Life Up, Light Up
Physiology

The 68th Annual Meeting of The Korean Physiological Society

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Aims and Scope

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

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Editorial Board

2016 대한생리학회 임원명단

고 문 강두희 강복순 김광진 김기순 김기환 김명석 김용근 김우겸 김 전
김종규 김종환 김중수 남숙현 민병일 박양생 박춘식 박형진 배선호
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심은보 배영민 오석배 우선희
이은희 진영호 차승규

이 사 강다원 강동묵 공인덕 곽지연 구용숙 권성춘 권혁일 김경년 김동욱
김민선 김보경 김상정 김선희 김성주 김성준 김세훈 김양인 김영미
김용운 김원재 김의용 김재호 김정훈 김종연 김진혁 김창주 김형찬
나승열 나창수 나흥식 남택상 류판동 박경표 박규상 박명규 박병림
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배재훈 배혜란 백은주 서덕준 서상원 서석효 서인석 송대규 신동민
신형철 안덕선 안도환 안동국 양훈모 연동수 염재범 염철호 오석배
우선희 우재석 윤신희 윤영욱 이경림 이덕주 이무열 이배환 이상목
이상진 이석호 이수환 이영만 이영호 이윤렬 이장헌 이종은 이지희
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Exhibited by

싸이텍코리아
바이오월드
환은바이오텍
글로케스

Contents

Invitation (초대의 글)

회원 여러분,

열대야가 지속되는 기상 이변 속에서도 모두 건강하시고 연구에 매진하고 계시리라 기대합니다. 더위가 기승을 부리는 계절에는 추운 겨울이 생각나고, 한파가 몰아치는 추운 날씨에는 더운 여름이 생각나는 것은 우리 모두의 공통점이라고 생각합니다. 그러나 폭염이 지속되는 자연의 섭리를 인간이 조절할 수 없기 때문에 우리의 의지대로 가능한 마음의 시원함을 얻도록 노력한다면 어려운 시기도 무난하게 넘어갈 수 있지 않을까요?

오는 11월 2 – 4일은 광주에서 제68차 대한생리학회가 개최됩니다. 이번 학회는 생리학회 공식학술지인 Korean Journal of Physiology & Pharmacology의 창간 20주년을 맞는 추계학술대회이며, “Life Up, Light Up Physiology”라는 슬로건으로 하여 다양한 주제의 심포지엄과 포스터 세션을 통하여 한 해 동안 연구한 새로운 결과들을 발표하고 토론하면서 풍성한 연구정보들을 접할 수 있는 자리를 마련하였습니다. 또한 이번 학술대회에서는 대학을 떠나신 원로 교수님들을 모시고 생리학의 발전사와 학회의 발전을 위한 고견을 접함으로 생리학 연구에 대한 마음의 자세를 재고할 뿐만 아니라 우리나라 생리학 발전을 위한 귀중한 시간이 될 것으로 생각합니다. 문화와 예술의 도시 빛고을 광주에서 이번 학술대회가 그 동안의 연구성과를 공유하고 새로운 정보를 교환함으로써 회원 여러분의 학문적 성장과 친목의 장이 됨과 아울러 신진연구자와 학생연구자들의 발전을 격려하고 희망을 선물할 수 있길 기대합니다.

이번 학술대회를 준비하기 위하여 노력하신 이사님들, 조선대학교 관계자 여러분을 비롯하여 물심양면으로 도움을 주신 많은 분들께 깊은 감사를 드립니다.

회원 여러분, 모두 건강하시고 행복하시길 기원합니다.

대한생리학회 회 장 **박 병 림**
대한생리학회 이사장 **방 효 원**

Schedule (일정표)

Wednesday, November 2

조선대학교 (해오름관, Hall A)

Time	Contents	
14:00-14:10	Welcome Address for Young Physiologists	
14:10-15:20	**Young Physiologists' session - 1 (Hall A)**	**Chair:** *박원선 (강원대)*
15:20-16:00	포스터 발표	
16:00-17:00	**Young Physiologists' session - 2 (Hall A)**	**Chair:** *차승규 (연세원주의대)*
17:00-17:20	**신진생리학자상 수상강연 (김선광, 경희대학교)**	**Chair:** *김성준 (서울대)*
17:30-18:10	**원로교수 특강 – 민병일 (경희대학교 명예교수)**	**Chair:** *김선희 (전북대)*
18:30-20:00	Welcome Reception (**솔마루 레스토랑 교직원 식당 3층**)	

Thursday, November 3

조선대학교 (해오름관)

Time	Contents		
08:30-08:55	Registration (Lobby 1층)		
08:55-11:00	해오름관 대공연장		
	Opening remarks		
	Symposium I: Neurophysiology of behavioral disorder		
11:00-11:15	Coffee break		
11:15-12:10	해오름관 대공연장		
	Plenary Lecture Renal renin producing cells: motors of the renin-angiotensin system	*Prof. Armin Kurtz (Regensburg Univ., Germany)*	
12:10-12:30	KJPP 현황 및 발전방안	*강동묵 (KJPP 편집위원장)*	
12:30-12:40	생리학 기본강의록 및 학습목표 구체화 작업	*방효원 (대한생리학회 이사장)*	
12:40-13:30	Student Center, Cafeteria		
	Lunch		
13:30-15:30	Poster Presentation (in parallel with poster oral presentation in Hall A)		
15:30-17:30	Hall A	Hall B	Hall C
	Symposium II Central regulation of metabolism and food intake	**Symposium III** Calcium signaling and Ca^{2+} channels	**Symposium IV** Muscle and heart
18:00-20:00	솔마루 레스토랑 교직원 식당 (솔마루 3층)		
	간친회 (Official Dinner)		

Friday, November 4

조선대학교 (해오름관)

Time	Contents		
	Hall A	Hall B	Hall C
09:30-11:30	**Symposium V** Optogenetics for physiology	**Symposium VI** Pacemaker mechanisms and motility	**Symposium VII** Neurophysiology in IBS
11:30-12:00	휴식 및 도시락 배부		
12:00-13:00	Hall A		
	General Assembly & Poster Presentation Awards		
13:00-13:10	Closing remark (Hall A)		

Venue Guide (학술대회장 안내)

