using digital imaging methods for Zn^{2+}, Ca^{2+}, reactive oxygen species (ROS), and mitochondrial membrane potential. Reproducible $[Zn^{2+}]_i$ increases were elicited by applying glutamate (100 μM) for 7 min at 30 min intervals. Pretreatment with a cell membrane-permeable Zn^{2+} chelator, N,N,N',N'-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN, 5 μM) for 2 min blocked glutamate-induced $[Zn^{2+}]_i$ response but it did not affect glutamate-induced $[Ca^{2+}]_i$ increase. Pretreatment with C3G (100 ng/ml to 1 mg/ml) for 30 min inhibited glutamate-induced $[Zn^{2+}]_i$ response in a concentration-dependent manner (IC_{50}=14.26 μg/ml). Treatment with the sulfhydryl oxidizing agent 2,2'-dithiodipyridine (30 μM) and H_2O_2 (100 μM) for 10 min, markedly induced an increase in $[Zn^{2+}]_i$ although they induced relatively a modest $[Ca^{2+}]_i$ increase. Pretreatment with the reducing agent dithiothreitol (50 μM) for 5 min blocked glutamate-induced $[Zn^{2+}]_i$ response markedly, but it did not affect glutamate-induced $[Ca^{2+}]_i$ response. Pretreament with C3G (15 μg/ml) for 30 min blocked glutamate, H_2O_2 and DTDP-induced generation of ROS. Pretreament with DTT (50 μM) for 5 min also blocked these generation of ROS. C3G significantly inhibited glutamate-induced mitochondrial depolarization. All these results suggest that cyanidin-3-glucoside inhibits glutamate-induced $[Zn^{2+}]_i$ increase by inhibition of calcium signaling, ROS formation, and mitochondrial depolarization in cultured rat hippocampal neurons.

Key Words: Flavonoid, Glutamate, Mitochondrial membrane potential, Reactive oxygen species, Zn^{2+}

NC-29

Molecular and Electrophysiological Identification of Autonomic Pelvic Ganglion Neurons Innervating the Urogenital System

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Pelvic ganglion (PG) innervating the urogenital system is unique among autonomic ganglia since both sympathetic and parasympathetic neurons are co-localized within the same ganglion capsule. To date, however, molecular and cellular signatures of each type of the PG neurons are not fully defined. In the present study, we combined electrophysiological recordings with single-cell RT-PCR analysis to associate expression of sympathetic and parasympathetic markers with firing patterns of the PG neurons from rats. Under the gramicidin-perforated patch-clamp configuration, PG neurons showed either phasic or tonic spike firing in response to a depolarizing current injection for 1 s. The phasic neurons could be further divided into the rapidly adapting phasic I neuron (34%) showing one or couple of spikes and then becoming silent and the slowly adapting phasic II neuron (21%) showing a burst of spikes and then becoming silent throughout an excitation. Conversely, the tonic neuron (45%) shows non-adapting spike firing and rise of spike frequency by increasing of stimulation strength. The phasic I (32±2 pF, 29±1 μm, n=57) and phasic II (44±3 pF, 30±2 μm, n=35) neurons have smaller capacitance and diameter when compared with the tonic

neurons (71±3 pF, 37±1 μm, n=74). Spike amplitude and duration were similar among the phasic I, phasic II, and tonic neurons. However, duration of afterhyperpolarization (AHP) was significantly longer in the phasic neurons than the tonic neurons although there was no significant difference in amplitude of AHP among three types of PG neurons. On average, AHP duration of the phasic I, phasic II and tonic neurons was 181±6, 171±5, and 137±6 ms, respectively. Single cell RT-PCR analysis revealed that the phasic I neuron expresses choline acetyltransferase (ChAT), whereas the tonic neuron does tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DβH). These data suggest that the phasic I and tonic neurons are parasympathetic and sympathetic, respectively. Interestingly, the phasic II neurons were found to express either ChAT or TH. In addition, 5-HT receptor 3A subunit and nNOS are expressed only in the phasic I neuron, whereas T-type α1H calcium channel and $GABA_A$ receptor β_2 subunit are in the tonic neuron. In conclusion, this study provides reliable criteria for identifying phenotypes of PG neurons, which is critical for understanding physiological and pathophysiological roles of each type of PG neurons in controlling the urogenital system.

Key Words: Pelvic ganglion (PG), Parasympathetic neuron, Sympathetic neuron, CaV3.2 T-type Ca^{2+} channel

NC-30(PO-15)

Combined Effects of Hematopoietic Progenitor Cell Mobilization from Bone Marrow by G-CSF and AMD3100, and Chemotaxis into the Brain using SDF-1α in Alzheimer's Disease Mouse Model

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Transplantation of bone marrow stem cells (BMSC) has been suggested as a potential therapeutic approach to prevent various neurodegenerative diseases. However, clinical attempts at exogenous BMSC injection for these disorders remain problematic. An alternative strategy is pharmacological-induced recruitment of endogenous BMSC into an injured site by systemic administration of growth factors or chemokines. The aim of the present study was therefore to examine the effects of combination therapy involving Granulocyte colony stimulating factor (G-CSF)/AMD3100 (CXCR4 antagonist) and stromal cell derived factor-1α (SDF-1α) on endogenous stem cell recruitment into damaged brain in an Alzheimer's disease (AD) mouse model. To mobilize BMSCs, G-CSF was injected in AD mice intraperitoneally, and boosted by AMD3100. Simultaneously, mice received intracerebral stereotaxic injection with SDF-1α to induce migration of mobilized endogenous BMSCs into brain. We found that the memory deficit in the AD mouse was significantly improved but amyloid β (Aβ) deposition was unchanged. Interestingly, microglial activation was increased with alternative activation of microglia to a neuroprotective phenotype and by generating a APP/PS1-GFP chimeric mouse we ascertained that the GFP positive microglia identified in the brain were BM derived. Additionally, increased hippocampal neurogenesis and improved memory was only observed in mice receiving G-CSF/AMD3100 and SDF-1α and not in controls receiving each treatment alone. These results suggest that SDF-1α is an effective adjuvant in inducing migration into brain of the endogenous stem cells, mobilized by G-CSF/AMD3100 and that the two can act synergistically to produce a therapeutic effect. This approach warrants further investigation as a potential therapeutic option for the treatment of AD patients in the future.

Key Words: Alzheimer's disease model, Granulocyte colony stimulating factor, Stromal cell derived factor, Stem cell mobilization, Bone marrow-derived hematopoietic progenitor cell, Bone marrow-derived microglia

MU-1

Changes in Vascular Contractility in TRPC3 KO Mouse

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Canonical transient receptor potential (TRPC) channels are Ca^{2+}-permeable nonselective cation channels that regulate ion homeostasis and intracellular Ca^{2+} signaling in numerous cell types. Important physiological functions such as vasoregulation and neuronal growth have been assigned to this class of ion channels. We investigated the role of TRPC3 channel in the modulationof vascular contractility. Myogenic tones, contractile responses, changes in $[Ca^{2+}]_i$, and releases of NO were measured. Transmural pressure-induced myogenic contraction was not different between TRPC3 WT and KO mice. Phenylephrine-induced contraction was slightly weaker in TRPC3 KO than TRPC3 WT mice in the 40 mmHg. Acetylcholine-induced vasorelaxation and changes in $[Ca^{2+}]_i$ was inhibited in TRPC3 KO mice. Pre-treatment with the selective TRPC3 inhibitor Pyr3 moderately decreased ACh-induced vasorelaxation of TRPC3 WT mice. Acetylcholine-induced NO release was smaller in arteries of TRPC3 KO than those in TRPC3 WT mouse. These results suggest that TRPC3 contributes to endothelial nitric oxide-mediated vasorelaxation in mouse mesenteric arteries.

Key Words: TRPC3, TRPC3 KO mouse, Vascular contractility, Nitric oxide

MU-2

Changes of Behavior and Protein Quantities According to the Sustained Stretching of Soleus Muscle in Rat

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Stretching of muscle is a general therapeutic maneuver, used to increase range of motion (ROM) by elongating structures that have adaptively shortened and become hypomobile for a long time. Several studies that have examined the effects of muscle stretching in a lengthened position suggested that the stretched muscle fibers increase the number of sarcomeres to maintain a normal passive length-tension relationship (Caiozzo et al., 2002). But it is not still clarified what kind of behavioral and molecular changes during sustained muscle stretch occurs. This study was performed to investigate the changes of protein expression and behavioral signs of favoring (weight shift to unaffected limb) according to the sustained stretching of soleus muscle by dorsiflexion using plaster cast in rat. *Sprague-Dawley* male rats (7 weeks) were divided into 7 groups; one was the normal group and 6 groups with sustained stretching of soleus muscle by combining fixed angles (30^o, 55^o) and various period (7, 14 and 21 days). We did animal behavior test to measure the changes in favoring and western blotting for the change of pro-apoptotic protein level (Bax) and TUNEL (terminal deoxynucleotidyl transferase nick end labeling) assay for measuring the apoptotic changes in isolated soleus muscle after sustained muscle stretch. In result, after the removing plaster cast, the group with fixed angle of 55^o showed significantly favoring of weight to unaffected limb at 7, 14 and 21 days than the group with fixed angle of 30^o did. In the normal group, Bax expression didn't change as time passed, but Bax expression increased in the sustained stretching groups (both 30^o and 55^o) significantly. And the number of TUNEL-positive myonuclei increased significantly at sustained stretching groups. We can conclude that sustained stretching of soleus muscle might induce the favoring of weight to unaffected side and it could be caused by apoptosis of stretched muscle confirmed by increased Bax expression and TUNEL-positive nuclei in soleus muscle. So, Bax can be considered to play an essential role in promoting the activation of apoptotic signaling cascades in sustained muscle stretch.

Key Words: Muscle stretching, Apoptosis, Bax

MU-3

Lipid-Raft-Related SNAP23 Contributes to the Regulation of Intracellular Cholesterol Exocytosis in Vascular Smooth Muscle Cell and the Progression of Hypertension in Rat

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Synaptosomal-associated protein of 23 kDa (SNAP23) is mainly localized at the plasma membrane and intracellular membrane. Recently, this protein, which is known as an important regulator in vesicle trafficking and exocytosis, was reported to be involved in various diseases including type 2 diabetes by reason of its involvement in lipid droplet formation and cholesterol transport control in plasma membrane. Moreover, SNAP23 has been shown to play a role in the translocation and fusion with plasma membrane and exo-cytic vesicle. However, the function of SNAP23 in the development and progression of hypertension has not reported. In this study, we investigated the function of lipid raft-related SNAP23 in hypertension progression. Lipid rafts were isolated and purified from vascular smooth muscle cells (VSMC) of spontaneous hypertensive (SHR) and Wistar-Kyoto (Wky) rats at 3, 7 and 12 weeks old using ultracentrifuge and sucrose density gradient. SNAP23 expressions in VSMC membrane and lipid rafts was analyzed using 1D-PAGE, LC-MS/MS and cell based ELISA.

SNAP23 expression level in the lipid raft was significantly high in Wky-VSMC compared with SHR rats at 3, 7 and 12 weeks old. In a colorimetric assay, total cholesterol level was increased in SHR-VSMC membrane. SNAP23 knockdown in VSMCs increased total cholesterol level in the cell membrane. Vesicle level was 43% higher in Wky than SHR rats and cholesterol level in serum also showed significant increment in Wky compared with SHR rat. SNAP23 knockdown in VSMCs decreased vesicle level and cholesterol level in serum. These results suggest that lipid raft-related SNAP23 is closely involved in the trafficking of intracellular cholesterol and may regulate cholesterol level in vessels under hypertension. This study may provide useful information for the prevention or treatment of hypertension.
Key Words: Hypertension, SNAP23, Cholesterol, Vesicle, Exocytosis

MU-4

78kD Glucose-Regulated Protein in the Lipid Rafts is involved in Platelet-Derived Growth Factor-Induced Proliferation in Hypertensive Rat Vascular Smooth Muscle Cells

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Lipid rafts are sphingolipid and cholesterol-rich domains of the plasma membrane that contains and regulate a variety of cell surface receptor-induced signaling and transport proteins. Thus, lipid rafts underlie vascular pathological processes including hypertension and atherosclerosis. Using 1-DE and LC-MS/MS, we analyzed the protein expression profiles of lipid rafts of vascular smooth muscle from spontaneously hypertensive (SHR) rats, as well as from age-matched normotensive Wistar-Kyoto (Wky) rats. The expressions of ten proteins were altered in SHR compared with Wky. Among these proteins, the 78kD glucose-regulated protein (GRP78) showed greater expression level in SHR than Wky. The GRP78, also referred to as immunoglobulin heavy chain binding protein (BiP), is a chaperon protein in the endoplasmic reticulum of all cell types. Recently, GRP78 is expressed on the cell surface of multiple types of tumor cells, where it functions as a signaling receptor for a wide variety of ligands and plays a critical role in tumor progression and metastasis. In this study, we thus hypothesized that expression of GRP78 in the lipid rafts from vascular smooth muscle cells of SHR rats (SHR-SMCs) and Wky rats (WKy-SMCs) is associated with the development of hypertension. Using sucrose density gradient, we fractionated lipid rafts from SHR-SMCs and Wky-SMCs and also confirmed that GRP78 existed in the lipid raft of SMCs. The increase of GRP78 expression in lipid raft of SHR-SMCs was founded in cell-based ELISA, lipid rafts fraction, and the Western blot analysis. In proliferation analysis of SHR-SMCs using BrdU incorporation assay, the knockdown of GRP78 in SHR-SMCs efficiently suppressed platelet-derived growth factor (PDGF)-induced proliferation. Our study demonstrated that the expression of GRP78 is raised higher in lipid rafts from SHR-VSMCs than Wky-VSMCs and GRP78 expressed in the lipid rafts is associated with the PDGF-induced proliferation in vascular smooth muscle cells. In conclusion, hypertensive vascular condition may lead to change of blood vessel cell events inducing proliferation. Therefore, these results suggest that GRP78 in the lipid rafts could be a candidate molecule in developing a drug for the prevention or treatment of hypertension.
Key Words: Lipid rafts, Vascular smooth muscle cells, Hypertension, Atherosclerosis, Cell surface

MU-5

Decreased Potassium Current in Arterial Myocytes of Angiotensin II-Induced Hypertensive Rats and Its Recovery by Exercise Training

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A moderate increase in extracellular $[K^+]$ ($[K^+]_e$) induces relaxation of small arteries via augmenting inwardly rectifying K^+ current (I_{Kir}). The K^+-vasodilation is one of the mechanisms for the regional control of blood flow in response to neuronal and skeletal muscle activity. Previously we reported that exercise training (ExT) upregulates I_{Kir} in smooth muscle cells of cerebral and skeletal arteries (CA and DFA). Here we investigated whether I_{Kir} and K^+-vasodilation are affected in these arteries of hypertensive rats with or without ExT. To establish hypertension, rats were implanted with osmopumps delivering either Ang II (125 ng/kg/min) or saline. At 4 weeks, smooth muscle cells of CA (CASMCs) and DFA (FASMCs) were isolated and then we voltage clamped to evaluate the Ba^{2+}-sensitive I_{Kir} and voltage-dependent K^+ current (I_{Kv}). The current density of both I_{Kir} and I_{Kv} (pA/pF) decreased in Ang II-treated hypertensive rats (AII-R). We trained rats with treadmill running for the latter two weeks (3rd and 4th weeks of Ang II application). ExT recovered I_{Kir} and I_{Kv} in CASMCs and FASMCs. At the same time depolarizing inward conductance (nonselective cation current) was also increased by ExT in CASMCs while not in FASMCs. This current was partly inhibited by amiloride, an inhibitor for ENaC and ASIC channel. The K^+-vasodilation was monitored by using video-analysis of pressurized artery. Consistent with the changes in I_{Kir}, vasodilation by adding 2 mM KCl was weakened in both DFA and CA from AII-R. The K^+-vasodilation of DFA but not of CA was recovered in AII-R with ExT. The decreased K^+ conductance might reflect an adaptive change of resistance arteries to prevent over-perfusion of vital organs such as brain. The recovery of I_{Kir} and I_{Kv} by ExT might be one of the mechanisms for the beneficial effects of regular exercise on hypertension.
Key Words: Hypertension, Exercise training, Angiotensin

II, Arterial smooth muscle cell

MU-6

Steam Distillation Extract of *Chrysanthemum boreale* Makino Inhibits Platelet-Derived Growth Factor-Stimulated Migration and Proliferation in Rat Aortic Smooth Muscle Cells

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Chrysanthemum (*chrysanthemum boreale* Makino), which belongs to compositae family, is perennial plants. It has been shown to have anti-oxidant activity, anti-microbial activity, anti-bacterial activity, and anti-tumor effects. However, its prevention effect on atherosclerosis has not been reported. Vascular smooth muscle cell migration and proliferation in atherosclerosis is induced by various inflammatory cytokines and growth factors. Especially, platelet-derived growth factor (PDGF) is known as most important growth factor. This study determined the role of a steam distillation extract of chrysanthemum in PDGF-stimulated proliferation and migration of rat aortic smooth muscle cells (RASMCs). PDGF-BB induced the migration and proliferation of RASMCs, which were inhibited by chrysanthemum steam distillation extract in a dose-dependent manner. Treatment with chrysanthemum steam distillation extract inhibited PDGF-BB-stimulated sprout outgrowth of aortic rings. Taken together, this study demonstrates that the steam distillation extract of chrysanthemum inhibits PDGF-BB-stimulated migration and proliferation in RASMCs, as well as sprout outgrowth. Therefore, this extract may be useful for prevention of vascular disease such as atherosclerosis.

Key Words: Chrysanthemum, Atherosclerosis, Vascular smooth muscle cells, Proliferation, Migration

MU-7

Beta Adrenergic Overstimulation Impaired Vascular Contractility via Actin-cytoskeleton Disorganization in Rabbit Cerebral Artery

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Beta adrenergic overstimulation may increase the vascular damage and stroke. However, the underlying mechanisms of beta adrenergic overstimulation in cerebrovascular dysfunctions are not well known. We investigated the possible cerebrovascular dysfunction response to isoproterenol induced beta-adrenergic overstimulation (ISO) in rabbit cerebral arteries (CAs). ISO was induced in six weeks aged male New Zealand white rabbit (0.8-1.0 kg) by 7-days isoproterenol injection (300 μg/kg/day). We investigated the alteration of protein expression in ISO treated CAs using 2DE proteomics and western blot analysis. Systemic properties of 2DE proteomics result were analyzed using bioinformatics software. ROS generation and following DNA damage were assessed to evaluate deteriorative effect of ISO on CAs. Intracellular Ca^{2+} level change and vascular contractile response to vasoactive drug, angiotensin II (Ang II), were assessed to evaluate functional alteration of ISO treated CAs. Ang II-induced ROS generation was assessed to evaluated involvement of ROS generation in CA contractility. Proteomic analysis revealed remarkably decreased expression of cytoskeleton organizing proteins (e.g. actin related protein 1A and 2, α-actin, capping protein Z beta, and vimentin) and anti-oxidative stress proteins (e.g. heat shock protein 9A and stress-induced-phosphoprotein 1) in ISO-CAs. As a cause of dysregulation of actin-cytoskeleton organization, we found decreased level of RhoA and ROCK1, which are major regulators of actin-cytoskeleton organization. As functional consequences of proteomic alteration, we found the decreased transient Ca^{2+} efflux and constriction response to angiotensin II and high K^+ in ISO-CAs. ISO also increased basal ROS generation and induced oxidative damage in CA; however, it decreased the Ang II-induced ROS generation rate. These results indicate that ISO disrupted actin cytoskeleton proteome network through down-regulation of RhoA/ROCK1 proteins and increased oxidative damage, which consequently led to contractile dysfunction in CA.

Key Words: Beta adrenergic receptor overstimulation, Cerebrovascular damage, 2DE-MALDI-TOF, Cytoskeleton proteins

MU-8

Possible Contribution of DJ-1 Protein to the Regulation of Vascular Smooth Muscle Reactivity

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DJ-1, a novel gene responsible for an autosomal recessive early-onset Parkinsonism, is known as multifunctional protein that plays essential roles in biological functions in various cellular compartments. However, the function of DJ-1 in vascular tone has remained unclear. The present study was designed to investigate involvement of DJ-1 in regulation of vascular smooth muscle tone with the thoracic aortas of DJ-1 knockout (KO) mice and corresponding wild- type (WT) control. The norepinephrine (NE)-induced

vascular contraction also showed increased tendency in DJ-1 KO compared with WT mice in the presence of endothelium, but not in the absence of endothelium. NE and KCl-induced vascular contractions revealed no significant difference between DJ-1 KO and WT mice in both absence of endothelium. Acetylcholine inhibited NE-elevated contraction in endothelium-intact aorta and this is significantly attenuated in KO compared with WT mice. Endothelial nitric oxide (NO) synthase expression and NO production decreased in KO mice compared with WT controls, which recovered in DJ-1-overexpressing cells. H_2O_2 production in endothelial cells is higher in KO than WT mice. These results demonstrate that DJ-1 protein may play an important role in the regulation of vascular smooth muscle tone, probably by inhibitory mechanism of endothelium-dependent relaxation through the H_2O_2-realted decrease of NO production. This study suggests that the deficiency of DJ-1 may be relevant to the development of pathophysiological condition such as hypertension.

Key Words: DJ-1, Vascular smooth muscle, Contraction, Relexation, Endothelium

MU-9

An Antibody against α-actinin 4 Raised by Immunization with Endothelial Cells Exerts an Inhibitory Effect of Endothelium-Related Vasodilation

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This study was designed to identify new vascular functional molecules expressed on plasma membrane of human umbilical vein endothelial cells (HUVECs) using a monoclonal antibody-based proteomics technology. Twenty two antibodies were developed from rat inoculated with HUVECs and their effects were determined by observing vascular reactivity. Among these antibodies, C-7 antibody significantly inhibited endothelium-dependent vasorelaxation in response to acetylcholine (ACh) but not by sodium nitroprusside or histamine. Moreover, C-7 antibody did not affect norepinephrine (NE)-induced contraction in both endothelium-intact and -denuded aorta. Proteomics study using an immunoprecipitation of C-7 antibody with biotinylated HUVECs showed that C-7 antibody bound to plasma membrane proteins corresponded to immunoglobulin heavy chain VHDJ region, chaperonin containing TCP1, and α-actinin 4. In immunocytochemical study, ACh receptor and α-actinin 4 were co-localized on the plasma membrane of HUVECs, which is increased by ACh but inhibited by pretreatment with C-7 antibody. These results demonstrate that monoclonal C-7 antibody may exert an inhibitory effect on endothelium-dependent vasorelaxation induced by ACh and this response may at least partially result from the inhibition of α-actinin 4. This study may provide useful information for development of a new technology for finding an unknown functional molecule from membrane of cells.

Key Words: Monoclonal antibody, Plasma membrane protein, a-actinin 4, Vasorelaxation, Proteomics

MU-10

Deficiency of DJ-1 Protein Elevates Vascular Smooth Muscle Cell Proliferation and Neointima Formation via ERK1/2-Mediated Cylcin D1 Pathway

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DJ-1 is a ubiquitously expressed multifunctional protein that plays essential roles in biological functions in a variety of cells, especially neuronal cells. However, the function in vascular system has not been reported. In the present study, we investigated the involvement of DJ-1 in the regulation of vascular remodeling with DJ-1-deficiency ($DJ\text{-}1^{-/-}$) mice and -overexpressed system. Cell growth and proliferation induced by platelet-derived growth factor (PDGF)-BB were significantly increased in aortic smooth muscle cells (SMC) from $DJ\text{-}1^{-/-}$ mice compared with that from wild ($DJ\text{-}1^{+/+}$) ones. Expression of cyclin D1 and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) were greater in aortic SMC from $DJ\text{-}1^{-/-}$ mice than in wild $DJ\text{-}1^{+/+}$ ones, which were reversed in the aortic SMC treated with ERK1/2 inhibitor and antioxidants, as well as that in cells subjected to DJ-1 overexpression. The sprout outgrowth of the aortic ring *ex vivo* was greater in $DJ\text{-}1^{-/-}$ mice than in $DJ\text{-}1^{+/+}$ ones. Carotid ligation elevated the formation of neointima, which was greater in the $DJ\text{-}1^{-/-}$ mice than in $DJ\text{-}1^{+/+}$ ones. These results indicate that DJ-1 may be involved in vascular SMC proliferation via the ERK1/2-mediated cyclin D1 pathway. Therefore, DJ-1 may play a critical role in modulating the risk of the vascular remodeling.

Key Words: DJ-1, Vascular smooth muscle cells, Proliferation, Neointima formation

MU-11

STIM1 Negatively Regulates the Calcium Release from the Sarcoplasmic Reticulum in Skeletal Myotubes

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Stromal interaction molecule 1 (STIM1) mediates store-operated Ca^{2+} entry (SOCE) via the hetero-oligomerization of STIM1s and Orai1s into puncta in skeletal muscle. However, the role(s) of STIM1 and SOCE in skeletal muscle function have not been identified. In the present study, to identify the roles of STIM1 and SOCE in skeletal muscle function, wild-type STIM1 and two STIM1 mutants (the Triple mutant, missing Ca^{2+}-sensing residues, and E136X, missing the C-terminus, which is the reverse mutation of the Triple mutant) were over-expressed in mouse primary skeletal myotubes. The wild-type STIM1 increased SOCE, while neither mutant had an effect on SOCE. Interestingly, puncta from endogenous STIM1 and Orai1 was detected without any stimulus during the differentiation of myoblasts to myotubes, and increased puncta formation was observed in the triple mutant as well as the wild-type, suggesting that, in skeletal muscle, the formation of puncta is part of the differentiation process and not the necessary and sufficient condition for SOCE initiation. On the other hand, the Triple mutant, but not E136X, decreased the gain of excitation-contraction coupling (ECC) in a dominant-negative manner without affecting the SR Ca^{2+} amount or resting Ca^{2+} level. STIM1 was co-immunoprecipitated with the dihydropyridine receptor (DHRP) in a Ca^{2+}-independent manner. These results suggest that STIM1 could be a negative regulator of skeletal ECC, possibly via its Ca^{2+}-independent C-terminal interaction with DHPR.
Key Words: DHPR, STIM1, SOCE

MU-12

Mitsugumin 53 Attenuates the Activity of Sarcoplasmic Reticulum Calcium ATPase 1 (SERCA1) in Skeletal Muscle

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Mitsugumin 53 (MG53) participates in membrane repair in skeletal muscle. However, role(s) of MG53 in unique functions of skeletal muscle has not been addressed although MG53 is only expressed in skeletal and cardiac muscle. In the present study, MG53-interacting proteins were searched among proteins mediating skeletal muscle contraction and relaxation using the binding assays of various MG53 domains and quadrupole time-of-flight mass spectrometry. MG53 interacts to sarcoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a) via its tripartite motif (TRIM) and PRY domains. The interaction was confirmed in rabbit skeletal muscle and mouse primary skeletal myotubes using co-immunoprecipitation and immunocytochemistry. MG53 knock-down in mouse primary skeletal myotubes increased Ca^{2+}-uptake through SERCA1a (more than 35%) in the presence of micromolar Ca^{2+} but not nanomolar Ca^{2+}, suggesting that MG53 attenuates SERCA1a activity possibly during skeletal muscle contraction or relaxation but not during resting period. Based on in-silico studies, the interaction of MG53 to SERCA1a could be mediated by unique ways compared with interactions by other proteins containing TRIM or PRY domains.
Key Words: SERCA, MG53, TRIM, PRY

MU-13

Effects of Bisphosphonates on Rat Cardiac Left-Ventricular Pressure (LVP)

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Background: Bisphosphonates are widely used to treat and prevent osteoporosis, which acts by inhibiting osteoclast-mediated bone-resorption. However, the recent studies are shown that bisphosphonates are associated with a risk of adverse cardiac events including atrial fibrillation or QT prolongation. The goal of this study was to investigate the effect of bisphosphonates, etidronate, alendronate and pamidronate, on the rat cardiac function by evaluating the LVP-related parameters. **Materials & Methods:** In this study, alendronate, etidronate and pamidronate are evaluated as test drugs. All drugs were purchased on Sigma-aldrich (MO, USA). Male Sprague-Dawley rat (6 weeks) hearts were retrogradely perfused with a Langendorff apparatus. After the stabilization of isolated hearts, systolic pressure (SP), developed pressure (DP), diastolic pressure/diastolic time (dP/dt), left ventricular end-diastolic pressure (LVEDP), the peak positive and negative differentials of pressure change with the time (+dP/dt and −dP/dt, respectively) and heart rate (HR) were monitored. The standard protocol consisted of a minimum 15 min stabilization period, followed by 4 periods of each 15 min perfusions for the vehicle only (control) and the test articles exposure at 3 ascending concentrations (1, 10 and 100 μM). **Results:** All bisphosphonates tested in these experiments caused concentration-dependent decrease of the left ventricular SP, DP and +/− dv/dt. The LVEDPs were significantly increased in the presence of alendronate or etidronate, although it was decreased in the case of pamidronate. The HRs were not changed in any concentrations of drugs. **Conclusion:** Based on the results, alendronate, etidronate and pamidronate increased the contractility of rat hearts at high doses than the therapeutic plasma concentrations.
Key Words: Osteoporosis, LVP, Langendorff, Bisphosphonate

MU-14

The Potential Role of Cortisol on Cardiac Contractility: Activation of PKC and Ouabain-Targeted Na^+/K^+ ATPase

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Although numerous studies revealed the impact of cortisol, produced in the adrenal gland and released to stress, on body but direct physiological effect of acute high dose of cortisol on heart function is still unknown. When perfused with cortisol (10 μM) in Lagnedorff system, there was no significant changes in heart rate ($p>0.05$) but coronary flow and left ventricular developing pressure (LVDP) was significantly decreased ($p<0.05$). In cardiomyocytes, cortisol treatment did not lead to increase in cytosolic and mitochondrial calcium, which are important regulators in cardiac pressure development and there was no significant change in the level of intracellular free zinc, which contents are decreased in stressful condition. In isolated mitochondria, cortisol treatment decreased ADP-mediated oxygen consumption rate (active state) and thereby reduced relative respiration rate. PKC was significantly phosphorylated by cortisol treatment and decrease in LVDP was attenuated in the presence of PKC peptide inhibitor (0.2 μM). Furthermore, the inhibitory effect of ouabain on Na^+/K^+ ATPase which is an important component of calcium regulation in heart was significantly suppressed by pretreatment of cortisol in isolated cardiomyocytes and hearts. Taken together, high dose of cortisol may influence on heart contractility through activation of PKC and suppression of calcium handling in rat heart.
Key Words: Cortisol, Hemodynamics, PKC, Ouabain, Left ventricular developing pressure, Na^+/K^+ ATPase, Coronary flow

MU-15(PO-16)

Myogenic Response in Rat Posterior Cerebral Arteries: Role of Endogenous ENaC and TRP Channels

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Mechano-gated ion channels are thought to mediate pressure-induced myogenic vasoconstriction in small resistance arteries. More recent findings have indicated that the transient receptor potential channels (TRPC) and epithelial sodium channels (ENaC) are involved mechanotransduction. The purpose of this study is to research the relationship between TRPC and ENaC. A previous work from our laboratory suggests that ENaC may be components of the mechanosensitive ion channels in rat posterior cerebral arteries (PCA); however, the specific ion channel proteins mediating myogenic constriction are unknown. In the present study, we find that, for the first time, ENaC was interact with TRPM4 but not TRPC6 by using immunoprecipitation assay and confocal microscopy. Treatment of a specific βENaC inhibitor, amiloride, a specific TRPM4 inhibitor, 9-phenanthrol, and a TRPC6 inhibitor, SKF96365, inhibited the pressure-induced myogenic reponse. Moreover, the myogenic response inhibited in rat PCA transfected with βENaC, TRPM4, and TRPC6 small interfering RNA, respectively. Cotreatment of amiloride and 9-phennthrol showed the similar inhibition effects on myogenic contraction treated single amiloride or 9-phenanthrol. In transfected PCA with βENaC or TRPM4 siRNA, inhibition of myogenic tones also were not affected by 9-phenanthrol or amiloride treatment, respectively. But, pressure-induced myogenic response was fully inhibited by treatment of amiloride, 9-phenanthrol, and SKF96365 at the same time, and by treatment SKF96365 in PCA transfected βENaC siRNA. These our results suggest that ENaC, TRPM4, and TRPC6 are must be important role as initiating pressure-induced myogenic response in rat PCA.
Key Words: ENaC, TRPM4, TRPC6, Immunoprecipitation, Myogenic response

MU-16

Metabolic Substrates Diminish the Anti-Adrenergic Effect of Insulin on Rat Left Ventricular Myocyte Contractility and Induce Arrhythmias