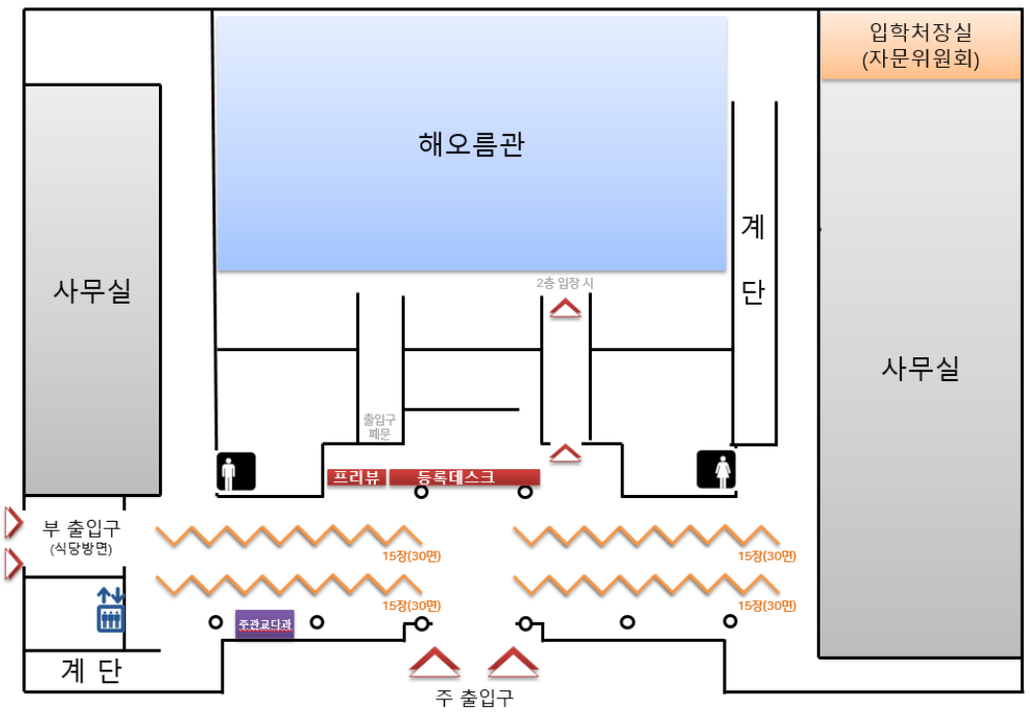
조선대학교 캠퍼스 맵

조선대학교 해오름관 및 학생식당

해오름관

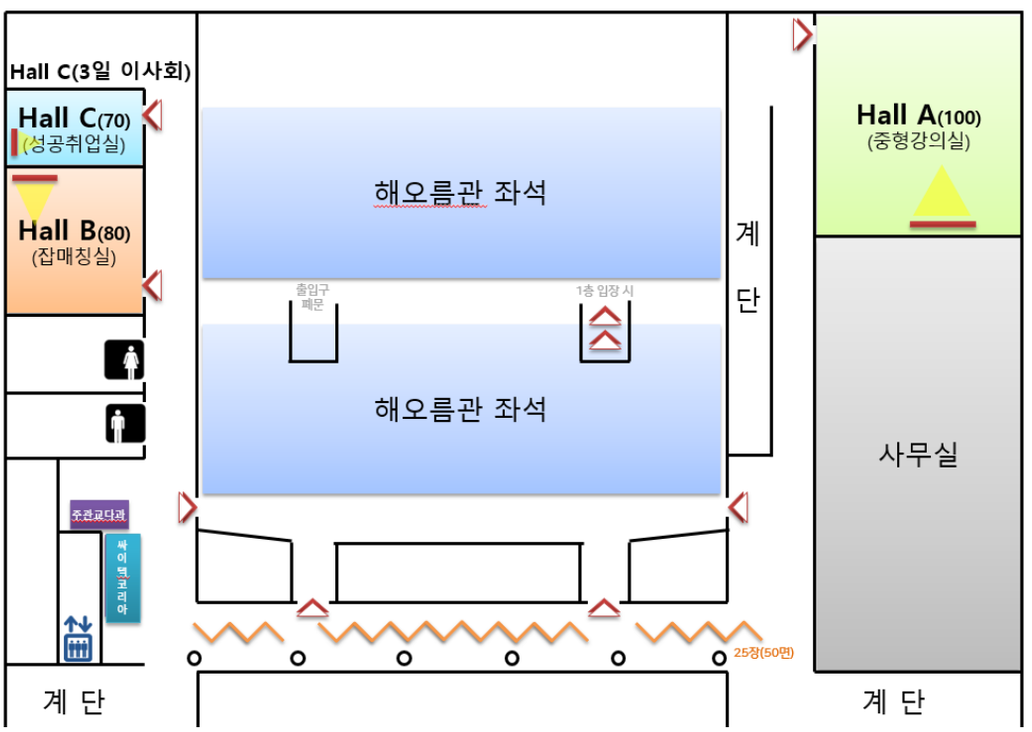
학생식당

해오름관 1층



등록데스크, 프리뷰룸, 포스터전시
자문위원회: 입학처장실 부속실 – 3일(목)11시~12시

해오름관 2층



Hall A, B, C, 포스터전시
후원사전시: (주)싸이텍코리아
이사회: Hall C – 3일(목)12시45분~13시 45분

Scientific Program (학술프로그램)

Young Physiologists' Day (Wednesday, November 2)

Time	Contents
14:00-14:10	Welcome Address for Young Physiologists
14:10-15:20	**Young Physiologists' Session - 1** **Chair:** *박원선 (강원대)*
14:10-14:20	CR6-interacting factor 1 inhibition damages vascular function by inhibiting the Sirt1-eNOS pathway *Shuyu Piao (Chungnam National University)*
14:20-14:30	Characteristics of mitochondrial calcium efflux regulation in permeabilized single ventricular myocytes *Jeong Hoon Lee (Ulsan University)*
14:30-14:40	Mechanism of hERG current inhibition by 4-hydroxynonenal (4-HNE), a polyunsaturated fatty acid-derived electrophile *Seong Woo Choi (Seoul National University)*
14:40-14:50	Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells *Hyoung Kyu Kim (Inje University)*
14:50-15:00	Ethanol inhibition of KCNQ2/3 channel is regulated by plasma membrane PI(4,5)P2 *Kwon-Woo Kim (DGIST)*
15:00-15:10	Neurosteroids-mediated actions on GnRH neuron in letrozole-induced polycystic ovarian syndrome (PCOS) mouse model *Pravin Bhattarai (Chonbuk National University)*
15:10-15:20	Analgesic effect of endogenous oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells *Rafael Taeho Han (Korea University)*
15:20-16:00	**포스터 발표**
16:00-17:00	**Young Physiologists' Session - 2** **Chair:** *차승규 (연세원주의대)*
16:00-16:10	Impaired myogenic responses in the skeletal arteries from denervated hind limbs and their recovery by exercise training; underlying changes of ion channel currents in the arterial myocytes *Ming Zhe Yin (Seoul National University)*
16:10-16:20	ATP release via gap junction hemichannels in rat atrial myocytes under shear stress *Joon-Chul Kim (Chungnam National University)*
16:20-16:30	Rapamycin ameliorates the portal hypertension, cardiovascular autonomic dysfunction, and alterations in the excitability of sympathetic and parasympathetic cardiac neurons in cirrhotic rats *Choong-Ku Lee (Yonsei University)*
16:30-16:40	A Study for Electrophysiological Characteristics of Neural Circuit between Ventral Tegmental Area and Medial Prefrontal Cortex in Rats *Yu Fan (Wonkwang University)*
16:40-16:50	Intrinsic plasticity amplifies long-term depression in parallel fibre input gain in cerebellar Purkinje cells *Hyun Geun Shim (Seoul National University)*
16:50-17:00	Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan *Yeon Hee Noh (Inje University)*
17:00-17:20	**신진생리학자상 수상강연** **Chair:** *김성준 (서울대)* Astrocytes-mediated synaptic rewiring in the primary somatosensory cortex: A novel mechanism for peripheral neuropathic pain *Sun Kwang Kim (KyungHee University)*
17:30-18:10	**원로교수 특강** **Chair:** *김선희 (전북대)* **민병일 (경희대학교 명예교수)**

Symposium (Thursday, November 3)

Contents	
Symposium I: Neurophysiology of behavioral disorder	**Organizer :** *김명환 (서울의대)*
Phospholipase Cγ1 abnormality for neuropsychiatric disorders	*김정훈 (POSTECH)*
Dysfunction of GABAergic transmission in the emotional disorder	*민선식 (을지대학교 의과대학)*
Epigenetic basis of Bdnf gene suppression by drugs of abuse in the ventral tegmental area	*구자욱 (한국뇌연구원)*
NMDAR dysfunction and autism spectrum disorder	*김은준 (KAIST)*
Symposium II: Central regulation of food intake and metabolism	**Organizer :** *손종우 (KAIST)*
Hypothalamic SF-1 regulates energy homeostasis and psychiatric behaviors	*김기우 (연세대학교 원주의과대학 약리학교실)*
Nutrient availability in astrocytes is linked to whole body energy metabolism	*김재근 (인천대학교 생명과학부)*
Divergence of food intake regulation by brain serotonin circuit	*손종우 (카이스트 생명과학과)*
Interplay between glucose and leptin signalling determines the strength of GABAergic synapses at POMC neurons	*이동근 (경상대학교 의과대학 생리학교실)*
Symposium III: Calcium Signaling and Ca^{2+} channels	**Organizer :** *신동민 (연세치대)*
A new aspect of SOCE in invertebrates	*박찬영 (UNIST)*
Does Ca^{2+} influx through CD20 determine the efficacy of anti-CD20 antibodies in B cell depletion?	*김주영 (연세대학교 의과대학 약리학교실)*
Dynamic phospholipid interaction of β2e subunit regulates the gating of voltage-gated Ca^{2+} channels	*서병창 (Department of Brain & Cognitive Sciences, DGIST)*
Ca^{2+} signaling and pacemaking in midbrain dopamine neurons	*박명규 (성균관의대 생리학교실)*
Symposium IV: Muscle and heart	**Organizer :** *전병화(충남의대), 이은희 (가톨릭의대)*
Tauroursodeoxycholic acid (TUDCA) attenuates pressure-overload induced cardiac remodeling by ameliorating endoplasmic reticulum stress (ERS)	*김도한 (광주과학기술원 생명과학부)*
A matricellular protein CCN5 reverses established cardiac fibrosis	*박우진 (광주과학기술원 생명과학부)*
P2A/HSP70 dynamically regulate hypertrophic response through modulating HDAC2 phosphorylation and its activity	*엄광현 (전남대학교 의과대학 약리학교실)*
Function of junctional proteins in Ca^{2+} dynamics	*우진석 (Dept. of Physiology, David Geffen School of Med., UCLA)*

Poster Oral Presentation (Thursday, November 3)

해오름관 Hall A

Time	Contents	Chairs : *진영호, 우선희*
13:30-13:40	PO-01: TRPC channels are a novel culprit for hepatic stellate cell activation and hepatic fibrosis	*Kyu-Hee Hwang (Yonsei University)*
13:40-13:50	PO-02: Down-regulation of NKCC and AE2 transporters in salivary gland cells by dexmedetomidine	*Minjeong Ji (Gachon University)*
13:50-14:00	PO-03: A Jumonji C (JmjC) domain-containing protein negatively regulates RANKL-mediated osteoclastogenesis	*Hye-Jin Kim (Seoul National University)*
14:00-14:10	PO-04: IDH2 Inhibition impairs endothelium-dependent vasomotor function via mitochondrial function in endothelial cells	*Sujeong Choi (Chungnam National University)*
14:10-14:20	PO-05: Computational analysis of cerebrovascular flow reserve using patient-specific medical images	*Eun Bo Shim (Kangwon National University)*
14:20-14:30	PO-06: WNK1 promotes renal tumor progression by TRPC6-NFAT pathway via activating phosphatidylinositol 4-kinase IIIa	*Ji-Hee Kim (Yonsei University)*
14:30-14:40	PO-07: Gross morphological properties of the primo-vascular system and its relations with the acupuncture meridian	*Chae Jeong Lim (Seoul National University)*
14:40-14:50	PO-08: Salt loading recruits Mg^{2+}-resistant extrasynaptic NMDA receptors in supraoptic nucleus neurons	*Kyung-Ah Park (Chungnam National University)*
14:50-15:00	PO-09: Low Intensity Ultrasound Decreases α-Synuclein Aggregation in PC12 Cells: Potential action on mitochondrial reactive oxygen species	*Mrigendra Bir Karmacharya (Inha University)*
15:00-15:10	PO-10: The maintenance ability and Ca^{2+} availability of skeletal muscle are enhanced by sildenafil	*Mei Huang (The Catholic University)*

Symposium (Friday, November 4)

Contents	
Symposium V: Optogenetics for physiology	**Organizer :** *강동묵 (성균관의대)*
Next generation optogenetics: tool development and applications	*허원도 (KAIST)*
Application of optogenetics for treatments of urological disorders	*서준교 (KIST)*
Optogenetic control of stress and mood disorders	*김대수 (KAIST)*
Optogenetic dissection of neurons in the basal forebrain	*김 태 (GIST)*
Symposium VI: Pacemaker mechanisms and motility	**Organizer :** *전제열 (조선의대)*
Properties of waxing and waning in the mouse small intestine	*Yoshihiko Kito (Saga University, Japan)*
Automaticity of interstitial cells of Cajal: A computational study	*염재범 (인제대학교)*
Hyperpolarization-activated cyclic nucleotide-gated channels are working as a pacemaker channels in colonic interstitial cells of Cajal	*최 석 (조선대학교)*
Electromechanical delay in human ventricle under various load conditions: simulation study	*임기무 (금오공대)*
The role of Korean medicines in gastrointestinal motility	*김병주 (부산대학교)*
Symposium VII: Neurophysiology in Institute for Basic Sciences (IBS)	**Organizer :** *박주민 (IBS)*
Parvalbumin expressing neurons, a link between homeostatic synaptic plasticity and cognitive dysfunction	*박주민 (Center for Cognition and Sociality, IBS)*
Regrowth of Serotonin Axons in the Adult Mouse Brain Following Injury	*진윤주 (Center for Cognition and Sociality, IBS)*
Whole-cell recordings in freely moving rodents	*이도윤 (Center for Cognition and Sociality, IBS)*
Astrocytes increase the activity of synaptic GluN2B NMDA receptors	*한정현 (Center for Neuroscience Imaging Research, IBS)*