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Background & Aim: Metabolic syndrome (MetS) is well established to be the precursor of various cardiovascular diseases and type 2 diabetes that increase the incidence of heart failure, sudden cardiac death or stroke. Insulin resistance in muscle is one of the key underlying mechanisms for the malignant effects of MetS. So far, investigation of metabolic substrates regulation of cardiac contractile function and insulin responsiveness is lacking. Therefore, we design to study whether supplementation of metabolic substrates *in vitro* affects basal and beta-adrenergic left ventricular (LV) myocyte contractility and changes insulin response in rat hearts. **Methods:** LV myocytes are isolated from SD rat hearts (10-12 weeks, males) using enzymatic dispersion technique in Langendorff perfusion system. LV myocyte contraction was assessed by analyzing the peak amplitude of sarcomere length (in μm) using a video-sarcomere detection system (IonOptix Corp, field stimulation at 2 Hz, 36±1°C). Metabolic substrates (oleic acid 200 μM, palmitic acid 100 μM, linolic acid 100 μM, lactate 1 mM, pyruvate 100 μM and carnitine 50 μM) are supplemented (termed nutrition full, NF solution) in normal tyrode (NT) solution. Insulin (1-10 nM) and isoprenaline (ISO, 10-50 nM) were used in the study. **Results:** Our results demonstrate that LV myocyte contraction is greater in NF (sarcomere shortening, 0.155±0.007 in NF vs. 0.094± 0.004 in NT, $P<0.0001$, n=19). ISO increased LV myocyte contraction in NT (0.097±0.01 in control and 0.155± 0.01 with ISO, $P<0.0001$, n=8) and in NF (0.156±0.014 in control and 0.267± 0.02 with ISO, P=0.01, n=6). Insulin pretreatment did not affect basal LV myocyte contraction in NT (0.077±0.004 in control and 0.078±0.01 with insulin,

P=0.6, n=8) but prevented ISO-induced positive inotropic effect in NT (0.082± 0.005 with insulin and 0.074±0.006 with insulin+ISO, P= 0.2, n=7). However, insulin failed to affect basal or ISO-increase in myocyte contraction in NF (0.157±0.011 with insulin and 0.274±0.02 with insulin+ISO in NF, P=0.01, n=5). Notably, ISO induced arrhythmia in NF in almost all the LV myocytes studied (72%). Antioxidant, NAC (N-Acety-L- cysteine 1 mM) did not reduce the arrhythmias (77%). **Conclusion:** Our results show that *in vitro* supplementation of metabolic substrates increased LV myocyte contractility but diminished the anti-adrenergic effect of insulin and induced arrhythmias. Downstream mechanisms of the lack of insulin response of cardiac inotropy with comprehensive metabolic substrates warrant further study.
Key Words: Insulin, Myocyte, Metabolic syndrome, Heart failure, Anti-adrenergic

MU-17

Protective Effect of Melatonin against TNF-α Toxicity in the L6 Myotubes

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Muscle atrophy with decreased cell number and cell size poses a serious concern to patients inflicted with inflammatory diseases. An increasing body of evidence implies that tumor necrosis factor alpha (TNF-α) plays a critical role in muscle atrophy in a number of these clinical settings. We hypothesize that reactive oxygen species (ROS) mediate TNF-α-induced muscle cell death and muscle cell hypotrophy. Recently, melatonin has attracted attention because of their free radical scavenging and antioxidant properties. The aim of this study was to evaluate the possible protective role of melatonin against TNF-α-induced muscle cell death and hypotrophy in rat L6 myotubes. To examine this, L6 myotubes were subjected to various concentrations of recombinant TNF-α for 24 hr. TNF-α at the concentration of 100 ng/mL induced ROS generation and decreased cell viability. Further analysis revealed that apoptosis, but not autophagy, may be important for TNF-α induced cell death mechanism. Melatonin significantly attenuated TNF-α-induced ROS generation and the apoptosis via its plasmalemmal receptor-independent signaling pathway. In addition, decreased muscle fiber diameter and increased muscle cell proteolysis by TNF-α was strongly ameliorated by treatment with melatonin. Taken together, these results suggest that TNF-α may mediate ROS-induced muscle cell death and cellular hypotrophy, and melatonin may be a useful tool as a protecting agent for muscle atrophy related with inflammatory diseases.
Key Words: Muscle atrophy, Muscle cell death, L6 myotube, TNF-α, Melatonin

MU-18(PO-17)

SR Ca^{2+} Channels are Altered in Cultured Vascular Smooth Muscle Cells

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Vascular smooth muscle cells can be modulated from a contractile phenotype to proliferative phenotype in atherosclerosis or hypertension and primary culture. Since phenotypic modulation may be associated with alterations of proteins which handle intracellular Ca^{2+} concentration, this study aimed to determine whether or not Ca^{2+} channels of the sarcoplasmic reticulum (SR) are altered during rat aortic smooth muscle cell (RASMC) culture. We used freshly dissociated RASMCs and cultured RASMCs in this study. RASMCs were dissociated from thoracic aorta by enzymatic digestion with collagenase and elastase. Cytosolic Ca^{2+} concentration was measured in fura-2-loaded cells using digital imaging. Ca^{2+} release was measured in permeabilized cell by beta-escin using the low affinity Ca^{2+} indicator, mag-fura-2/AM. To confirm the expressions of IP_3-receptor and ryanodine-receptor, western blot and immunocytochemistry were performed. Cytosolic Ca^{2+} was markedly increased by caffeine in freshly dissociated RASMCs, but not in cultured RASMCs. In permeabilized freshly dissociated RASMCs, we observed Ca^{2+} release from the SR by caffeine, ryanodine, or Ca^{2+}. However Ca^{2+} release was not observed in permeabilized cultured RASMCs. Ryanodine-receptor proteins were detected in freshly dissociated RASMCs, but not in cultured RASMCs using immunocytochemistry. Inositol triphosphate (IP_3) stimulated Ca^{2+} release from SR in both types of cells, but the time constants of Ca^{2+} release were different from each other. Expression of IP_3-receptor type 1 protein was increased in cultured RASMCs. In addition, IP_3-receptor type 2 and type 3 were not expressed in fresh RASMCs but expressed in cultured RASMCs. The above results provide direct evidence that cell culture alters Ca^{2+} channels of SR in RASMCs. Ryanodine-receptor is disappeared and IP_3-receptor subtypes are altered during culture.
Key Words: Vascular smooth muscle cell, Proliferation, Inositol 1,4,5-trisphosphate receptor, Ca^{2+} imaging

MU-19

Hypertrophy in Skeletal Myotubes Induced by Junctophilin-2 Mutant, Y141H, Involves an Increase in Store-operated Calcium Entry via Orai1

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Junctophilins (JPs) play an important role in the formation of junctional membrane complexes (JMC) in striated muscle by physically linking the transverse-tubule and sarcoplasmic reticulum (SR) membranes. Researchers have found five JP2 mutants in humans with hypertrophic cardiomyopathy. Among these, Y141H and S165F are associated with severely altered Ca^{2+} signaling in cardiomyocytes. We previously reported that S165F also induced both hypertrophy and altered intracellular Ca^{2+} signaling in mouse skeletal myotubes. In the present study, we attempted to identify the dominant-negative role(s) of Y141H in primary mouse skeletal myotubes. Consistent with S165F, Y141H led to hypertrophy and altered Ca^{2+} signaling (a decrease in the gain of excitation-contraction coupling and an increase in the resting level of myoplasmic Ca^{2+}). However, unlike S165F, neither ryanodine receptor 1-mediated Ca^{2+} release from the SR nor the phosphorylation of the mutated JP2 by protein kinase C was related to the altered Ca^{2+} signaling by Y141H. Instead, abnormal JMC and increased SOCE via Orai1 were found, suggesting that the hypertrophy caused by Y141H progressed differently from S165F. Therefore JP2 can be linked to skeletal muscle hypertrophy via various Ca^{2+} signaling pathways, and SOCE could be one of the causes of altered Ca^{2+} signaling observed in muscle hypertrophy.

Key Words: Junctophilins, Protein kinase C, Muscle hypertrophy, SOCE

MU-20

Impaired Endurance Exercise Capacity in AQP3 Knock-out Mice

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Aquaporin 3 (AQP-3) is an aquaglyceroporin that facilitates the rapid transport of glycerol across the cell membrane as well as water. Although the major water channel in skeletal muscle is known to be AQP4, skeletal muscle also expresses AQP3. However, the function of AQP3 in skeletal muscle fat metabolism during exercise is currently unknown. Here we investigated the capacity of acute endurance exercise in wild-type and AQP3 knock-out CD1 mice and compared metabolic parameters in plasma, liver and skeletal muscle. In response to a single bout of exhausting treadmill running at the moderate intensity (~60% of maximal intensity), AQP3 knock-out mice exhibited early fatigue and decrease in exercise performance and resistance to fatigue. The average exercise duration of AQP3 knock-out mice was reduced to 37±5% of that of wild-type mice. After exhausting exercise, plasma and liver glycerol levels of AQP3 knock-out mice were significantly reduced compared with those of wild-type mice. Interestingly, glycerol levels in hamstring muscles of AQP3 knock-out mice were higher than wild-type mice, implying the decreased glycerol efflux from skeletal muscle into blood and subsequently into liver for the constant energy supply through gluconeogenesis during the prolonged endurance exercise. These results suggest that AQP3 plays a role in skeletal muscle fat metabolism and influence the endurance exercise capacity.

Key Words: AQP3, Glycerol, Skeletal muscle, Acute endurance exercise

MU-21(PO-18)

Mutual Regulation between STIM1 and NFATc3 to Induce C2C12 Myoblast Differentiation

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We studied the pro-myogenic effects of STIM1-activated store-operated Ca^{2+} entry (SOCE) and calcineurin/NFAT signaling in differentiating C2C12 myoblast. Especially, we focused on the mutual regulation between STIM1 and NFAT transcription factors to induce C2C12 differentiation. Among 4-subtypes of NFAT (NFATc1-c4), NFATc3 expression (mRNA and proteins) was increased within 1-day of differentiation, while STIM1 increased continuously during C2C12 differentiation. In C2C12 myoblasts, mutual regulation between NFATc3 and STIM1 was observed. Overexpression or silencing of NFATc3 (siNFATc3) increased or decreased STIM1 protein, SOCE activity, and degree of myogenesis, respectively. Overexpression or silencing of STIM1 (siSTIM1) increased or decreased NFATc3 protein, NFAT transcription activity, and degree of myogenesis, respectively. Our results suggest that positive feedback regulation between STIM1 and NFATc3 is an important mechanism to induced C2C12 myogenesis.

Key Words: C2C12, SOCE, STIM1, NFATc3, Myoblast differentiation

MU-22

Oleanolic Acid Accentuates Atrial Natriuretic Peptide Secretion in Beating Rat Atria

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The heart is an endocrine organ involved in the regulation of blood pressure and body fluid homeostasis through the secretion of atrial natriuretic peptide (ANP). Oleanolic acid (OA) is a naturally occurring triterpenoid, widely distributed

in medicinal plants. The purpose of present study shows that OA induces subacute effects on the ANP synthesis and secretion. OA (30 mg/kg/day) was administrated orally in Sprague-Dawley rats; Atrial dynamics, pulse pressure, and ANP secretion were measured. To identify the effects of OA on the stretch-induced changes in the atrial secretory and contractile function were tested. The present study shows that atrial stretch-induced increase in ANP secretion is accentuated in atria from rats treated with OA. To investigate the effects of OA on the ANP secretion by activation of β_1-AR and mAChR, isoproterenol and acetylcholine (ACh) were treated in the atria from OA orally administrated rats. Oral intake of OA was significantly accentuated the ACh-induced increase of ANP secretion. These results suggested that OA accentuated the increase of ANP secretion induced by muscarinic receptor. In conclusion, these results demonstrate that subacute effects of OA were an accentuation of plasma ANP levels and atrial ANP secretion via enhancement of ANP synthesis in beating rat atria.

Key Words: Oleanolic acid, Atrial natriuretic peptide, Stretch, Muscarinic acetylcholine receptor

MU-23

Mechanism of the 5-HT2A Receptor-Mediated Vasoconstriction in Rat Mesenteric Artery: Receptor-Specific Roles of Caveolae, Src Tyrosine Kinase, and Kv Channels

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Although important roles of serotonin (5-hydroxytryptamine, 5-HT) 5-HT_{2A} receptor (5-HT_{2A}R) under various physiologic and pathologic conditions are increasingly recognized, precise signaling pathway of the 5-HT_{2A}R is unclear. Moreover, recent studies suggest differential roles of caveolae, cSrc tyrosine kinase, and voltage-gated K^+ channel (Kv) among G-protein-coupled receptors including 5-HT_{2A}R and α-adrenoceptor. Isometric arterial tension measurement, nystatin-perforated patch-clamp technique, electron microscopy, and Western blotting analysis were used for examining roles of caveolae, Src tyrosine kinase, PKC, and Kv in the 5-HT_{2A}R and α-adrenoceptor-mediated vasoconstriction in rat mesenteric artery. 5-HT-induced vasoconstriction was inhibited by blocking voltage-gated Ca^{2+} channels or by high KCl pretreatment, indicating that E_m depolarization contributes to 5-HT-induced vasoconstriction. The E_m depolarization was attributed by Kv inhibition, and the 5-HT_{2A}R mediated the 5-HT-induced Kv inhibition and vasoconstriction. Pretreatment of cSrc tyrosine kinase inhibitor PP_2 almost completely prevented the 5-HT_{2A}R-mediated Kv inhibition and vasoconstriction. On the contrary, vasoconstriction by α_1-adrenoceptor was relatively less inhibited by PP_2, but was markedly inhibited by PKC inhibitior chelerythrine. Inhibition of PKC did not affect the 5-HT_{2A}R-mediated responses. The K_V inhibitor 4-aminopyridine failed to evoke additional vasoconstriction after 5-HT-induced vasoconstriction, verifying that K_V inhibition mediates the 5-HT effect. Disruption of caveolae by methyl-β-cyclodextrin not only inhibited 5-HT-induced vasoconstriction but also rescued 4-aminopyridyne-induced vasoconstriction after vasoconstriction by 5-HT, indicating that integrity of caveolae is required for the 5-HT-induced K_V inhibition. Accordingly, methyl-β-cyclodextrin treatment prevented the 5-HT_{2A}R-mediated Kv inhibition. However, vasoconstriction by α_1-adrenoceptor was not inhibited by methyl-β-cyclodextrin treatment. Western blot analysis revealed that cSrc tyrosine kinase is phosphorylated by 5-HT, but not by norepinephrine. The 5-HT-induced cSrc tyrosine kinase phosphorylation was also inhibited by methyl-β-cyclodextrin treatment. We conclude that caveolae-dependent cSrc tyrosine kinase activation and the subsequent Kv inhibition is the main signaling of the 5-HT_{2A}R-mediated vasoconstrciton, whereas caveolae-independent PKC activation largely contributes to the a-adrenoceptor-mediated vasoconstriction in rat mesenteric artery.