Young Physiologists' Session

S 27 YP-01

CR6-interacting factor 1 inhibition damages vascular function by inhibiting the Sirt1-eNOS pathway
Shuyu Piao[1], Harsha Nagar[1]*, Saet-byel Jung[2]*, Min Jeong Ryu[3]*, Su-jeong Choi[1], Sung-Ho Jun[1], Hee-Jung Song[4], Shin Kwang Kang[5], Minho Shong[5], Dong Woon Kim[6], Kaikobad Irani[7], Byeong Hwa Jeon[1], Gi Ryang Kweon[3], Cuk-Seong Kim[1]
[1]Department of Medical Science & Physiology, [2]Department of Endocrinology, [3]Department of Biochemistry, [4]Department of Neurology, [5]Department of Thoracic and Cardiovascular Surgery, [6]Department of Anatomy, School of Medicine, Chungnam National University, Republic of Korea, [7]Division of Cardiovascular Medicine, Department of Internal Medicine, University of Iowa Carver College of Medicine, USA

S 27 YP-02

Characteristics of mitochondrial calcium efflux regulation in permeabilized single ventricular myocytes
Jeong Hoon Lee, Duong Duc Pham, Ga Yul Kim, Ji Yeon Song, Ji Eun Kim, Chae Hun Leem
Department of Physiology, College of Medicine, Ulsan University, Seoul, Korea

S 27 YP-03

Mechanism of hERG current inhibition by 4-hydroxynonenal (4-HNE), a polyunsaturated fatty acid-derived electrophile
Seong Woo Choi[1], Hyang-Ae Lee[1,2], Yin-Hua Zhang[1], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, Korea, [2]Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea

S 28 YP-04

Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells
Tae Hee Ko, Hye Jin Heo, Hyoung Kyu Kim, Jae Boum Youm, Sung Woo Cho, In-Sung Song, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee and Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea.

S 28 YP-05

Ethanol inhibition of KCNQ2/3 channel is regulated by plasma membrane PI(4,5)P2
Kwon-Woo Kim, Dongil Keum and Byung-Chang Suh*
Department of Brain & Cognitive Science, DGIST, Daegu, Korea

S 28 YP-06

Neurosteroids-mediated actions on GnRH neuron in letrozole-induced polycystic ovarian syndrome (PCOS) mouse model
Pravin Bhattarai[1], Santosh Rijal[1], Dong Hyu Cho[2] and Seong Kyu Han[1]
[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, [2]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine

S 28 YP-07

Analgesic effect of endogenous oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells
Rafael Taeho Han[1]*, Hanbyul Kim[2]*, Youngbum Kim[1],
Kiyeon Park[2], Pareum Lee[2], Heung Sik Na[1], Seogbae Oh[2]#
[1]Neuroscience Research Institute and Department of Physiology, Korea University College of Medicine, Seoul, Korea
[2]National Research Laboratory for Pain, Dental Research Institute and Department of Physiology, School of Dentistry, Seoul National University, Seoul, Korea

S 29 YP-08

Impaired myogenic responses in the skeletal arteries from denervated hind limbs and their recovery by exercise training; underlying changes of ion channel currents in the arterial myocytes
Ming Zhe Yin[1,2], Eun Young Seo[1,2], Hae Jin Kim[1,2], Yin Hua Zhang[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S 29 YP-09

ATP release via gap junction hemichannels in rat atrial myocytes under shear stress
Joon-Chul Kim, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Korea

S 30 YP-10

Rapamycin ameliorates the portal hypertension, cardiovascular autonomic dysfunction, and alterations in the excitability of sympathetic and parasympathetic cardiac neurons in cirrhotic rats
Choong-Ku Lee & Seong-Woo Jeong
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 30 YP-11

A Study for Electrophysiological Characteristics of Neural Circuit between Ventral Tegmental Area and Medial Prefrontal Cortex in Rats
Yu Fan[1], Ho Koo[2], Sang Hu Han[2], Se Jin Moon[2], Jae Hyo Kim[1], Byung Rim Park[2], Min Sun Kim[2]
[1]Department of Meridian & Acupoint, College of Korean Medicine, Wonkwang University, Iksan, Korea, [2]Department of Physiology, Wonkwang University School of Medicine, Iksan, Korea

S 30 YP-12

Intrinsic plasticity amplifies long-term depression in parallel fibre input gain in cerebellar Purkinje cells
Hyun Geun Shim[1,2,4], Dong Cheol Jang[1,3,4], Sang Jeong Kim[1,2]
[1]Department of Physiology, [2]Department of Biomedical Science, Seoul National University College of Medicine, [3]Department of Brain and Cognitive Science, College of Science, Seoul National University, Seoul, Republic of Korea, [4]These authors contributed equally to this work

S 31 YP-13

Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan
Dang Van Cuong[1,#], Hyoung Kyu Kim[1,2,#], Yeon Hee Noh[1], Jubert Marquez[1], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], and Jin Han[1,*]
[1]National Research Laboratory for Mitochondrial Signaling, Department of Physiology, Department of Health Sciences and Technology, BK21 Project Team, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, [2]Department of Integrated Biomedical Science, College of Medicine, Inje University, Busan 47392, Korea

Poster Oral Presentation

S 31 PO-01

TRPC channels are a novel culprit for hepatic stellate cell activation and hepatic fibrosis
Kyu-Hee Hwang[1,2], Ji-Hee Kim[1,2], Soo-Jin Kim[1,2], Seong-Woo Jeong[1,2,3], In Deok Kong[1,2,3], Kyu-Sang Park[1,2,3] and Seung-Kuy Cha[1,2,3]
Departments of [1]Physiology and [2]Global Medical Science, and [3]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, South Korea

S 31 PO-02

Down-regulation of NKCC and AE2 transporters in salivary gland cells by dexmedetomidine
Minjeong Ji, Wanhee Suk, Jeong Hee Hong*
Department of Physiology, College of Medicine, Gachon University, Lee Gil Ya Cancer and Diabetes Institute, 155 Getbeolro, Yeonsu-gu, Incheon, 21999, South Korea

S 32 PO-03

A Jumonji C (JmjC) domain-containing protein negatively regulates RANKL-mediated osteoclastogenesis
Seon-Young Kim[1], Hye-Jin Kim[1], Jong-Wan Park[2], Yang-Sook Chun[1,2]
[1]Department of Physiology, and [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 32 PO-04

IDH2 Inhibition impairs endothelium-dependent vasomotor function via mitochondrial function in endothelial cells
Sujeong Choi[1,7]*, Harsha Nagar[1,7]*, Shuyu Piao [1,7], Saet-byel Jung[2], Hyun-Woo Kim[1], Shin Kwang Kang[3], Jun Wan Lee[4], Jin Hyup Lee[5], Jeen-Woo Park[6], Byeong Hwa Jeon[1], Hee-Jung Song[7+], Cuk-Seong Kim[1,7+]
[1]Department of Medical Science & Physiology, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea; [2]Department of Endocrinology, [3]Department of Thoracic and Cardiovascular Surgery, [4]Emergency ICU, Regional Emergency Center, [7]Department of Neurology, School of Medicine, Chungnam National University Hospital, Daejeon 301-721, Republic of Korea; [5]Department of Food and Biotechnology, Korea University, Sejong 339-700, Republic of Korea; [6]School of Life Sciences, College of Natural Science, Kyungbook National University, Taegu 702-701, Republic of Korea; [7]Department of Medical sciences, College of Natural Science, Chungnam National University Hospital, Daejeon 301-721, Republic of Korea

S 32 PO-05

Computational analysis of cerebrovascular flow reserve using patient-specific medical images
Ajin Ryu[1], Kyoung-Min Lee[2], Eun Bo Shim[1]
[1]Department of Mechanical and Biomedical Engineering Kangwon National University, [2]Department of Neurology, Seoul National University Hospital

S 33 PO-06

WNK1 promotes renal tumor progression by TRPC6-NFAT pathway via activating phosphatidylinositol 4-kinase IIIα
Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Minseob Eom[3], Seong-Woo Jeong[1,4], In Deok Kong[1,2,4], Kyu-Sang Park[1,2,4] and Seung-Kuy Cha[1,2,4]
Departments of [1]Physiology, [2]Global Medical Science, [3]Pathology, and [4]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea

S 33 PO-07

Gross morphological properties of the primo-vascular system and its relations with the acupuncture meridian
Chae Jeong Lim, So Yeong Lee, Pan Dong Ryu
Department of Veterinary Pharmacology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea

S 33 PO-08

Salt loading recruits Mg^{2+}-resistant extrasynaptic NMDA receptors in supraoptic nucleus neurons
Kyung-Ah Park, Chiranjivi Neupane, and Jin Bong Park
Department of Physiology and Biomedicine, School of Medicine, Chungnam National University, Republic of Korea

S 34 PO-09 Low Intensity Ultrasound Decreases α-Synuclein Aggregation in PC12 Cells: Potential action on mitochondrial reactive oxygen species
Mrigendra Bir Karmacharya[1], Binika Hada[2], Byung Hyune Choi[2]*, and So Ra Park[1]*
[1]Department of Physiology and Biophysics, Inha University College of Medicine, Incheon, 22212, South Korea, [2]Department of Biomedical Sciences, Inha University College of Medicine, Incheon, 22212, South Korea

S 34 PO-10 The maintenance ability and Ca^{2+} availability of skeletal muscle are enhanced by sildenafil
Mei Huang[1], Keon Jin Lee[1], Kyung-Jin Kim[2], Mi Kyoung Ahn[1], Deok-Soo Han[1], Chung-Hyun Cho[2], Do Han Kim[3], and Eun Hui Lee[1]*
[1]Department of Physiology, College of Medicine, The Catholic University, Korea, [2]Department of Pharmacology, Seoul National University College of Medicine, Korea, [3]School of Life Sciences and Systems Biology Research Center, Gwangju Institute of Science and Technology, Gwangju, Korea

Young Physiologist Award Lecture

S 35 Astrocytes-mediated synaptic rewiring in the primary somatosensory cortex: A novel mechanism for peripheral neuropathic pain
Sun Kwang Kim
Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul 02447, Korea

Plenary Lecture

S 36 Renal renin producing cells: motors of the renin angiotensin system
Armin Kurtz
Institute of Physiology, University of Regensburg, Germany

Symposium

Symposium I: Neurophysiology of behavioral disorder

S 37 S-I-1 Phospholipase Cγ1 abnormality for neuropsychiatric disorders
Joung Hun Kim
Department of Life Sciences, POSTECH (Pohang University of Science & Technology)

S 37 S-I-2 Dysfunction of GABAergic transmission in the emotional disorder
Sun Seek Min
Department of Physiology and Biophysics, School of Medicine, Eulji University

S 37 S-I-3 Epigenetic basis of Bdnf gene suppression by drugs of abuse in the ventral tegmental area
Ja Wook Koo[1,2], Michelle S. Mazei-Robison[1], Quincey LaPlant[1], Ezekiell Mouzon[1], Mary Kay Lobo[3], David M. Dietz[4], Scott J. Russo[1], Rachael L. Neve[5], Yasmin L. Hurd[1], and Eric J. Nestler[1]
[1]Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA, [2]Department of Neural development and disease, KBRI, Daegu 700-300, Korea, [3]Department of Anatomy and Neurobiology, University of Maryland, Baltimore, MD 21201, USA, [4]Department of Pharmacology and Toxicology, SUNY at Buffalo, Buffalo, NY 14214, USA, [5]Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA

S 37 S-I-4 NMDA receptor dysfunction and autism spectrum disorders
Eunjoon Kim
Center for Synaptic Brain Dysfunctions, Institute for Basic Science (IBS), and Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea

Symposium II: Central regulation of food intake and metabolism

S 38 S-II-1 Hypothalamic SF-1 regulates energy homeostasis and psychiatric behaviors
Ann W. Kinyua, Dong Joo Yang, Dong-Whee Son, My Khanh Q. Huynh, Jae-Won Choi, Chang Mann Ko, Ki Woo Kim
Department of Pharmacology and Global Medical Science, Wonju College of Medicine, Yonsei University, Korea

S 38 S-II-2 Nutrient availability in astrocytes is linked to whole body energy metabolism
Jae Geun Kim
Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Korea

S 38 S-II-3 Divergence of food intake regulation by brain serotonin circuit
Jong-Woo Sohn[1], Chen Liu[2], Eun-Seon Yoo[1], Seahyung Park[1], Kevin W. Williams[2], Joel K. Elmquist[2]
[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, [2]Division of Hypothalamic Research, Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas

S 38 S-II-4 Interplay between glucose and leptin signalling determines the strength of GABAergic synapses at POMC neurons
Dong Kun Lee[1], Jae Hoon Jeong[2], Sung-Kun Chun[2], Streamson Chua, Jr.[3], Young-Hwan Jo[3]
[1]Department of Physiology, Institute of Health Sciences, Gyeongsang National University School of Medicine, Korea, [2]Division of Endocrinology, Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, USA, [3]Department of Molecular Pharmacology, Albert Einstein College of Medicine of Yeshiva University, USA

Symposium III: Calcium signaling and Ca^{2+} channels

S 39 S-III-1 A new aspect of SOCE in invertebrates
Kyu Min Kim[1], Tharaka Wijerathne[2], Kyu Pil Lee[2], Chan Young Park[1]
[1]Department of Biological Sciences, Ulsan National Institute of Science and Technology (UNIST), Ulsan and [2]Department of Physiology, College of Veterinary medicine, Chungnam National University, Daejun, Korea

S 39 S-III-2 Does Ca^{2+} influx through CD20 determine the efficacy of anti-CD20 antibodies in B cell depletion?
Woo Heo[1], Jinu Lee[2], Min Goo Lee[1], Joo Young Kim[1]
[1]Department of Pharmacology and Brain Korea 21 Plus Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea, [2]College of Pharmacy, Yonsei Institute of Pharmaceutical Sciences, Yonsei University, Incheon 406-840, Korea