Key Words: Serotonin, Voltage-gated K^+ channels, 5-HT_{2A} receptor, Caveolae, Src tyrosine kinase

SC-1

Effects of DA-6034 on Induction of Ca^{2+} Signaling in Exocrine Gland Cells

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DA-6034, a eupatilin derivative of flavonoid, has shown potent effects on the protection of gastric mucousa and induced the increases in fluid and glycoprotein secretion in human and rat corneal and conjunctival cells, suggesting that it might be considered as a drug for the treatment of dry eye. However, whether DA-6034 induces Ca^{2+} signaling and its underlying mechanism in exocrine gland cells are not known. In the present study, we investigated the mechanism for actions of DA-6034 in Ca^{2+} signaling pathways of human corneal epithelial cells and mouse salivary gland cells. DA-6034 activated Ca^{2+}-activated Cl^- channel gating and increased intracellular calcium concentrations ($[Ca^{2+}]_i$) in human conjunctival cells and mouse salivary gland cells. $[Ca^{2+}]_i$ increases of DA-6034 was dependent on the Ca^{2+} entry form extracellular and Ca^{2+} release of intracellular Ca^{2+} stores. Interestingly, these effects of DA-6034 were not related to phospholipase C/1, 4, 5-trisinositolphosphate pathway. These results suggest that DA-6034 induces Ca^{2+} signaling in exocrine gland cells and has a possibility of drug against dry eye and mouth.
Key Words: DA-6034, Calcium signaling, Exocrine gland

SC-2(PO-19)

Role of Mitochondrial Ca^{2+} Uniporter in Mitochondrial pH Gradient and Metabolism-Secretion Coupling in INS-1E Cells

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The dominant signaling factor in insulin exocytosis is ATP/ADP ratio which determines plasmalemmal K^+ conductance and voltage-sensitive Ca^{2+} influx. In pancreatic β-cells, ATP synthesis strongly relies on oxidative phosphorylation that can be accelerated by mitochondrial Ca^{2+} influx. A major route of Ca^{2+} influx is mitochondrial Ca^{2+} uniporter (MCU), which is very recently discovered to be encoded by *Ccdc109a* gene. Here, we took the advantage of molecular identification of MCU to investigate the bioenergetic mechanism and physiological role of MCU in nutrient-induced insulin secretion of clonal beta cell INS-1E. Confocal microscopic imaging system was used to measure mitochondrial matrix Ca^{2+} ($[Ca^{2+}]_{mito}$) and pH (pH_{mito}) after overexpressing ratio pericam (RP_{mit}) and mitoAlpHi, respectively. Fluorescence microplate reader was used to measure mitochondrial membrane potential ($\Delta\Psi_{mito}$) after loading JC-1 dye. ATP production and insulin secretion were measured by using bioluminescence assay and immunoassay kit, respectively. In permeabilised cells with staphylococcal α-hemolysin toxin, knockdown of MCU by siRNA (siMCU) treatment delayed and attenuated $[Ca^{2+}]_{mito}$ rise in response to extramitochondrial Ca^{2+} (500 nM) addition. Interestingly, succinate-induced pH_{mito} alkalinisation was markedly reduced in MCU-silenced cells. In addition, knockdown of MCU ablated slow acidifying response of pH_{mito} in response to extramitochondrial Ca^{2+} which might be related to Ca^{2+} efflux. However, hyperpolarisation of $\Delta\Psi_{mito}$ induced by succinate was not significantly altered by silencing MCU. In intact INS-1E cells, knockdown of MCU prominently decreased glucose-induced alkalinisation of pH_{mito}, whereas it did not affect $\Delta\Psi_{mito}$. Nutrient-induced ATP production was decreased in siMCU-treated cells. Consistently, insulin secretion stimulated by glucose was severely impaired by knockdown of MCU. Taken together, Ca^{2+} influx through MCU plays an important role to increase mitochondrial chemical (pH) gradient which may be critical for mitochondrial ATP generation and metabolism-secretion coupling.
Key Words: Mitochondrial calcium uniporter, Mitochondrial pH gradient, Mitochondrial membrane potential, Mitochondrial ATP production, Metabolism-secretion coupling

SY-1

Cilostazol Attenuated Catecholamine Mediated Myocardial Remodeling against Restrained Stress Following Myocardial Infarction Model in Rat

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Introduction: Principal actions of Cilostazol, a phosphodiesterase III inhibitor are inhibition of platelet aggravation and vasodilation. But some studies demonstrated pleotrophic effect of Cilostazol including reducing oxygen free radial, chronotrophic effect and inhibition of intracellular Ca^{2+} associated catecholamine secretion. We aimed to verify effect of Cilostazol on catecholamine mediated myocardial remodeling in myocardial infarction (MI) with restrained stress (ST) rat model. **Methods:** Male Sprague-Dawley rats (n=15) were equally divided into three groups: control, MI + ST, MI + ST with Cilostazol (5.0 mg/day/kg). We assessed ischemia-stress derived myocardial remodeling with measurement of LV systolic ejection fraction (LVEF) at immediate post MI + ST and follow up, LV mass by heart weight/body weight ratio and tissue BNP expression by western blot. Serum level of epinephrine and norepinephrine was also measured. **Results:** There was no significant difference in mean LVEF at immediate post MI + ST (45.6 ± 9.3 vs 48.3 ± 10.9). But Cilostazol group showed significant improvement of LVEF at follow up exam (66.9 ± 14.3 vs 47.0 ± 17.1, $p < 0.05$). LV mass and tissue BNP expression were significant lower in Cilostazol group ($p < 0.05$). Significant lower serum level of epinephrine and norepinephrine was observed in Cilostazol group ($p < 0.05$). **Conclusion:** The data in this study suggested that Cilostazol ameliorated ischemia-stress derived myocardial remodeling and mechanism of myocardial protection might be associated with inhibition of catecholamine secretion.

Key Words: Cilostazol, Myocardial infarction, Stress, Systolic ejection fraction, Catecholamine

SY-2

Repeated Stimulation of Peripheral P2Y1 Receptors Facilitate Capsaicin and Acidic pH-Induced Thermal Nociception via Phosphorylation of TRPV1 in Rats

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$P2Y_1$ receptor is Gq-coupled receptor located in sensory neurons, and there are several studies reporting the role of $P2Y_1$ receptors in sensory transduction and pain perception. We have recently demonstrated that injection of ATP diluted in acidic pH saline acts synergistically via $P2Y_1$ and TRPV1 receptors to produce thermal hyperalgesia (TH). Although these results suggested a possible interaction between $P2Y_1$ and TRPV1, the precise role of these receptors and the nature of their synergism in the development of TH remains unclear. This study was designed to investigate the potential role of peripheral $P2Y_1$ and the relationship between $P2Y_1$ and TRPV1 on TH. MRS2365, a selective $P2Y_1$ receptor agonist, was intraplantarly injected to stimulate peripheral $P2Y_1$ receptors. Neither single injection nor repeated injection of MRS2365 had any affect on basal responses to noxious heat. However, rats that received repeated injections of MRS2365 showed a significant augmentation of capsaicin-induced TH as well as the development of TH after the injection of acidic saline. This facilitated TH was completely reversed by injection of the $P2Y_1$ antagonist, MRS2500 or the TRPV1 antagoinst, AMG-9810. To examine the functional activity of TRPV1 in the peripheral tissue, we performed a western blot assay of hind paw lysates targeting PKC dependent phosphorylation of TRPV1. Repeated intraplantar injection of MRS2365, significantly increased the expression of phosphorylated TRPV1. Moreover, administration of the PKC inhibitor, chelerythrine, significantly blocked the increase in TRPV1-mediated TH after repetitive $P2Y_1$ activation. These results demonstrate that repeated stimulation of peripheral $P2Y_1$ receptors functionally activates TRPV1 via PKC-dependent phosphorylation, which ultimately contributes to the development or enhancement of thermal hyperalgesia.

Key Words: $P2Y_1$, Thermal hyperalgesia, TRPV1, PKC, Phosphorylation

SY-3

Migration in CD3+ T Cells Isolated from DJ-1 Deficient Mice is Involved in the SDF-1/CXCR4 -Mediated ERK1/2 Pathway

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Inflammation is an important role in atherosclerosis pathogenesis. Recently, it has been indicated that stromal-derived factor (SDF)-1/CXCR4 axis is in the recruitment of inflammatory cell during atherosclerosis process. DJ-1 protein, a multifunctional protein scavenging reactive oxidative stress, is reported as a possible regulator of inflammatory responses. Nevertheless, the role of DJ-1 in SDF-1/CXCR4 axis-mediated atherosclerosis has not been investigated. In the present study, we have tested the hypothesis that activation of CD3+ T cell migration contributes to atherosclerosis pathogenesis in DJ-1 knockout (KO) mice. Our result showed the level of migration and ERK1/2 activation in CD3+ T cells from DJ-1 KO mice increased compared with those from control. But the expression level of SDF-1 receptor CXCR4 was not elevated in the cells from

DJ-1 KO mice. In *in vivo* test, CD3 + T cells were predominant on neointimal plaque in DJ-1 KO mice. Moreover, CD3 + T cell from DJ-1 KO mice showed increased expression of IFN-γ and IL-17 after differentiation into Th-1 and Th17, compared to those from wild type. Collectively, these results suggest that DJ-1 protein may act as a key player in atherosclerosis pathogenesis.
Key Words: Stromal-derived factor (SDF)-1, CXCR4, DJ-1, CD3+ T cell migration, Atherosclerosis pathogenesis

SY-4(PO-23)

Angiotensin III Stimulates High Atrial Stretch-Induced Atrial Natriuretic Peptide Secretion via AT2R/PI3K/Akt/eNOS/GC/PKG

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Angiotensin II (Ang II) and Angiotensin-(1-7) [Ang-(1-7)] are bioactive peptides produced in renin-angiotensin system (RAS) and play a pivotal role in cardiovascular physiology. Recently, we have reported that Ang II has an opposite effect to Ang-(1-7) on atrial natriuretic peptide (ANP) secretion via Ang II type 1 (AT1) receptor and Mas receptor, respectively. However, the role of Angiotensin III (Ang III) is still unknown. The aim of present study is to investigate the modulatory role of Ang III in ANP secretion and to find out the signaling mechanism of Ang III. The ANP secretion was increased by 70% at high atrial stretch condition (2.5 cm). Ang III augmented ANP secretion in a dose-dependent manner. The stimulated effect of Ang III on stretch-induced ANP secretion was blocked by the pretreatment of AT2R antagonist but not by AT1R or Mas receptor antagonist. Pretreatment with inhibitor of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), nitric oxide synthase (eNOS), guanylyl cyclase (GC), or cGMP-dependent protein kinase (PKG) blocked the augmentation of high atrial stretch-induced ANP secretion by Ang III. Ang III did not influence pulse pressure and ECF translocation at high atrial stretch condition. In anesthetized rats, acute infusion of Ang III increased blood pressure and plasma ANP level, which were attenuated by the pretreatment with losartan. These results suggest that Ang III may stimulate ANP secretion via AT2R, PI3K, Akt, eNOS, GC and PKG at high atrial stretch condition. Supported by the National Research Foundation (2012-0009322).
Key Words: Renin-angiotensin system, Atrial natriuretic peptide, Angiotensin III, AT2 receptor

SY-5

Odor Discrimination in the Main Olfactory Bulb of Anesthetized Dogs

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Efficient odor discrimination of mammals is crucial for the survival. Main olfactory bulb (MOB) is known as one of the key parts of mammals' olfactory system and we recently showed the possibility of inferring odorants by extra cellular single unit recording in rats' MOB. However, the attempt for studying species which have superior olfactory capabilities, especially canine, has been rarely reported. In spite of its outstanding abilities, the psychological reluctances and difficulties from anatomical structure have hindered the intensive electrophysiological research. Consequentially, most of studies for canine olfaction have been limited in behavioral experiments. Here, using a multi-channel single neuron recording system, we present the neural activity changes in mitral/tufted cell layer of dogs' MOB (n=4; AP +2.20-2.30 cm, ML 2.5-3.5 mm, DV 2.7-2.8cm, downward angle 10^{o}-20^{o} from brain surface) following odor presentation. Two odorants (350 ppm; isoamylacetate (IAA), 1,7 octadiene (1,7 oct)) and blank (control) were used as stimuli. Just one inhalation was enough to evoke robust neural activities in the MOB and it didn't alter respiration rate (10.57±0.3 times/min). Maximum likelihood (ML) decoding based on the Gaussian distribution showed 80-100% inferring accuracy for both odorants. Our result suggests the possibility of creating a sensory neuron decoding-based brain machine interface (BMI) system for the discrimination of various odorants. We believe this type of BMI neurotechnology could be applicable for various scent industries. Supported by Brain Research Center (BRC, 2012K001127), Ministry of Knowledge Economy (MKE, 10033634-2012) and National Research Foundation of Korea (NRF, 2012-0005787).
Key Words: Olfaction, Dog, Odorant, Decoding, BMI

SY-6

Angiotensin II Type 2 Receptor Agonist Stimulates Stretch-Induced ANP Secretion via PI 3K/PKG/NO Pathway

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Angiotensin II (Ang II) effects as controlling blood pressure and volume in the cardiovascular system. It binds to two receptors, Ang II type 1 (AT1) and type 2 (AT2) receptor. It is already known that the AT1 receptor mediates the major cardiovascular effects of Ang II. However, effects mediated by AT2 receptor are still controversial. The aim of present study is to define the effect of AT2 receptor agonist, CGP42112A on stretch-induced ANP secretion and its mechanism using *in vitro* perfusion experiments. CGP42112A (10^{-8} M, 10^{-7} M, 10^{-6} M) stimulated stretch-induced ANP secretion from isolated perfused rat atria. However, atrial

contractility and the translocation of extracellular fluid (ECF) did not change. The augmented effect of CGP42112A on stretch-induced ANP secretion was attenuated by pretreatment with AT2 receptor antagonist and inhibitor for phosphoinositol 3-kinase (PI3K), protein kinase G (PKG) or NO. However antagonist for AT1 or Mas receptor augmented the effect of CGP42112A on stretch-ANP secretion. In diabetic rats, the response of stretch-induced ANP secretion and concentration to CGP42112A augmented. AT2 receptor mRNA and protein level were up-regulated in diabetic rat atria. Therefore, we suggest that AT2 receptor agonist stimulates stretch-induced ANP secretion through PI3K/PKG/NO pathway. Further studies about cross-talk between AT1 and AT2, or Mas receptors on ANP secretion are needed. Supported by the National Research Foundation (2012-0009322)
Key Words: Renin-angiotensin system, ANP, Diabetes

SY-7

Sustained Hypertension Causes Insulin Resistance Mediated by Oxidative Stress and ROS

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It is already known that cardiovascular disorders and diabetes mellitus are closely related. However, there are a few reports mentioned hypertension can induce diabetes or related disorders. The purpose of this study is to evaluate whether elder hypertensive rats has diabetes mellitus or related disorders, and its mechanisms. Two-kidney, one-clip hypertensive (2K1C) rats were used 24 weeks after surgery. Insulin resistance was observed in 2K1C rats compared to age-matched sham rats, but fasting blood glucose level had no change. Insulin to glucose in isolated β cells was decreased in 2K1C rats compared with sham rats. 4 weeks after surgery, water, captopril (25 mg/kg) or α-lipoic acid (50 mg/kg) was feed orally for 20 weeks. Oral glucose tolerance test (OGTT) showed that only in α-lipoic acid-treated group insulin resistance was improved without change in blood pressure. In captopril-treated group, blood pressure was decreased without change in insulin resistance. Immunohistochemistry showed that number of β cells were decreased in pancreas tissue in 2K1C rat feed with water, whereas the number is increased in 2K1C rats feed with captopril or α-lipoic acid, similar as sham rats. Therefore, we suggest that sustained hypertension may induce insulin resistance by β cell dysfunction. This effect may mediated by oxidantive stress and ROS. Supported by the National Research Foundation (2012-0009322).
Key Words: Hypertension, Insulin resistance, Oxidative stress, ROS

SY-8

Amphetamine Regulates ERM Proteins Signaling in the Nucleus Accumbens Core via GSK3β

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The ezrin-radixin-moesin (ERM) proteins have been implicated not only in cell-shape determination but also in cellular signaling pathway. We have previously showed that cocaine decreases phosphorylation levels of these proteins in the nucleus accumbens (NAcc), an important brain area mediating addictive behaviors. Here we further revealed that the phosphorylation levels of ERM were decreased in the NAcc core, but not in the shell, by a single injection of amphetamine (AMPH) (2 mg/kg, IP). When LiCl (100 mg/kg, IP) was co-administered with AMPH (2 mg/kg, IP), the decreases of phosphorylation levels by AMPH for both GSK3β and ERM were not present in the NAcc core. In contrast, S9 peptide (0.5 or 5.0 μg/μl), a supposed GSK3β activator, decreased phosphorylation levels for both GSK and ERM in this site. Together, these results suggest that psychomotor stimulants like AMPH regulate phosphorylation levels of ERM in the NAcc core possibly via GSK3β signaling pathway.
Key Words: Amphetamine, ERM, Nucleus accumbens, GSK3β, LiCl

SY-9

Human Giant Congenital Melanocytic Nevus Possessed Potential Proteomic Alteration Leading to Melanotumorigenesis

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Background: Giant congenital melanocytic nevus (GCMN) is a pigment cell malformation. It is a distress to patients for two reasons. One is disfigurement and the other is possibility of malignant change. But the underlying mechanism of development of GCMN and melanotumorigenesis in GCMN were unknown. The aim of this study was to identify the proteomic alterations and associated functional pathways in GCMN to answer the concerns. **Results:** Ten GCMN and normal skin samples were collected from patients. A proteomic comparison of normal skin (n = 3) and GCMN (n = 3) was carried out using 1D-LC MS/MS analysis. Relative levels of selected proteins were validated using western blotting analysis. The related biological processes of differentially expressed proteins were an-

alyzed using the bioinformatics tools. Among the total proteins in GCMN, expressions of 48 proteins were significantly ($p < 0.05$) down- (4 proteins) or upregulated (42 proteins) compared to normal skin. Importantly, 31% of upregulated proteins were implicated with various cancers and five proteins were related with melanoma. Differentially expressed proteins in GCMN were involved in biological process of 'neurotrophin signaling', 'melanosome', and 'downregulated of MTA-3 in ER-negative breast tumors'. Especially, increases in the expression of members of the 14-3-3 protein family appeared be associated with key cellular biological functions in GCMN. Western blot analysis confirmed the upregulation of 14-3-3epsilon, tau and prohibitin in GCMN. **Conclusion:** These finding suggest that GCMN possessed potential proteomic alteration leading to melanotumorigenesis and the intensive alteration of 14-3-3 family proteins could be a key regulator of GCMN biological pathway remodeling.