S 39 S-III-3 Interplay of β2e and Ca^{2+} promotes inactivation of CaV channels in a calmodulin-independent manner
Byung-Chang Suh
Department of Brain and Cognitive Sciences, DGIST, Daegu 42988, Korea

S 39 S-III-4 Ca^{2+} signaling and pacemaking in midbrain dopamine neurons
Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, 440-746, Korea

Symposium IV: Muscle and heart

S 40 S-IV-1 Tauroursodeoxycholic acid (TUDCA) attenuates pressure-overload induced cardiac remodeling by ameliorating endoplasmic reticulum stress (ERS)
Shilpa Rani, Pradeep Kumar Sreenivasaiah, Jin Ock Kim and Do Han Kim
School of Life Sciences, Gwangju Institute of Science and Technology (GIST)

S 40 S-IV-2 The Matricellular Protein CCN5 Reverses Established Cardiac Fibrosis
Woo Jin Park
Gwangju Institute of Science and Technology, Gwagnju, Republick of Korea

S 41 S-IV-3 Mechanisms of hypoxic vasoconstriction in rat femoral arteryProtein phosphatase 2A/heat shock protein 70 dynamically regulates phosphorylation of histone deacetylase 2 and its activity in cardiac hypertrophy
Hyun-Ki Min*, Somy Yoon*, Duk-Hwa Kwon, Sera Shin, Taewon Kook, Hosouk Joung, Seung Hoon Jeong, Hyun Kook, Gwang Hyeon Eom
Department of Pharmacology, Chonnam National University Medical School, Gwagnju 61469, Republick of Korea

S 41 S-IV-4 Function of junctional proteins in Ca^{2+} dynamics
Jin Seok Woo[1], Sonal Srikanth[1], Miyuki Nishi[2], Peipei Ping[1], Hiroshi Takeshima[2], and Yousang Gwack[1]
[1]Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA; [2]Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto 606-8501, Japan

Symposium V: Optogenetics for physiology

S 41 S-V-1 Next generation optogenetics: tool development and applications
Won Do Heo
Department of Biological Sciences, KAIST & Center for Cognition and Sociality, IBS, Korea

S 41 S-V-2 Application of optogenetics for treatments of urological disorders
Jun-Kyo F. Suh
Center for Bionics, Korea Institute of Science and Technology (KIST), Seoul, Korea

S 42 S-V-3 Optogenetic control of stress and mood disorders
Daesoo Kim
Behavioral Genetics Lab, Department of Biological Sciences, Korea Advanced Institute of Science & Technology (KAIST), Daejeon 305-701, Korea

S 42 S-V-4 Optogenetic dissection of neurons in the basal forebrain
Tae Kim
Department of Biomedical Science & Engineering (BMSE), Gwangju Institute of Science and Technology (GIST), Gwangju 61005, Korea

Symposium VI: Pacemaker mechanisms and motility

S 43 S-VI-1 Properties of waxing and waning in the mouse small intestine
Yoshihiko Kito
Department of Pharmacology, Faculty of Medicine, Saga University, Nabeshima, Saga, 849-8501, JAPAN

S 43 S-VI-2 Automaticity of interstitial cells of Cajal: A computational study
Jae Boum Youm[1,2], Haifeng Zheng[2], Mei Hong Zhu[2], Tae Sik Sung[2], Kenton M. Sanders[2], Sang Don Koh[2]
[1]Cardiovascular and Metabolic Disease Center (CMDC), Department of Physiology, College of Medicine, Inje University, Busan, South Korea, [2]Department of Physiology and Cell Biology, University of Nevada, School of Medicine, Reno, Nevada, USA

S 43 S-VI-3 Hyperpolarization-activated cyclic nucleotide-gated channels are working as a pacemaker channels in colonic interstitial cells of Cajal
Seok Choi, Chansik Hong, Jae Yeoul Jun
Department of Physiology, College of Medicine, Chosun University

S 44 S-VI-4 The Role of Korean Medicines in GI motility
Hyun Jung Kim, Yoon Ah Byun, Byung Joo Kim
Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine

Symposium VII: Neurophysiology in Institute for Basic Sciences (IBS)

S 44 S-VII-1 Cortical parvalbumin inhibitory interneurons and Homeostatic dysfunction in Schizophrenia model
Jae Jin Shin[1,2] , Yong Gyu Kim[2,3], Soo Yong Kim[2,3], CukChan Lee[2], Joo min Park[4], Sang Jeong Kim[1,2,3]
[1]Department of Brain and Cognitive Science, College of Natural Science, Seoul National University, Seoul 110-794, Korea, [2]Department of Physiology, College of Medicine, Seoul National University, Seoul 110-799, Korea, [3]Department of Biomedical Science, College of Medicine, Seoul National University, Seoul 110-700, [4]Center for Cognition and Sociality, Institute for Basic Science (IBS), Daejeon 305-338, Korea

S 44 S-VII-2 Regrowth of serotonin axons in the adult mouse brain following injury
Yunju Jin[1], Sarah E. Dougherty[1], Kevin Wood[2], Landy Sun[1], Robert H. Cudmore[1], Aya Abdalla[2], Geetha Kannan[1,3], Mikhail Pletnikov[1,3], Parastoo Hashemi[2], David J. Linden[1]
[1]Solomon H. Snyder Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore MD [2]Department of Chemistry, Wayne State University, Detroit, MI [3]Department of Psychiatry and Department of Molecular and Comparative Pathobiology, The Johns Hopkins University School of Medicine; Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore MD

S 45 S-VII-3 Whole-cell recordings in freely moving rodents
Doyun Lee
Center for Cognition and Sociality, Institute for Basic Science (IBS), Daejeon 305-338, Korea

S 45 S-VII-4 Astrocytes increase the activity of synaptic GluN2B NMDA receptors
Junghyun Hahn*, Xianhong Wang and Marta Margeta
Department of Pathology, University of California San Francisco, San Francisco, CA, USA. *Current address: IBS-CNIR, Sungkyunkwan University, Suwon, 16419, Korea

Poster Presentation (Poster Oral Presentation)

P01: Diet, Phytochemicals and Stress Physiology

S 46 P01-01 Rg3-enriched Korean Red Ginseng improves stability of blood pressure in spontaneously hypertensive rats
Harsha Nagar[1], Sujeong Choi[1], Saet-byel Jung[2], Sungju Jee[3], Jae Young Moon[4], Kwang-sun Suh[5], Shin Kwang Kang[6], Byeong Hwa Jeon[1], Hee-Jung Song[9], Cuk-Seong Kim[1]
[1]Department of Medical Science & Physiology, School of Medicine, Chungnam National University, Daejeon, Korea; [2]Department of Endocrinology, [3]Department of Rehabilitation Medicine, [4]Department of Internal Medicine, [5]Department of Pathology, [6]Department of Thoracic and Cardiovascular Surgery, [9]Department of Neurology, School of Medicine, Chungnam National University, Daejeon, Korea