Key Words: Giant congenital melanocytic nevi, Melanotumorigenesis, Proteomics, 14-3-3epsilon, 14-3-3tau, Systemic analysis

SY-10

Expression of IL-34 in Human Adipose Tissues and Its Role in the Pathogenesis of Obesity-Related Diseases

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Objectives: Interleukin-34 (IL-34) is a recently identified alternative ligand for colony stimulating factor (CSF)-1R. Like CSF-1, IL-34 stimulates phosphorylation of ERK1/2 in human monocytes and promotes the formation of the granulocyte-macrophage progenitor and megakaryocyte progenitor in human bone marrow cultures. Through the receptor CSF-1R, IL-34 as well as CSF-1 serves as the key regulator of the differentiation, proliferation, and survival of the mononuclear phagocyte lineage cells. In the present study, we investigated the expression of IL-34 in human adipose tissues and its role in the pathogenesis of obesity related diseases such as insulin resistance and type 2 diabetes. **Methods:** In total, 19 non-diabetic obese women, 19 type 2 diabetic women undergoing gastric bypass surgery, and 27 normal-weight women undergoing gynecological surgery (total 65 women) were enrolled. Their anthropometric variables, abdominal fat distribution and metabolic parameters, serum IL-34 concentrations, and IL-34 mRNA expressions in abdominal subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were measured. In addition, the expression and secretion of IL-34 were measured in differentiated human adipocytes treated with conditioned media, which is collected during the culture of human monocytes, macrophages, or LPS-activated macrophages. **Results:** In human adipose tissues, IL-34 was expressed in adipocytes as well as in non-adipocytes. The IL-34 mRNA level was significantly higher in VAT than in SAT. The expression levels of IL-34 mRNA in the two fat depots were not significantly different in obese and type 2 diabetic subjects compared to the normal weight control subjects. However, serum IL-34 concentrations were significantly higher in obese subjects than in the controls. In addition, serum IL-34 levels showed significant positive correlations with an insulin resistance index and other insulin resistance-related metabolic parameters. Furthermore, in differentiated human adipocytes, IL-34 mRNA expression and protein secretion were markedly increased by the treatment with conditioned media. **Conclusions:** Adipose tissue IL-34 may play an important role in the pathogenesis of insulin resistance and may contribute to the metabolic abnormalities associated with obesity.

Key Words: IL-34, Adipose tissue, Obesity, Diabetes, CSF-1R

SY-11

Non-Silent Story on Synonymous Sites in Voltage-Gated Ion Channel Genes

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Synonymous mutations are usually referred to as "silent", but increasing evidence shows that they are not neutral in a wide range of organisms. We looked into the relationship between synonymous codon usage bias and residue importance of voltage-gated ion channel proteins in mice, rats, and humans. We tested whether translationally optimal codons are associated with transmembrane or channel-forming regions, i.e., the sites that are particularly likely to be involved in the closing and opening of an ion channel. Our hypothesis is that translationally optimal codons are preferred at the sites within transmembrane domains or channel-forming regions in voltage-gated ion channel genes to avoid mistranslation-induced protein misfolding or loss-of-function. Using the Mantel-Haenszel procedure, which applies to categorical data, we found that translationally optimal codons are more likely to be used at transmembrane residues and the residues involved in channel-forming. We also found that the conservation level at synonymous sites in the transmembrane region is significantly higher than that in the non-transmembrane region. This study provides evidence that synonymous sites in voltage-gated ion channel genes are not neutral. Silent mutations at channel-related sites may lead to dysfunction of the ion channel.

Key Words: Voltage-gated ion channel, Synonymous mutation, Transmembrane, Mistranslation, Optimal codon, Translational selection

SY-12

Green Tea Extract Co-Administered with a Polymer Effectively Prevents Alcoholic Liver Damage by Prolonged Inhibition of Alcohol Absorption in Mice

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Aims: Alcohol toxicity can induce multiple organ dysfunction, including liver. Gallated catechins (GC), the components of green tea extract (GTE), have been known to inhibit intestinal lipid absorption. The present study was designed to investigate the inhibitory effect of GC on the absorption of the lipid-soluble ethanol in normal mice. In addition, the effectiveness of prolonging the GC-mediated effect was evaluated as a means of preventing alcoholic liver damage. **Methods:** GTE was administered orally immediately or 90 min before ethanol administration and the blood ethanol and acetaldehyde levels were measured. Binge ethanol administration (by gavage every 6 hr for 24 hr) was used to induce acute liver injury, and GTE was administered 90 min prior to every ethanol administration. **Results:** When GTE, but not GC-decreased GTE, was administered immediately before ethanol intake, the blood ethanol and acetaldehyde levels were significantly lower than the control. On the other hand, GTE had no effect when GTE was administered 90 min before ethanol intake. When GTE was co-administered with the polymer polyethylene glycol (PEG) or poly-γ-glutamate (PGA) 90 min before ethanol intake, the lowering effect of GTE on the blood ethanol and acetaldehyde levels was maintained in contrast to the GTE-alone-treated group. After binge ethanol administration, liver weight decreased, and serum ALT and AST levels were elevated. Additionally, histopathological changes, such as macrovesicular steatosis and necrosis, were induced in the liver, together with reactive oxygen species generation. When GTE+PEG or GTE+PGA, but not GTE alone, was administered 90 min before the ethanol, acute liver injury was ameliorated. **Conclusions:** These findings support the development of GTE+PEG or GTE+PGA as an inhibitor of intestinal alcohol absorption for the preventative treatment of acute alcohol toxicity.
Key Words: Green tea extract, Gallated catechin, Alcohol absorption, Alcohol toxicity, Polyethylene glycol, Poly-γ-glutamate

SY-13

Effect of *Atractylodes macrocephala* on Hypertonic Stress-Induced Water Channel Protein Expression in Renal Collecting Duct Cells

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Edema is a symptom that results from the abnormal accumulation of fluid in the body. The cause of edema is related to the level of aquaporin (AQP)2 protein expression, which regulates the reabsorption of water in the kidney. Edema is caused by overexpression of the AQP2 protein when the concentration of Na+ in the blood increases. The rhizome of *Atractylodes macrocephala* has been used in traditional Oriental medicine as a diuretic drug; however, the mechanism responsible for the diuretic effect of the aqueous extract from A. macrocephala rhizomes (AAM) has not yet been identified. We examined the effect of the AAM on the regulation of water channels in the mouse inner medullary collecting duct (mIMCD)-3 cells under hypertonic stress. In the mIMCD3 cells, the AAM attenuates a hypertonicity-induced increase in AQP2 expression as well as the trafficking of AQP2 to the apical plasma membrane. Hypertonicity is associated with Sgk1 phosphorylation; however, the AAM attenuates hypertonic stress-induced glucocorticoid-inducible protein kinase (Sgk)1 phophorylation. Tonicity-responsive enhancer binding protein (TonEBP) is a transcription factor known to play a central role in cellular homeostasis by regulating the expression of some proteins, including AQP2. Western immunoblot analysis demonstrated that the protein and mRNA expression levels of TonEBP also decrease after AAM treatment. These results suggest that the AAM has a diuretic effect by suppressing water reabsorption via the downregulation of the sgk-TonEBP-AQP2 signaling pathway.
Key Words: Edema, Aquaporin-2, Hypertonicity, mIMCD-3, *Atractylodes macrocephala*

SY-14

Beneficial Effect of Combination with Korean Red Ginseng and Morus Alba in Metabolic Syndrome

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Objectives: *Korean red ginseng* and *Morus alba* L. are used as a traditional treatment for diabetes. This study was designed to elucidate whether combination with *Korean red ginseng* and *Morus alba* L. (MPM) ameliorates metabolic syndrome in fructose-fed rats. **Methods:** Animals were divided into four groups; Control receiving tap water, fructose-fed, rosiglitazone-treated fructose-fed rats, and MPM-treated fructose-fed rats both receiving supplemented with 60% fructose (n=10). The MPM or rosiglitazone groups initially received a high-fructose (HF) diet alone for 8 weeks, with supplementation with MPM or rosiglitazone occurring during the final 6 weeks. **Results:** MPM and rosiglitazone,

synthetic PPARγ agonist, treatment significantly prevented the increase in fasting serum glucose, leptin, triglyceride, and low density lipoprotein in the HF group when comparing with the control group. MPM and rosiglitazone also led to an increase in high density lipoprotein level in the HF group. The administration of MPM and rosiglitazone prevented the development of the metabolic disturbances such as impaired glucose tolerance, and blood pressure. MPM suppressed increased expressions of endothelin-1 (ET-1) in HF rat aorta. In addition, MPM significantly increased IR-β and PPAR-γ expression in muscle. **Conclusions:** Based on these results, we suggest that the administration of MPM improves metabolic syndrome through the alteration in lipid profiles and suppression of insulin resistant and blood pressure.
Key Words: Korean red ginseng, Morus alba, Metabolic syndrome, Hyperlipidemia, Hypertension

SY-15

The Relationship of Exercise and Brachial-Ankle Pulse Wave Velocity from the Patients with Stroke

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Exercise can induce functional changes the cardiovascular system and decrease for the cardiovascular risk factors and symtoms after stroke. We investigated that functional recovery of movement and brachial-ankle pulse wave velocity (BAPWV) in stroke patients is closely related compared with those control groups. However, the change of BAPWV after stroke with the approach of neuromuscular facilitation by physiotherapists has not been fully understood. Therefore, we examed the change of BAPWV after stroke according to functional recovery of movement by neuromuscular treatment. Subjects were 17 male and 19 female, of whom 22 healthy volunteers and 14 suffered from impairment of the cerebrovascular system. Exercise was performed at 6 days per week for 4 weeks. BAPWV was measured on their bilateral upper and lower extremities. Grip strength was evaluated both affected and nonaffected side. In analysis of BAPWV, grip strength was evaluated baseline, after two and four weeks. The BAPWV was significantly higher on baseline with after 4 weeks of the stroke patients. Furthermore, BAPWV was significant decreases on nonaffected side, and affected side after physical exercise. grip strength was significant increases on nonaffected side, and affected side after physical exercise. Relationship of BAPWV and grip strength showed a correlation in affected side. The present results suggested that Exercise decreases the BAPWV on affected side on stroke.
Key Words: Exercise, Brachial-ankle pulse wave velocity, Stroke

SY-16

Effects of Therapeutic Exercise on Standing Balance in Patients with Incomplete Spinal Cord Injury

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A recent study has been also demonstrated hippotherapy to be an effective intervention aimed at improving hip and trunk stability. However, there is no comparative study that has tested standing balance after therapeutic exercise in incomplete cervical spinal cord injury. Therefore, the purpose of this study examined the effect static standing balance of therapeutic exercise in patients with incomplete cervical spinal cord injury. In the present study, there were eleven patients with incomplete cervical spinal cord injury. We have applied therapeutic exercise for 30 minutes. All participants were evaluated each session. Each session included the assessment of static standing balance using interactive balance system (IBS). Standing balance parametars, including of F1, F2-4, F5-6, F7-8, weight distribution index (WDI), and stability index (SI) by methods of eight standing position, were recorded and analyzed. F1 and WDI were significantly improved in group of therapeutic exercise. But, F7-8 was statistically no significant in group of therapeutic exercise. In stability, all measurement was statistically significant improvement result in exercise group. These results suggested that therapeutic exercise of stability on static standing balance after spinal cord injury which may support additional effect to rehabilitation in patients with incomplete cervical spinal cord injury.
Key Words: Incomplete Spinal cord injury, Therapeutic exercise, Standing Balance

SY-17

Analysis of Body Components for Taekwondo Athletes Compared to Nonathletes

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It is well known that the difference in individual characteristics showed between somatotypes and body components. However, few studies have investigated differences in body components and gender in taekwondo athletes compared

to nonathletes. The present study is to analyze the body components and skin-fold thickness of taekwondo athletes compared to nonathletes, and to contribute to current research on rehabilitation and its application and significance. The fat body mass and impedance were significantly decreased in the taekwondo athlete compared with the nonathletic groups. The same values of the men group showed significantly greater decreases than those of the women in both the nonathlete and taekwondo groups. But, the lean body mass, basal metabolic rate (BMR), and total body water were significantly increased in the taekwondo athlete compared with the nonathletic groups. In terms of gender, the values of the men in both the nonathlete and taekwondo groups showed significantly greater increases than those of the women of both groups. However, there were no significant differences between the men and women skin-fold thickness data for all participants. Therefore, these results, in part, found that there was a difference in body components between taekwondo athletes and nonathletes. Also, when applying physical stimuli such as electrotherapy, ultrasound, and heat, the body components of each patient for healthy life need to be carefully considered.

Key Words: Body components, Skin-fold thickness, Taekwondo athletes

SY-18

Analysis of Posture Alignment through the Crutch Gait in Normal Subjects

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Change of posture alignment according to various types of crutch gait, however, are not fully understood. This study examined changes on posture alignment during the noncrutch, swing-to, swing-through, two-point, and four-point crutch gaits in 23 healthy volunteers. Superior, anterior, and lateral view due to a static state were measured during gait with or without a crutch, respectively. The superior view in the male group (noncrutch dynamic gait, two-point gait, four-point gait) indicated significantly differences in posture alignment shoulder area and female group was shoulder, ear-shoulder area at the between crutch gait and noncrutch dynamic gait in right side. The superior view in the male group indicated statistically significant differences in posture alignment shoulder, ear-shoulder area and female group was shoulder area at the between crutch gait and noncrutch dynamic gait in left side. The anterior view in the male group indicated statistically significant differences in body alignment hand, right knee area and female group was right knee area at the between crutch gait and noncrutch static gait, respectively. Furthermore, the anterior view in the male group indicated statistically significant differences in posture alignment shoulder, hand area and female group was right knee area at the between crutch gait and noncrutch dynamic gait in right side. Except for crutch gait and noncrutch dynamic gait in left side, the lateral view in the both group [(noncrutch static gait, swing-to gait, swing-through gait) and (noncrutch dynamic gait, two-point gait, four-point gait)] indicated statistically significant differences in posture alignment neck area, respectively. These results suggest that posture alignment on various types of gait with or without a crutch was changed in normal subjects.

Key Words: Posture alignment, Crutch gait, Normal subjects

SY-19

The Change of Physical Activities of the Joint due to Excessive and Repetitive Exercise by Athletes

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Studies have reported that physical activity is increasingly being encouraged as an essential part of a healthy lifestyle; thus, sports and preventable injury are becoming an important public health issue. Using an isokinetic test, we evaluated physical activities such as muscular activities and dynamic stability of the knee joint of skilled female athletes. The subjects were 42 females aged 24.5±0.9 years old, weighing 61.5±1.1 kg; they were 169.6±1.3 cm in height and had a body mass index (BMI) of 18.2±0.5. We used a Biodex 3 System Pro® as the isokinetic dynamometer. The extensor in the dominant leg of the jumping group showed significantly higher strength than its counterpart in the cutting group. However, knee flexors showed no significant difference between groups. Furthermore, the H:Q ratios of the jumping group (nondominant: 45.4±2.3%, dominant: 45.0±1.2%) and the cutting group (nondominant: 48.1±1.9%, dominant: 51.5±1.6%) were lower than 60%. In addition, the ratio of dominance in the jumping group was significantly lower than in the cutting group. These results suggest that skilled female athletes who perform excessive and repetitive jumping actions need to be more aware of their risk of developing anterior cruciate ligament injury, and they need a more specific therapeutic program for this injury.

Key Words: Anterior cruciate ligament injury, Muscular characteristics, Exercise

SY-20

Modification of Central Sweating Thresohold and Peripheral Sweating Function Induced by Long-Term Physical

Activity in Relative Active Heating

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The study sought to determine the modification of central sudomotor mechanisms and sweat gland function by endurance physical activity. Fifteen sedentary (control) and 20 long-term physical activity (trainee) subjects (VO_{2max}, 43.2±5.1 vs. 59.4±3.7 ml/kg/min; body mass index, 23.5±3.7 vs. 17.3±2.5; body fat, 22.5±4.8 vs. 16.3±2.9%; $p<0.001$) sat in a chair in a relaxed posture for 60 min and then performed 30 min running tests at 60% VO_{2max} (active heating, AH) in a thermoneutral climate chamber (temperature, 25±0.5°C, 60±3% relative humidity). The tympanic temperature (Tty), local skin temperatures (chest, upper arm, thigh, and leg) and sweating rate, activated sweat gland density were measured during AH. The Tty threshold for sweating was lower in trainee than control ($p<0.05$) and sweat onset time on local parts (chest, abdomen, back and thigh) was shortened in trainee ($p<0.001$). The local sweat volume, activated sweat gland on torso (chest, abdomen, upper back, lower back) and limb (upper arm, forearm, thigh, leg), activated single sweat gland output and whole body sweat loss volume were significantly higher in trainee than control subjects. In conclusion, the Tty threshold for sweating was lower in trainee by changing the central sudomotor drive and increases-regulated responsiveness of peripheral sweating function.