S 46 P01-02

Prunella vulgaris attenuates diabetic nephropathy by suppressing renal fibrosis and inflammation
Seung Namgung[1,2], Jung Joo Yoon[1,2], HyeYoom Kim[1,2], Da Hye Jeong[1,2], Yun Jung Lee[1,2], Dae Gill Kang[1,2], Ho Sub Lee[1,2]*
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, Wonkwang University, 460, Iksan-daero, Iksan, Jeonbuk 54538, Republic of Korea

S 46 P01-03

Ligustilide attenuates vascular inflammation and activates Nrf2/HO-1 induction, NO synthesis in HUVECs
EunSik Choi[1,2], ByungHyuk Han[1,2], You Mee Ahn[1,2], Xian Jun Jin[1,2], Yun Jung Lee[1,2], Ho Sub Lee[1,2], Dae Gill Kang[1,2]*
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, Wonkwang University, 460, Iksan-daero, Iksan, Jeonbuk 54538, Republic of Korea

S 46 P01-04

The effect of dietary fatty acid composition on food intake and hypothalamus gene expressions in mice
Mi Jang, Yong-Woon Kim, So-Young Park, and Jong-Yeon Kim
Department of Physiology, School of Medicine, Yeungnam University, Daegu 42415, Korea

S 47 P01-05

Effects of the methanolic extract of *Schisandra chinensis* fruit and γ-schisandrin on transient receptor potential vanilloid 3
Yuran Nam[1,2], Joo Hyun Nam[1,2]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 38066,Republic of Korea, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine,Goyang 10326, Republic of Korea

S 47 P01-06

Agrimonia pilosa leaf extract accelerates skin barrier restoration by activation of transient receptor potential vanilloid 3 (TRPV3)
Hyun Jong Kim[1,2], Joo Hyun Nam[1,2]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 38066, Republic of Korea, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang 10326, Republic of Korea

S 48 P01-07

Foeniculum vulgare extract and its constituent, *trans*-anethole, inhibit UV-induced melanogenesis *via* ORAI1 channel inhibition
Mi-Ok Lee[1], Dong-Ung Lee[2], Joo Hyun Nam[1,3]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 38066, Republic of Korea, [2]Division of Bioscience, Dongguk University, Gyeongju 780-714, Republic of Korea, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang 10326, Republic of Korea

S 48 P01-08

Skin protective effect of guava leaves against UV-induced melanogenesis *via* inhibition of ORAI1 channel and tyrosinase activity
Joo Hyun Nam[1,2], Yung Kyu Kim[1], Woo Kyung Kim[2,3]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju, Korea; [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang, Korea; [3]Department of Internal Medicine, Graduate School of Medicine, Dongguk University, Goyang, Korea

S 48 P01-09

Anti-allergic effect of Oleamide isolated from *Arctium lappa L.*
Sung Ryul Lee[1], Dae Yun Seo[1], Hyoung Kyu Kim[1], Jae Boum Youm[1], Se Chan Kang[2]*, Jin Han[1]*
[1]Cardiovascular & Metabolic Disease Center, College of Medicine, Inje University, [2]Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University

S 48 P01-10

Crinum asiaticum var. japonicum Baker extract inhibits adipocyte differentiation and adipogenesis
Sung Ryul Lee[1], Dae Yun Seo[1], Hyoung Kyu Kim[1], Jae Boum Youm[1], Se Chan Kang[2]*, Jin Han[1]*
[1]Cardiovascular & Metabolic Disease Center, College of Medicine, Inje University, [2]Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University

S 49 P01-11

Cynanchum wilfordii extract attenuates an atherogenic diet with fructose-induced liver damage
Sung Ryul Lee[1], Dae Yun Seo[1], HyoungKyu Kim[1], Jae Boum Youm[1], Se Chan Kang[2]*, Jin Han[1]*
[1]Cardiovascular & Metabolic Disease Center, College of Medicine, Inje University, [2]Department of Oriental Medicine Biotechnology, College of Life Sciences, Kyung Hee University

S 49 P01-12

Bee venom and bee venom derived phospholipase A_2: Their analgesic effects in oxaliplatin-induced neuropathic pain in mice
Woojin Kim, Dongxing Li, Ji Hwan Lee, Heera Yoon, Hyunsu Bae, Sun Kwang Kim
Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul 02447, Korea

S 50 P01-13

Effect of *Imperatae Rhizomaon* on the mechanism of nitric oxide-mediated vasorelaxation
HyeYoom Kim[1,2], You Mee Ahn[1,2], Xian Jun Jin[1,2], MiHyeon Hong[1,2], Yun Jung Lee[1,2], Dae Gill Kang[1,2], Ho Sub Lee[1,2]*
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, Wonkwang University, 460, Iksan-daero, Iksan, Jeonbuk 54538, Republic of Korea

S 50 P01-14 Protective effect of betulinic acid on early atherosclerosis in diabetic apolipoprotein-E knockout mice
Jung Joo Yoon[1,2], Seung Namgung[1,2], Da Hye Jeong[1,2], Yun Jung Lee[1,2], Dae Gill Kang[1,2], Ho Sub Lee[1,2]*
[1]College of Oriental Medicine and Professional Graduate School of Oriental Medicine, [2]Hanbang Body-fluid Research Center, Wonkwang University, 460, Iksan-daero, Iksan, Jeonbuk 54538, Republic of Korea

P02: Endocrine and Metabolic Physiology

S 50 P02-01 Angiotensin-(4-8), an active mediator of renin-angiotensin system, suppresses ANP secretion via angiotensin type 1 receptor
Hoang Thi Ai Phuong, Lamei Yu, Byung Mun Park, Suhn Hee Kim
Department of Physiology, Research Institute for Endocrine Sciences, Chonbuk National University Medical School, Jeonju, Korea

S 51 P02-02 Anthropometry-based estimation of the body heat capacity for individuals aged 7-69 y: the Size Korea Survey 2010
Duong Duc Pham, Jeong Hoon Lee, Young Boum Lee, GaYul Kim, Ji Yeon Song, Ji Eun Kim, Chae Hun Leem*
Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

S 51 P02-03 Exposure to Bisphenol-A affects neurotransmitter-mediated responses on GnRH neurons in mice
Janardhan P. Bhattarai, Thi Thanh Hoang Nguyen, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

S 51 P02-04(YP-01) CR6-interacting factor 1 inhibition damages vascular function by inhibiting the Sirt1-eNOS pathway
Shuyu Piao [1], Harsha Nagar[1]*, Saet-byel Jung[2]*, Min Jeong Ryu[3]*, Su-jeong Choi[1], Sung-Ho Jun[1], Hee-Jung Song[4], Shin Kwang Kang[5], Minho Shong[2], Dong Woon Kim[6], Kaikobad Irani[7], Byeong Hwa Jeon[1], Gi Ryang Kweon[3], Cuk-Seong Kim[1]
[1]Department of Medical Science & Physiology, [2]Department of Endocrinology, [3]Department of Biochemistry, [4]Department of Neurology, [5]Department of Thoracic and Cardiovascular Surgery, [6]Department of Anatomy, School of Medicine, Chungnam National University, Republic of Korea, [7]Division of Cardiovascular Medicine, Department of Internal Medicine, University of Iowa Carver College of Medicine, USA