Key Words: Central sweating threshold, Peripheral sweating function, Long-term physical activity, Active heating, Temperature

SY-21

Aged Garlic Extract Enhances Exercise-Mediated Improvement of Metabolic Parameters and Inflammatory Factors in High Fat Diet-Induced Obese Rats

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Aged garlic extract (AGE) is known to have a protective effect against immune system, endothelial function, oxidative stress and inflammation. We investigated the effects of exercise with and without aged garlic extract treatment on body weight, lipid profiles, inflammatory cytokines, and oxidative stress markers in high-fat diet (HFD)-induced obese rats. Forty-five Sprague-Dawley rats were fed either a HFD (HFD, n=40) or a normal diet (ND, n=5) for 6 weeks and thereafter randomized into ND (n=5), HFD (n=10), HFD with AGE (n=10), HFD with Exercise (n=10), or HFD with Exercise+AGE (n=10) for 4 weeks. AGE was administered at a dose of 2.86 g/kg · body weight, orally. Exercise consisted of running 15-60 min 5 days/week with gradually increasing intensity. AGE ($p<0.01$), Exercise, and Exercise +AGE ($p<0.001$) attenuated body weight gain and food efficiency ratio compared to HFD. Visceral fat and liver weight gain were attenuated ($p<0.05$) with all three interventions with a greater effect on visceral fat in the Exercise +AGE than AGE ($p<0.001$). In reducing visceral fat ($p<0.001$), epididymal fat ($p<0.01$) and liver weight ($p<0.001$), Exercise+AGE was effective, but exercise showed a stronger suppressive effect than AGE. Exercise+AGE showed further additive effects on reducing visceral fat and liver weight ($p<0.001$). AGE significantly attenuated the increase in total cholesterol and low-density lipoprotein-cholesterol compared with HFD ($p<0.05$). Exercise+AGE attenuated the increase in triglycerides compared with HFD ($p<0.05$). Exercise group significantly decrease in C-reactive protein ($p<0.001$). These results suggest that AGE supplementation and exercise alone have anti-obesity, cholesterol lowering, and anti-inflammatory effects, but the combined intervention is more effective in reducing weight gain and triglycerides levels than either intervention alone.

Key Words: Aged garlic extract, Exercise, High fat diet, Obesity, Metabolic parameters

SY-22

The Effect of Aerobic and Resistance Exercise on Cardiac Morphological Alteration in OLETF Rats

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Cardiac remodeling is characterized by changed shape, size and function in left ventricular and commonly divided into physiological or pathological state by volume overload or pressure overload. Type 2 diabetes mellitus (T2DM), which is caused due to lack of insulin action, is one of most important risk factor of cardiovascular disease and pathological cardiac remodeling condition that results of mortality and morbidity. Although recent researches are focused on the exercise T2DM heart by exercise, the effect of aerobic and resistance exercise-induced cardiac structural alterations mechanism in diabetic hearts are yet to be fully elucidated. Accordingly, the aim of our study was undertaken to compare the effects of aerobic and resistance exercise on cardiac structural and functional alteration in diabetic rats. At the age of 25 weeks, Otsuka Long-Evans Tokushima fatty (OLEFT) rats were divided into aerobic exercise (EXA, n=8), resistance exercise (EXR, n=8), sedentary control (SED, n=8) groups. EXA groups were treated for 30～60 minutes at 10～20 m/min on the treadmill and EXR groups climbed ladder inclined at 85 degrees with weights attached to their tails. Both of exercise was performed 5 days/week for 12 weeks. After exercise, intraperitoneal glucose tolerance tests, lipid metabolism studies and echocardiography were measured. We observed

significantly improved glucose uptake, lipid metabolism in EXA and EXR rats compared to SED rats. EXA and EXR rats significantly reduced wall thickness (intraventicular septum, posterior wall) and increased LV chamber size (LVIDd, EDV) in diabetes. Cardiac performance (SV, CO) significantly increased in two exercise groups compared with SED rats. RWT and LVMI were reduced in EXA and EXR rats compared with SED rats. Our results suggested that T2DM shows thickened wall and diminished LV chamber, indicating as concentric remodeling and exercise training confers recovery against diabetes-induced morphological heart change. Further studies will be required to reveal the molecular mechanism of aerobic and resistance exercise-induced protection or recovery from cardiac damages in diabetes.

Key Words: Type 2 diabetes (T2DM), Aerobic exercise, Resistance exercise, Heart, Relative wall stress, LV mass index

SY-23

The Mechanical Stress into the Intervertebral Disc Generates the Sensory Signals and Impaired Behavioral Patterns

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The mechanism and stereotypical behaviors of the low-back pain (LBP) are rarely understood. Based on some studies using neurotracer, it was found that afferent fibers are innervated into the low-back structures including muscles, ligaments and intervertebral disc. We examined whether intervertebral discs (IVDs) themselves deliver sensory inputs such as mechanical stimulation and further may be another nociceptive source to influence animals' behavior. After the injury with needle punctures into IVDs (L4-L5 and L5-L6 IVDs) in SD rats (male, 250-270 g), we measured the behavioral changes which were categorized as weight loads, secondary hyperalgesia and rearing in open filed. Punctured IVD group showed the increased dependence into forelimbs, reduced mechanical threshold in hindlimbs and decreased duration and number of rearing. Compared to sham group, punctured group have such impaired behavior patterns between days 5 and 14 after surgery. These results implicate that IVDs could be a critical source of low back pain. Using *in vivo* single-nerve recording, we explored whether the afferent fibers innervated into IVDs in naïve rats might respond to mechanical stimuli and pressure. With the application of von-frey filaments (2-6-10-26 g), the neuronal excitations were displayed in the annulus fibrus and anterior longitudinal ligament. Together, the pressure (100-300 mmHg) into IVDs with saline-solution evoked neuronal activities. The present study suggests that the sensory information from IVDs could be transmitted to spinal cord and has the possibility to produce low back pain.

Key Words: Intervertebral discs, Weight bearing, Rearing, Sencodary hyperalgesia, In vivo single nerve recording

SY-24

Effects of Age on Pain Responses in the Experimental Animal Model with Knee Arthritis

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Arthritis is the most common chronic disease leading to debilitation. Arthritis increases with aging and produces spontaneous pain and exaggerated painful responses to external stimuli. Clinical manifestations of arthritis in adults may be different from those in young or old cohorts. Because young adult animals have been most frequently used in the experimental setting, pain responses in animals may not exactly reflect the clinical situation that occurs in middle-age or elderly adults. However, the effect of age on pain responses in the animal models of arthritis, which are usually used to study chronic inflammatory pain, has not been evaluated. Accordingly, in the present study, we investigated the effects of age on pain behaviors in commonly used arthritis model. Male Sprague-Dawley rats were divided into 4 groups based on their ages: (1) young rats (4-5 weeks old); (2) adult rats (7-8 weeks old); (3) middle-age rats (6 months old); (4) old rats (over 18 months old) groups. To induce arthritis, rats were injected with the Monosodium iodoacetate (MIA; 4 mg/50 μl) in the right knee joint under enflurane anesthesia (1-2%). To check that MIA injection produced arthritis, the diameters of the right and left knee joints were measured before and after injections. The degree of weight bearing using the weight bearing device and the incapacitance tester were measured to assess non-evoked pain behaviors. Paw withdrawal threshold to mechanical stimulation on both hindlimbs were also assessed. To assess the histological changes of knee joint, hematoxylin and eosin staining was used at 2 weeks after MIA injection. Old rats displayed less reduction in withdrawal threshold than other age groups. Withdrawal threshold of middle-age rats was reduced less than either adult or young rats. Knee joint diameters significantly increased after MIA injections. No significant age-related differences in knee joint edema as % increase in diameter were detected. Adult or young rats displayed more reduction of weight load in two tests and more severe articular-cartilage deterioration than either old or middle-age groups. This study demonstrated that young and adult rats developed more robust behavioral signs of pain and cartilage deterioration than old and middle-age rats and old rats. Therefore, age factor should be considered for the interpretation of pain related behaviors.

Key Words: Rat, Monosodium iodoacetate (MIA), Age, Inflammatory pain

SY-25

Suppression of Epinephrine Derived from Adrenal Medulla Potentiates the Analgesic Effect of Corticosterone Supplementation in Mouse Formalin Test

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Corticosterone has been clinically used to treat inflammatory diseases and produces potent anti-inflammatory and analgesic effects. Epinephrine is mainly synthesized by the enzyme phenylethanolamine N-methyltransferase (PNMT) from adrenal medulla and works as an endogenous ligand to adrenergic receptor, which can affect peripheral nociception. Recently it was also reported that adrenal gland-derived epinephrine was increased by exogenous injection of corticosterone. However, it is not clear whether change of peripheral epinephrine affects corticosterone-induced antinociceptive effect. This study was designed to examine whether exogenous corticosterone- induced antinociceptive effect is modulated by three methods for suppression of adrenal medulla-derived epinephrine, inhibition of PNMT enzyme activity using DCMB (PNMT inhibitor), blocking of peripheral adrenergic receptors (phentolamine: α-adrenoceptor antagonist and propranolol: β-adrenoceptor antagonist), surgical elimination of adrenal gland (adrenalectomy) in formalin-induced pain model in mice. Corticosterone was diluted in 10% dimethyl sulfoxide (DMSO) and the other drugs were diluted in saline. All drugs were administered by intraperitoneal injection. Supplementation of corticosterone at low doses (5 and 25 mg/kg) did not produce an antinociceptive effect, whereas a high dose of corticosterone (50 mg/kg) significantly reduced pain responses in late phase. The pre-treatment of DCMB (10 mg/kg) caused a leftward shift in the dose-response curve for corticosterone alone treatment. The pre-injection of propranolol (5 mg/kg), but not phentolamine, also enhanced corticosterone- induced anti-nociceptive effect. Moreover, low dose of corticosterone dramatically reduced pain responses in adrenalectomized animals. These results demonstrated that suppression of epinephrine derived from adrenal medulla could potentiate corticosterone-induced anti-nociceptive effect in inflammatory pain condition.

Key Words: Corticosterone, Epinephrine, PNMT, Adrenalectomy, Formalin test

SY-26

Transplantation of Human Umbilical Cord Blood or Amniotic Epithelial Stem Cells Alleviates Mechanical Allodynia after Spinal Cord Injury in Rats

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Stem cell therapy is a potential treatment for spinal cord injury (SCI) and a variety of different stem cell types have been grafted into humans suffering from spinal cord trauma or into animal models of spinal injury. Although several studies have reported functional motor improvement after transplantation of stem cells into injured spinal cord, the benefit of these cells for treating SCI-induced neuropathic pain is not clear. In this study, we investigated the therapeutic effect of transplanting human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) or amniotic epithelial stem cells (hAESCs) on SCI-induced mechanical allodynia (MA) and thermal hyperalgesia (TH) in T13 spinal cord hemisected rats. Two weeks after SCI, hUCB-MSCs or hAESCs were transplanted around the spinal cord lesion site and behavioral tests were performed to evaluate changes in SCI-induced MA and TH. Immunohistochemical and western blot analyses were also performed to evaluate possible therapeutic effects on SCI-induced inflammation and the nociceptive related phosphorylation of the NMDA NR1 receptor subunit. While transplantation of hUCB-MSCs showed a tendency to reduce MA, transplantation of hAESCs significantly reduced MA. Neither hUCB-MSCs nor hAESCs transplantation had any effect on SCI-induced TH. Transplantation of hAESCs also significantly reduced the SCI-induced increase in NMDA receptor NR1 subunit phosphorylation (pNR1) expression in the spinal cord. Both hUCB-MSCs and hAESCs reduced the SCI-induced increase in spinal cord expression of the microglial marker, F4/80, but not the increased expression of GFAP or iNOS. Taken together these findings demonstrate that the transplantation of hAESCs into the injured spinal cord can suppress mechanical allodynia and this effect seems to be closely associated with the modulation of spinal cord microglia activity and NR1 phosphorylation.

Key Words: Spinal cord injury, Stem cells, Mechanical allodynia, Glia, NMDA receptor

SY-27(PO-20)

Deficiency of Interleukin-10 Induces Dilated Cardiomyopathy through Distorted Extracellular Matrix Regulation

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Inflammation and oxidative stress in the myocardium is as-

sociated with adverse heart remodeling such as ventricular dilatation and fibrosis and leads to heart failure. Interleukin (IL)-10 is a potent anti-inflammatory cytokines and inhibits the synthesis of proinflammatory cytokines implicated in heart diseases. Since IL-10 has anti-inflammatory and antioxidant effects, we hypothesized that deficiency of IL-10 may produce a harmful effect in heart. In this study, the role of IL-10 in heart was examined in male C57BL/6 wild-type and IL-10 knockout (KO) mice. Gene expression of beta-myosin heavy chain (β-MHC), B-type natriuretic peptide (BNP), and atrial natriuretic peptide (ANP) was increased in IL-10 KO mice. Deficiency of IL-10 also increased gene expression and protein level of transforming growth factor beta (TGF-β). Plasma level of ANP and BNP also increased in IL-10 KO mice. Gene expression of matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitors of metalloproteinases (TIMP)-1, and TIMP-2 was increased by deficiency IL-10. Protein expression of MMP-2 also increased in IL-10 KO mice. Deficiency of IL-10 had no effect on mRNA level of collagen I and collagen III. Gene expression of cytokine interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) was increased in IL-10 KO mice. Gene expression of superoxide dismutase (SOD) and glutathione peroxidase (GPX) was increased in IL-10 KO mice. Protein level of nitrotyrosine was increased in IL-10 KO mice. While phosphorylation of AKT and p38 were increased in IL-10 KO, deficiency of IL-10 decreased phosphorylation of extracellular signal-regulated kinases (ERK). Ventricular dimension measured by ultrasonography was increased in IL-10 knockout mice. Fractional shortening and ejection fraction tended to be reduced in IL-10 KO mice. These results suggest that deficiency of IL-10 induces inflammation and oxidative stress in heart leading to distorted extracellular matrix regulation and dilated cardiomyopathy in mice.

Key Words: Interleukin-10, Heart

SY-28

Sigma-1 Receptor Modulates Spinal NADPH Oxidase Activation, Leading to Induction of the Chronic Neuropathic Pain

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We previously determined that spinal sigma-1 receptors (Sig-1Rs) play a critical role in the induction of mechanical allodynia in chronic constriction injury (CCI) rats. The present study identified the potential role of spinal NADPH oxidase (NOX)-induced reactive oxygen species (ROS) in this process. Neuropathic pain was produced by CCI of the right sciatic nerve in rats. The Sig-1R antagonist BD1047 or NOX inhibitor apocynin was administered intrathecally twice daily from postoperative days 0 to 5 (induction phase of neuropathic pain) or from days 15 to 20 (maintenance phase). Mechanical allodynia was evaluated using a von Frey filament. Western blot and immunohistochemistry were performed to assess the changes in not only NOX activity but also 8-hydroxyguanosine (8-OHG) and heme oxygenase-1 (HO-1) expression, markers of the ROS-induced oxidative damage, in spinal dorsal horn. BD1047 administration on induction phase significantly attenuated CCI-induced mechanical allodynia and increase of NOX activity. In addition, CCI-induced increase of 8-OHG and HO-1 expression were significantly diminished by BD1047 treatment. Apocynin administration on induction phase also reduced CCI-induced mechanical allodynia. In contrast, BD1047 or apocynin treatment on maintenance phase had no effect on mechanical allodynia and NOX activation. These results demonstrate that spinal Sig-1R plays a critical role in the induction of mechanical allodynia in CCI rats through modulation of the NOX activation ultimately resulting in a ROS-induced oxidative damage.

Key Words: Sigma-1 receptor, NADPH oxidase, ROS, Chronic neuropathic pain, Mechanical allodynia

SY-29

Sigma-1 Receptor Mediates Intracellular Calcium Level of Cultured Astrocyte in Rats

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Previously we have demonstrated that the spinal sigma-1 receptor (Sig-1 R) plays a critical role in the acute and chronic pain. However role of Sig-1 R in astrocyte, another critical pain modulator, has not been investigated. We designed this study to determine the role of Sig-1 R in cultured astrocyte using fluorometric calcium imaging technique. Primary astrocyte cultures were prepared from 1 day old newborn Sprague-Dawley rat cerebral cortices. Double immunocytochemistry revealed that the Sig-1 R was co-localized with glial fibrillary acidic protein (GFAP, an astrocyte marker)-positive cells. Treatment with Sig-1 R agonist, PRE-084 dose dependently (0.1, 1, 10, and 20 μM) increased intracellular calcium concentration. This increased intracellular calcium level was blocked by the pretreatment of selective Sig-1 R antagonist, BD-1047 (10 μM). In a further study, ethylene glycol tetraacetic acid (EGTA, 1.5 mM), extracellular calcium chelator, and thapsigargin (1 μM), calcium ATPase inhibitor, antagonized Sig-1 R agonist-induced effect. The results of this study suggest that the Sig-1 R of astrocyte may have a potential to modulate cell signaling pathway via the mediation of intracellular calcium level.

Key Words: Sigma-1 receptor, Astrocyte, Calcium, Fura-2

SY-30

Change of Digital Infrared Thermal Imaging and of Topography by Traction from the Herniated Nucleus Pulposus

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The study was to investigate the difference of symmetry between normal and patients with herniated nucleus pulposus (HNP) using topography and digital infrared thermal imaging (DITI). The change of symmetry after lumber-traction in patients with HNP was also compared by topography and DITI. Traction in continuous mode brought a noticeable change in contralateral body symmetry and pain severity. This study would be the first to explain the correlation of DITI, topography, and pain in patients with HNP treated with or without traction. Topography was twenty-five controls, nineteen patients with lumbar disc herniations at L4-5, L5-S1 levels. And DITI were sixteen controls, seventeen patients with lumbar disc herniations. Patients with lumbar HNP had been diagnosed by magnetic resonance imaging. The changes in skin temperature were measured at the surface of body using thermographic imaging. Topography in the controls and the patients with HNP upper trunk, middle lower trunk, gluteal regions showed significant differences between after traction treatment to be restored to controls. DITI in the controls and the patients with HNP upper trunk, middle lower trunk showed significant differences between after traction treatment to be restored to controls, but gluteal regions had no significant differences between controls, patients with HNP and also after traction treatment to be restored to controls. We found for the first time DITI and topography of had a correlation. Each of the significant differences between topography and DITI, but there was no correlation. The present results suggested that continuous traction is important for patients with HNP to body temperature and to keep a body balance.

Key Words: Traction, Patients, Topography, DITI

SY-31(PO-21)

Heat Acclimation Affects Circulating Levels of Prostaglandin E2 and Cyclooxygenase-2 in Humans

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We examined serum levels of prostaglandin E_2 (PGE_2), cyclooxygenase (COX)-2, and orexin before and after heat acclimation (HA) to test the hypothesis that decreased body temperature due to HA reduces circulating levels of these key thermoregulatory molecules. Nine healthy human male volunteers were recruited (age, 21.6 ± 2.1 years). The subjects were exposed to half-body immersion in hot water (42 ± 0.5°C) at the same time of day (2-5 pm) on alternate days for 3 weeks. The HA protocol included 10 bouts of 30 min immersion. All experiments were performed in an automated climate chamber (temperature, 26.0 ± 0.5°C; relative humidity, 60 ± 3.0%; air velocity, $<$ 1 m/sec). Tympanic and skin temperatures were measured, and mean body temperatures were calculated. The difference in body weight was used to estimate total sweat loss. Blood levels of PGE_2, COX-2, and orexin were analyzed before and after HA. Body temperature decreased significantly ($P < 0.05$) after HA, whereas sweat volume increased significantly ($P < 0.01$). Blood PGE_2, COX-2, and orexin concentrations decreased significantly compared to those at pre-acclimation ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). Our data suggest that decreased basal body temperature after HA was associated with decreases in fever-related molecules, such as PGE_2 and COX-2, and the thermogenesis-related molecule orexin.

Key Words: Heat-acclimatization, PGE_2, COX-2, Orexin, Body temperature

SY-32

The Peripheral Opioid Receptors in Knee Joint have Inhibitory Effects on Carrageenan-Induced Nociceptive Electrophysiology and Behavior

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The opioid drugs are well known to play an important role in treating the pain from the central. Also, opioid drugs have severe side effects as much as the obvious effect. But, recent study, it was found that the opioid receptor in the terminal that exists. So, side effects are lower peripheral than central treatment. Opioid receptors by binding to G protein-coupled receptors have been known to inhibit adenylyl cyclase. This is also related to inhibition of Ca^{2+} liberation and hyperpolarization of K^+. Here we examined whether the peripheral opioid receptor (subtype delta) could contribute to relieve pain behavioral signs (the reduction of weight load and in-vivo single nerve recording signal) induced by arthritis. 1) behavior test Carrageenan (1%, 50 μl/200 g) was injected into the knee joint space to induce arthritis in 7 weeks male SD rats. On 4hr after the induction of arthritis, we intra-articularly injected SNC80 (opioid delta agonist; 0.4 mg/80 μl) and saline (50 μl/200 g) and measured consecutively the peak value of weight load at pre, 4, 6, 8 hr and 1 day. 2) In-vivo single nerve recoring We used 9weeks male SD rats and same method of behavior test

induction model. On 2 hr after the induction of arthritis, we started to operate on right knee. After operation, we recorded saphenous nerve. Articular cavity was injected SNC80 (opioid delta agonist; 0.4 mg/80 μl) through catheter and saline (50 μl/200 g) and measured conduction velocity and time course consecutively the peak value of signal by stimuli of von-frey (2,6,26 and 60 g) at base_1, base_2, at 10-min intervals up to 30 minutes after drug injection, and 1 hr. We found that SN80 at dose of 0.4 mg reversed significantly the reduction of weight load on 6 and 8 hr. Taken together, our results indicate that peripheral opioid delta agonist in knee joint are involved in attenuating the arthritic pain behavior induced by Carrgneenan (1%) in rats. Peripheral opioid delta receptor can be potential target for therapeutic treatment to relieve pain associated with inflammation or peripheral joint/tissue damage.

Key Words: Opioid-delta agonist, In-vivo single nerve recording, Weight bearing test

SY-33

Spinal Microglia Suppresses Development of Contralateral Mechanical Allodynia via Interleukin-1β in Peripheral Inflammatory Pain Model

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Mirror-image pain (MIP) is an abnormal phenomenon in which damage on one side of the body also results in pain from corresponding contralateral healthy area. However, it is not clear which mechanism is involved in development of MIP. Interleukin-1β (IL-1β) in spinal cord, which is well known to be increased in peripheral inflammation, is mainly released from microglia. Because spinal IL-1β can modulate activity of neighboring neurons and other glial cells via IL-1 receptor, the present study was designed to evaluate whether microglial IL-1β modulates development of MIP in peripheral inflammatory pain model. After 2% carrageenan was injected into left hind-paw of rats, mechanical allodynia (MA) was estimated at each time point using von Frey filament. Immunohistochemistry and western blot assay were used to determine the changes of Iba-1, a marker of microglia, and IL-1β expression. Contralateral MA was developed day 5, but not day 0 after carrageenan injection in intrathecally (i.t.) saline-treated rats. However, Interestingly, i.t. pretreatment of IL-1 receptor antagonist (IL-1ra) on day 0 caused the temporary induction of contralateral MA. Moreover, i.t. pretreatment of minocycline, a selective inhibitor of microglia, dose-dependently resulted in facilitation of carrageenan-induced contralateral MA. IL-1β immunoreactive cells were double stained with Iba-1-positive cells, and IL-1β expression was also increased 2 hours after carrageenan injection. In addition, minocycline significantly decreased the expression of Iba-1 and IL-1β in negative correlation with the development of contralateral MA. These results demonstrated that the increase of spinal microglial IL-1β activation could suppress the induction of contralateral MA in peripheral inflammatory pain model, suggesting a novel possibility that microglial IL-1β plays an important role in the regulation of induction time of MIP.

Key Words: Mirror image pain, Microglia, IL-1β, Inflammatory pain

SY-34

A Deficit in Epidermal Filaggrin is Crucial for Pruritic Atopic Dermatitis in Rodent Models

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Epidermal filaggrin (filament-aggregating protein) is a major structural protein that plays a key role in the skin barrier function. Now, it is being accepted that loss-of-function mutations in filaggrin is one of major predisposing factors for atopic dermatitis. In recent, we demonstrated that neonatal capsaicin treatment into rat pups led to atopic dermatitis later in life and its pathogenesis was closely related to the down-regulation of filaggrin. However, the same treatment into mouse pups did not cause dermatitis or pruritus. In the present study, we examined whether the lack of atopic dermatitis-like signs in mice following neonatal capsaicin treatment was related to the expression of filaggrin. Capsaicin (50, 100 or 250 mg/kg, s.c.) was given to mouse pups within 48h after birth, and then scratching behavior, dermatitis and histological changes, including the expression of filaggrin, of the skin were examined. Similar to rats, neonatal capsaicin treatment caused both the reduction of TRPV1 mRNA in the DRG and the increase of pain threshold later in life in mice. Nevertheless, all the mice treated with each of 3 doses of capsaicin neither exhibited scratching behavior nor dermatitis throughout the 6-week experimental period. In contrast to the capsaicin-treated rats whose filaggrin immunoreactivity (ir) was completely abolished in the skin, it was still well preserved in the capsaicin-treated mice as much as control animals. Consistently, histological changes in the skin, such as epidermal hyperplasia, and increased number of mast cells which are common in rats treated with capsaicin, were not observed in the capsaicin-treated mice. The data presented here suggest that a difference in the expression of filaggrin following capsaicin treatment between the rat and mouse determines the presence of atopic dermatitis.

Key Words: Filaggrin, Atopic dermatitis, Pruritus, Itch, Capsaicin

SY-35(PO-22)

Characterization of Gene Expression Variants in Human Breast Cancer

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Functional and geneti discrimination between normal and cancer is important subjects for target therapy of the cancer. One of several ways to find the cancerous properties is selection of up- or down-regulated genes in the cancer compared with the normal cells. However, because of individual cancer heterogeneity, it is difficult to find these changed expressions across breast cancer samples. In this study, we hypotheses that each breast cancer undergoes individual adaptation process during cancer development. Under the assumption, heterogeneous gene expression patterns across the breast cancer samples can be interpreted as not a deregulation but a individual adaptation process. To prove the hypothesis, we investigated gene expression profiles, representing 64 normal and 64 breast cancer samples. Comparison of the gene expression profiles from the breast cancer samples to those extracted from the normal breast tissue revealed that there were cancer specific variant genes whose expression profiles were heterogeneous in the breast cancer but not in the normal samples. Indeed, these expression signatures were not observed in any other cancer types. Functionally, cancer-related genes were also significantly enriched in the set of the cancer variant genes. In conclusion, we proposed a new approach to detect distinct molecular signature for the breast cancer, and the results demonstrated that the heterogeneous gene expressions that were appeared in breast cancer are key signatures that discriminate cancer and normal samples.

Key Words: Breast cancer, Gene expression, Expression heterogeneity, Microarray

SY-36

Role of NMDA Receptor Subunit NR2B in Neuropathic Pain after Peripheral Nerve Injury in a Rat Model

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N-methyl-D-aspartate receptors (NMDAR) are generally known to be involved in synaptic plasticity and long-term potentiation (LTP) in hippocampus. Development of pathological pain is believed to be based on central sensitization of spinal dorsal horn cells which shares common mechanisms with LTP. Inhibition of NMDA receptors leads to reduction of pathological pain following peripheral nerve injury, but also causes intolerable side effects, which cannot be generally used as an analgesic drug. NR2B, one of the NMDAR subunits, is selectively located in the superficial lamina of spinal cord and forebrain where pain information is processed and transmitted from periphery to brain. Moreover, NR2B antagonists were report about its analgesic effect in several experimental pain models, but in some reports was not. So, this study was explored the effective stage of NR2B antagonist on neuropathic pain and temporal profile of NR2B expression in the spinal cord after peripheral nerve injury. 180-200 g Sprague-dawley (SD) rats were used in this study. L5 spinal nerve of the rats was tied under inhalation anesthesia (SNL model). Quadrants of L4 and L5 spinal cord were got from SNL operated rats and normal rats. Temporal expression of NR2B and its phosphorylation forms was quantified after SNL by western blotting. To evaluate the analgesic efficacy of NR2B antagonists, ifenprodil and RO 25-6981 were injected intrathecally in the acute phase and the chronic phases after nerve injury. Paw withdrawal threshold to mechanical stimulation with a series of von Frey filaments was assessed after drug injection. NR2B protein was more increased in the acute phase than the chronic phase at L5 spinal segment. Moreover, NR2B antagonists were also elevated paw withdrawal threshold more efficiently in the acute phase than in the chronic phase. Phosphorylation of Ser1303 was increased and maintained from acute phase to chronic phase after spinal nerve ligation but not Tyr1472. These results suggest that NR2B is involved in the development of neuropathic pain and NR2B and its signal transduction pathway are possible therapeutic targets for chronic pathological pain.

Key Words: Nerve injury, Glutamate, NMDAR NR2B, Mechanical allodynia, ser1303

SY-37

The Analgesic Effect of TENS on Central Neuropathic Pain after Spinal Cord Injury in Rats

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Spinal cord injuries (SCI) may result in central neuropathic pain which is extensively diffused with various qualities, including burning sensation, allodynia and hyperalgesia. Transcutaneous electrical nerve stimulation (TENS) is commonly used to treat pain that is applied with surface electrodes to the skin. On the basis of clinical experience, TENS is beneficial providing it is administered at a sufficiently strong intensity, close to the site of pain, but the stimulation parameters that would best achieve the therapeutic effect are still unclear. The aim of this study was to test the effect of TENS on neuropathic pain and to compare the effects of different stimulation strategies to alleviate mechanical allodynia after SCI. Spinal cord injury was made at T12 by using aneurysm clip in Sprague-Dawley rats (n=48) exerting a closing force 30 g to the spinal cord for 30 seconds under isoflurance anesthesia. Two groups received either high-frequency (HF) or low-frequency (LF) TENS on the back at level of the lesion. Electrodes positioned on the skin overlying either the right or left paraspinal musculature. Two additional groups received HF or LF TENS to dorsal and plantar aspect of ankle in the right

or left hind limbs. Control group received no TENS intervention. TENS was delivered 30 min. Mechanical allodynia was assessed by using a von Frey hair applied to the hind paw after 30, 45, 60 and 75 min after TENS application. In case of TENS application to hindlim, HF and LF TENS slightly reduced mechanical allodynia. However, when applied at the lesion site both HF- and LF TENS to at level to lesion site significantly reduced mechanical allodynia for 45 -75 min after treatment. The analgesic effect of HF TENS was more prominent ($p < 0.05$) and long lasted up to75 min after TENS application. Both HF and LF TENS significantly decreased c-fos expression in spinal dorsal horn at L4-5. The decrease of c-fos expression was more prominent in HF TENS group. This analgesic effect of LF- and HF TENS were reversed by pre-treated naloxone with 3 mg/kg and 6 mg/kg, a mu-opioid receptor antagonist 15 min before TENS application, respectively. These results demonstrated that the TENS applied at the level of the lesion is more effective to control neuropathic pain in SCI rat when applied to the site of tests. Both LH and HF TENS produce their effects by activation of opioid receptors in the spinal cord, respectively. Thus, TENS could be a useful tool to manage pain in people with SCI.

Key Words: Neuropathic pain, TENS, Spinal cord injury, Mechanical allodynia

SY-38

The Change of Sensory Threshold by Transcutaneous Electrical Nerve Stimulation from the Elderly People

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It is generally known that transcutaneous electrical nerve stimulation (TENS) decreases the threshold of pain and release of catecholamine by mechanical and chemical stimulation. However, there have been no studies to find the change in the sensory threshold and time-dependent stimulus by TENS on the body regions. The present study was to examine the difference of sensory threshold by electrical stimulation at low back and scapulodorsal and knee joint regions in the elderly people. The sensory threshold was significantly increased in the lower back compared with the shoulder and knee joint regions. The sensory threshold tended to increase in the older age group, increasing particularly in subjects in their 80 s. The change of the sensory threshold was significantly associated with a time-dependent manner, increasing after stimulation from 5, 10, and 15 min compared with 0 min for all ages. Furthermore, the sensory threshold in response to the application of TENS revealed a time-dependent response, with the sensory threshold tending to increase with age, in particular, in the lower back region. Therefore, these results at least partially suggest that the change of sensory threshold is associated with age, and that needed of the development of senile specialized physical therapy.

Key Words: Sensory threshold, Transcutaneous electrical nerve stimulation, Elderly people

SY-39

Angiotensin-(1-9) Stimulates Atrial Natriuretic Peptide Secretion via AT2R

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Renin-angiotensin system (RAS) plays an essential role in cardiovascular homeostasis. The peptide hormone angiotensin II (Ang II), generated by angiotensin converting enzyme (ACE), mainly regulates cardiovascular system via its receptors. More recently, we have reported that Ang-(1-7) stimulates ANP secretion via Mas receptor. However, Ang-(1-9), converted from Ang I by ACE 2, is still unknown. The aim of present study is to determine whether Ang-(1-9) stimulates ANP secretion using isolated perfused beating atria and to find out its signaling pathway. Ang-(1-9) augmented ANP secretion and concentration in a dose-dependent manner. Ang-(1-9)-induced ANP secretion (about 60% increase by 3 μM) was attenuated by the pretreatment with Ang II type 2 receptor (AT2R) antagonist but not by AT1R or Mas receptor antagonist. However, Ang-(1-9) did not influence atrial contractility and ECF translocation. These results suggest that Ang-(1-9) stimulates ANP secretion via AT2R. We try to do further study to find out the signaling mechanism involved these responses.

Key Words: Renin-angiotensin system, Atrial natriuretic peptide, Angiotensin-(1-9), AT2 receptor

SY-40

Optogenetic Mapping of Local Inhibitory Circuitry in Cerebellum

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Using high-speed optogenetic mapping, the spatial organ-

ization of local inhibitory circuits formed by cerebellar interneurons was examined. Transgenic mice expressing Channelrhodopsin-2 exclusively in molecular layer interneurons allowed us to focally photostimulate these neurons, while measuring resulting responses in postsynaptic Purkinje cells. This approach revealed that interneurons converge upon Purkinje cells over a broad area and that at least 7 interneurons form functional synapses with a single Purkinje cell. The number of converging interneurons was reduced by treatment with gap junction blockers, revealing that electrical synapses between interneurons contribute substantially to the spatial convergence. Remarkably, gap junction blockers affected convergence in sagittal cerebellar slices but not in coronal slices, indicating sagittal polarization of electrical coupling between interneurons. Lateral inhibitions from adjacent interneurons also contribute temporal timing of spike generation as well as confining spatial precision of interneuron AP generation. We conclude that electrical and chemical synapses spatially coordinate interneurons in the cerebellum and may serve this function in other brain regions.

Key Words: Optogenetic mapping, NOS-ChR2 transgenic mice, Inhibitory circuits, Electrical synapse, Chemical synase

SY-41

Localization of the Subthalamic Nucleus using the High Frequency Background Activity in Parkinson's Disease Patients

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We have examined the efficiency of the background high frequency activity of the microelectrode recording in the localization of the subthalamic nucleus. For this, we retrospectively studied 83 microelectrode recordings from 39 Parkinson's patients who did bilateral deep brain stimulation surgery. The high frequency (>1,000 Hz) background activity of the de-spiked and de-artifacted one second microelectrode showed subthalamic nucleus specific pattern that was higher in the subthalamic nucleus than the other nearby regions. We also analyzed the localization error that was calculated by substracting the subthalamic nucleus boundary by the intraoperative decision from the boundary by the high frequency background activity. This localization error was compared with medical outcomes: Unified Parkinson's Disease Rating Scale I, II, III, IV and Levodopa equivalent dosage. Our analysis found that eight patients in Unified Parkinson's Disease Rating Scale IV and four patients in Levodopa equivalent dosage had significant localization error in the left-ventral and left-dorsal boundary, respectively ($p<0.05$). We also explored that the pattern of high frequency activity power change using the the slope at the boundary in the high frequency activity trace. The pattern was compared with other deep brain stimulation target, especially the globus pallidus. Our result showed that the high frequency background activity pattern was subthalamic nucleus specific functionally and anatomically. We suggest that the high frequency background activity was very powerful to verify and localize the subthalamic nucleus. The process using one second microelectrode is simple and can be adapted to real time processing.

Key Words: Parkinson disease, Subthalamic nuclues, Microelectrode recording, Localization, High frequency background activity

SY-42

Activation of Sigma-1 Receptor Mediates Mechanical Allodynia via Phosphorylation of p38 MAP Kinasein mice and Chronic Constriction Injury Rats

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The direct activation of spinal sigma-1 receptor (Sig-1R) produces mechanical allodynia (MA) and thermal hyperalgesia (TH) in mice. In addition, the blockade of Sig-1R prevents the induction of MA, but not TH in chronic constriction injury (CCI)-induced neuropathic rats. The present study was designed to investigate whether the increase of spinal p38 MAPK phosphorylation mediates Sig-1R-induced MA or TH in mice and the induction of MA in neuropathic rats. MA and TH were evaluated using von Frey filaments and a hot-plate apparatus, respectively. Neuropathic pain was produced by CCI of the right sciatic nerve in rats. Western blot assay and immunohistochemistry were performed to determine the changes of p-p38 MAPK expression and to examine the effect of SB203580, p38 MAPK inhibitor on NMDA receptor GluN1 subunit phosphorylation (pGluN1) in spinal cord. Intrathecal (i.t.) injection of PRE084, a selective Sig-1R agonist into naïve mice time-dependently increased expression of p38 MAPK phosphorylation, which was reversed by pretreatment of BD1047, a Sig-1R antagonist. In addition, i.t. pretreatment with SB203580 dose-dependently prevented PRE084-induced MA, whereas TH induction was not affected. SB203580 injection also blocked PRE084-induced increase pGluN1 expression. In CCI rats, i.t. injection of BD1047 during the induction phase (postoperative days 0 to 5) reduced CCI-induced increase in p38 MAPK phosphorylation. Furthermore, i.t. SB203580 treatment during the induction phase suppressed development of CCI-induced MA, but not TH. These results demonstrate that increase of spinal p38 MAPK phosphorylation is closely associated with induction of Sig-1R mediated MA, but not TH. Moreover, Sig-1R-induced modulation of p38 MAPK phosphorylation plays an important role to induction but not maintenance of MA in neuropathic pain rats.

Key Words: Sigma-1 receptor, p38 MAPK, Mechanical allodynia, Neuropathic pain

SY-43(PO-24)

Electrophysiological Evidences of Stochastic Galvanic Vestibular Stimulation on the Substantia Nigra Pars Reticulata in Hemiparkinsonian Rats

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The vestibular system is known to influence various areas of central nervous system (CNS) including the basal ganglia and cortex. A few clinical trials using stochastic vestibular stimulation (SVS) showed the possibility as therapeutic options for alleviating symptoms of Parkinson's disease recently. However, it is not sufficient to explain how neuronal activities in the brain may be changed by SVS. This study examined possible mechanisms SVS through electrophysiological evidences in 6-hydroxydopamine hemi-lesioned rats. We focused on the electrophysiological changes in the substantia nigra pars reticular (SNr), a main output center of the basal ganglia, after the galvanic stochastic stimulation on the ipsilateral, contralateral, or bilateral horizontal semicircular canal to the lesioned hemisphere. Single-unit recording of the ipsilesional SNr in urethane-anaesthetized rats revealed that slow oscillation (<1 Hz) of SNr spike trains significantly increased following dopamine cell lesion. These slow oscillations were remarkably reduced by the ipsilesional, contralesional or bilateral SVS. The neuronal activity of SNr after SVS did not show a consistent tendency such as increase or decrease according to the location of stimulation, nevertheless the contralateral or bilateral SVS to hemi-lesioned side tended to increase neuronal activity comparing with the ipsilateral SVS, on the other hand, the ipsilateral SVS showed the decreasing tendency relatively. Theses electrophysiological evidences may help to understand mechanisms and develop more efficient methods of the SVS therapy for Parkinson's disease. [This study was supported by a grant from the Korea Basic Science Institute (KBSI).
Key Words: Stochastic vestibular stimulation, Parkinson's disease, Substantia nigra, Electrophysiological evidence

SY-44

The Increase of c-Fos Expression in the Nucleus of the Solitary Tract by the FII: Salt Taste Enhancement

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Salt is very important chemical for body metabolism, but high average salt intake could cause some problems in cardiovascular system. Lowering the amount of salt is a very important issue in keeping good health. In this study, we tested the potential of a salt substitute (FII) which was extracted from a traditional Korean soy sauce. We compared the c-Fos-like immunoreactivity (FLI) in the nucleus of the solitary tract by stimulating the rat tongue with various taste solutions including Control, 0.5 M NaCl only, 0.4% FII only, 0.4% FII plus 0.5 M NaCl, and 1.0 M NaCl only. The responses to 0.5 M KCl and 0.3 M NH_4Cl solutions were also compared. We found that the FLI-positive cell numbers by the taste stimulation with Control, 0.4% FII, and 0.5 M NaCl were not significantly different, but the cell numbers by 0.4 % FII + 0.5 M NaCl, 1.0 M NaCl, 0.5 M KCl, and 0.3 M NH_4Cl were significantly different from control ($p<0.001$). In particular, the higher FLI-positive cell numbers by low concentration FII-NaCl compound solution were promising result for salt enhancement of FII, since the FII contains only few ten mM of NaCl, KCl, and NH4Cl in the solution. From these results, we suggest that the FII may lower the salt intake as a salt substitute. This study was supported by Korea Food Research Institute (E0111501).
Key Words: Salt, Nucleus of the solitary tract, c-Fos, Chorda tympani, FII

SY-45

Microinjection of Ghrelin into the Nucleus Accumbens Core Enhances the Increase of Amphetamine-Induced Locomotion

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Ghrelin is a peptide hormone releasing from the stomach and known to increase food appetite. Ghrelin receptors are expressed in several brain areas, including the nucleus accumbens (NAcc), a region known to be important for processing the incentive motivational and locomotor activating properties of psychostimulant drugs. The existence of ghrelin receptors in the NAcc allows us to investigate what roles ghrelin may play in mediating locomotor activating effects of psychomotor stimulants in this site. Therefore, we examined the effect of ghrelin directly microinjected into the NAcc on amphetamine-induced locomotor activity in the rat. Ghrelin (0.1, 0.5 μg/side) was bilaterally microinjected into the NAcc core immediately followed by saline or amphetamine (1 mg/kg, i.p.) injection, then rats' locomotor activity was measured for 1 hour. In a dose-dependent manner, ghrelin in the NAcc core enhances the increase of amphetamine-induced locmotor activity, while ghrelin alone produces no significant change in basal locomotor activity in this site. These results suggest that ghrelin, a food appetite-increasing hormone, may have a distinct role in the NAcc to regulate psychomotor stimulants-induced locomotor activity.
Key Words: Ghrelin, Amphetamine, Nucleus accumbens, Locomotion, Addiction

SY-46(PO-25)

The Role of Protein Tyrosine Phosphatase Receptor T in Behavior

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Receptor protein tyrosine phosphatase receptor T (PTPRT), a receptor-like Protein tyrosine phosphatase (PTP), has a brain specific expression pattern in central nervous system and the function of the brain-specific PTPRT is reported to regulate neuronal synapse formation and could transfer extracellular signal into the cell by controlling catalytic activity related with juxta-membrane domain. The effect of the PTPRT dysfunction in CNS on behavior has yet to be investigated. Therefore, we investigated synaptic plasticities and behaviors of two different PTPRT mutant male mice; PTPRTe and PTPRTj which amino acide in extracellular ecto domain or juxtamembrane domain were mutated to histidine by point-mutation, respectively. As results, PTPRTe showed increased immobility time in forced swimming test (FST) ($p < .001$). Sucrose preference test during 24 hrs, PTPRTe showed a less preference of sucrose than wild type (WT) mice. PTPRTj mice showed more a prolonged latency from 2nd day of the acquisition phase in Morris water mase (MWM). PTPRTj mice also showed impaired memory in retention test. There was a significant decease in total number of Y-maze arms entries and alternation score. While WT showed a increased immobility time after a 8 hrs restraint stress, PTPRTj showed no change in immobility time in FST. More interestingly, both PTPRTe and PTPRTj mice showed a slower velocity in all behavior tests which we measured. In additions, both PTPRTe and PTPRTj showed the impairments of long-term potentiation. These results suggest that PTPRTe mutant has a depression-like behavior with hypoactivity, while dysfunction of PTPRTi may be related to learning impairments by attention deficit with hypoactivity.

Key Words: PTPRT, Depression, Attention deficiency disorder, Hypoactivity

SY-47

Retinal Ganglion Cell (RGC) Responses of *rd1* Retina with Symmetric and Asymmetric Biphasic Current Pulse for Epiretinal Stimulation

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Retinal prosthesis has been developed for the patients with retinitis pigmentosa (RP) and age related macular degeneration (AMD), and is regarded as the most feasible method to restore vision. Extracting optimal electrical stimulation parameters for the retinal prosthesis is one of the most important elements. In our previous study, we used both voltage and current pulse as electrical stimulus, and proposed optimal stimulus range for wild-type and *rd1* retina. Here, we used charge balanced biphasic current pulse and we tested if modulation of symmetric and asymmetric pulse could efficiently evoke RGC responses and if anodic (or cathodic) phase-1st biphasic pulse could make any difference in evoking RGC responses. The well-known animal model for RP, *rd1* (*Pde6b*^{rd1}) mice at postnatal 8 to 9 weeks were used. From the ex-vivo retinal preparation (n=15), retinal patches were placed ganglion cell layer down onto 8 × 8 MEA and RGC responses were recorded while applying electrical stimuli (epiretinal stimulation configuration). For the asymmetric pulse, 1st phase of the pulse is the same with symmetric pulse but the amplitude of 2nd phase of the pulse is less than 10 μA and charge balanced condition is satisfied by lengthening the duration of the pulse. For intensity (or duration) modulation, duration (or amplitude) of the pulse was fixed to 500 μs (30 μA), changing the intensities (or duration) from 2 to 60 μA, 60 to 1,000 μs. Fifty identical pulses were applied with 1 Hz frequency. The electrically-evoked RGC spikes response was defined as positive when the number of RGC spikes for 400 ms after stimulus was 1.3 times higher than that for 400 ms before stimulus in post-stimulus time histogram. The RGC response curve was fitted with sigmoidal function using Matlab. RGC responses were well modulated both with symmetric and asymmetric pulse regardless of amplitude or duration modulation. But the response pattern of amplitude modulation and duration modulation are different; in cathodic phase-1st pulse, the RGC spikes are easily evoked with lesser amplitude and duration range and the response curves saturated at 40 μA (800 μs). In anodic phase-1st pulse, the evoked RGC spikes response curves show linear relationship with pulse amplitude or pulse duration modulation and the response curves do not saturate. In duration modulation curve, cathodic phase-1st pulse has strengths over anodic phase-1st pulse in terms of lower threshold duration level (65.2 μs vs. 88.5 μs) and more efficient RGC spikes-evoking capability. In our experiment, cathodic phase-1st pulse is apparently more efficient than the anodic phase-1st pulse, especially in duration modulation experiment.

Key Words: Retinal ganglion cell, *rd1* mice, Biphasic current pulse

Exhibition (전시)

No	업체명 URL	대표전화	주요취급품목	기술제휴 회사명	부스위치
1	InkorScience www.inkor.co.kr	031-478-0703	Microscope, 이미징장비, cell counter, ES/iPS cell culture Reagents	LogosBiosystems, EVOS, Rrprocell	8
2	KOMABIOTECH www.komabiotech.co.kr	02-579-8787	Drug discovery service, Luminex/MAGPIX, Multiplex kits, Cell Analyzer, Assay kits, Inhibitors, Antibodies, Biochemicals	Merck Millipore, Tocris, Cell Biolabs, Alomone labs 등	9
3	(주)라이프텍 www.lifetechinc.co.kr	02-456-8566	생리학/약리학/생물학/신경과학용 실험 기자재	ADInstruments.Ltd	10,11
4	(주)싸이텍코리아	02-986-4413	Multi Myograph System, Patch Clamp system, Live Cell Imaging system, Electrophysiology Instruments	DMT, MDC, Fluxion	15,16
5	Carl Zeiss Co., Ltd/ 환은바이오텍 www.zeiss.co.kr /microscopy	02-3140-2600/ 051-751-6113	이미징 장비 - 광학현미경, 시스템현미경, 레이저 컨포칼 현미경, 현미경 전용 카메라, 이미징 소프트웨어 등	Carl Zeiss	2,3
6	(주)에스엠텍	042-824-4413	제진대, 광학테이블, 레이저, 광학마운트		1
7	다이아텍코리아(주) www.biomedms.co.kr	02-6910-0145	프로테오믹스 분석, 생체물질정량분석, 생물학적의약품 분석		13
8	YK 성형외과 www.yk5151.com	02-542-5151	미용성형(눈, 코, 가슴확대[축소]), 지방흡입(이식), 안면윤곽축소술, Facelift		4
9	제노믹원 www.genomicone.co.kr	055-384-9579	연구용 시약, 소모품, 기초장비	ATTO, QSP, LPS solution, Enzymics	6,7
10	태신바이오사이언스 www.taeshinbio.co.kr	031-563-0158	Ultra Niltrile Glove, Ultra Cooling Block, Nihon Freezer, 실험대		12
11	Park Systems Corp. www.parkafm.com	031-546-6800	원자현미경(Atomic Force Microscope)		5
12	글로케스	051-582-2366	기초연구장비, 연구용소모품	OHAUS	14

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