S 51 P02-05 Effect of fibroblast growth factor 23 on osteoblastic differentiation and mineralization of D1 mesenchymal stem cells
Da-Gyo Oh[1], Kyeong-Lok Park[2], Do-Whan Ahn[1]
Departments of [1]Physiology and [2]Dentistry, Kosin University College of Medicine

S 52 P02-06 Direct effects of neurosteroid on gonadotropin releasing hormone neurons
Janardhan Prasad Bhattarai[1], Dong Hyu Cho[2] and Seong Kyu Han[1]
[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju [2]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine, Jeonju, Korea

S 52 P02-07(YP-06) Neurosteroids-mediated actions on GnRH neuron in letrozole-induced polycystic ovarian syndrome (PCOS) mouse model
Pravin Bhattarai[1], Santosh Rijal[1], Dong Hyu Cho[2] and Seong Kyu Han[1]
[1]Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, [2]Department of Obstetrics and Gynecology, Chonbuk National University Hospital and School of Medicine

S 52 P02-08 Postnatal exposure of TCDD affects gonadotropin releasing hormone neuronal activities in mice.
Pravin Bhattarai, Seong Kyu Han
Department of Oral Physiology, School of Dentistry & Institute of Oral Bioscience, Chonbuk National University, Jeonju

S 53 P02-09 Alkalinization by Phosphate Uptake via PiT-1/2 Participates in High Phosphate-induced Oxidative Stress and Defective Insulin Secretion
Tuyet Thi Nguyen, Xianglan Quan, Shanhua Xu, Ranjan Das, Seung-Kuy Cha, Seong-Woo Jeong, In Deok Kong, Kyu-Sang Park
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 53 P02-10 VEGF-A expressing adipose tissue shows rapid beiging, enhanced survival after transplantation and confers metabolic improvements on the host in an IL4-independent manner
Min Kim[1], Jiyoung Park[2], Philipp E. Scherer[3], Jin Han[1]
[1]Cardiovascular and Metabolic Disease Center, Inje University,633-165, Gaegeum-Dong, Busanjin-Gu, Busan 614-735, Korea; [2]Department of Biological Sciences, School of Life Sciences, Ulsan National Institute of Science and Technology, 50 UNIST St., Ulsan, 689-798, Korea; [3]Touchstone Diabetes Center, Departments of Internal Medicine and 3Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA

P03: Exercise and Applied Physiolog

S 53 P03-01 Relative muscular strength is associated with reduced estimated glomerular filtration rate (eGFR) in community-dwelling young and middle-aged adults
Jae Seung Chang[1,2], Tae-ho Kim[1,2] and In Deok Kong[1,2]
[1]Department of Physiology, Yonsei University Wonju College of Medicine, [2]Yonsei Institute of Sports Science & Exercise Medicine, Yonsei University

S 54 P03-02 Exercise training normalizes excitability of hypothalamic paraventricular neurons (PVN) in the rats with heart failure
Yiming Shen[1], Chae Jeong Lim[1], Heow Won Lee[1],
So Yeong Lee[1], Seong Kyu Han[2], Pan Dong Ryu[1]
[1]Department of Veterinary Pharmacology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Korea; [2]Department of Oral Physiology, School of Dentistry, Chonbuk National University, Jeonju, Korea

S 54 P03-03 Ursolic acid supplement attenuates exercise-induced cardiac damage biomarkers in resistance-trained men
Dae Yun Seo[1], Hyun Suk Bang[2], Hyo-Bum Kwak[3], Min Kim[1], Sung Ryul Lee[1], Jin, Han[1]
[1]National Research Laboratory for Mitochondrial Signaling, Department of Physiology, Department of Health Sciences and Technology, BK21 Project Team, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, 633-165, Bokji-ro 75, Busan jin-gu, Busan, 47392, Republic of Korea, [2]Division of Humanities and Social Science, POSTECH, Pohang, 77 Cheongam-Ro, Nam-Gu, Pohang, 37673, Republic of Korea, [3]Department of Kinesiology, Inha University, 100 Inha-ro, Nam-gu Incheon 22212, Republic of Korea

S 54 P03-04 The Effect of Bio-active Materials Coated Fabric on Mitochondria
Donghee Lee, Hyemi Bae, Young-Won Kim, Misuk Yang, Inja Lim, Hyoweon Bang, Jae-Hong Ko
Department of Physiology, College of Medicine, Chung-Ang University, Seoul 156-756, Korea

P04: Ion Channels and Transporters

S 55 P04-01 Mitochondrial substrate dependent changes of mitochondrial function
Ji Yeon Song, Jeong Hoon Lee, Doung Duc Pham, Ga Yul Kim, Ji Eun Kim, Chae Hun Leem
Department of physiology, College of Medicine, Ulsan University, Seoul, Korea

S 55 P04-02 Identification of PKD2L1 phosphorylation sites and the regulation of PKD2L1 channel activation by cAMP signaling pathway
Eunice YJ Park, Kotdaji Ha, Insuk So
Department of Physiology, Seoul National University, College of Medicine, Seoul 110-799, Republic of Korea

S 55 P04-03 The changes of voltage-dependent K^+ channel activity during early phase of diabetes in the mesenteric arterial smooth muscle
Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, 200-701, South Korea

S 56 P04-04 Transient receptor potential canonical 4 (TRPC4) channel regulation by phosphotiesterase 5 inhibitor via the cyclic guanosine 3'5'-monophosphate
Jinhong Wie[1], Seung Joo Jeong[1], Mee Ree Chae[2], Jong Kwan Park[3], Sung Won Lee[2], and Insuk So[1]*
[1]Department of Physiology, [3]Department of Pharmacology, College of Medicine, Seoul 110-799, Republic of Korea; [2]Department of Urology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Korea

S 56 P04-05 Maxi-K channel (BKCa) activity veils the myogenic response of mesenteric artery in rats
Eun Young Seo, Ming Zhe Yun, Yin Hua Zhang, Hae Young Yoo, Sung Joon Kim
Department of Physiology and Department of Biomedical Sciences, Seoul National University College of Medicine

S 56 P04-06 Differential modulation of TASK-2 and TREK channel activity by pyrazole compounds
Hyun Jong Kim[1,3], Joohan Woo[2], Joo Hyun Nam[1,3]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju 38066, Republic of Korea, [2]Department of Physiology, Seoul National University College of Medicine, Seoul 03080, Republic of Korea, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang 10326, Republic of Korea

S 57 P04-07 Paliperidone, the active metabolite of risperidone, inhibits cloned hERG potassium channels
Hong Joon Lee[1], Jin-Sung Choi[2], Sang June Hahn[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea; [2]College of Pharmacy, Integrated Research Institute of Pharmaceutical, The Catholic University of Korea, Gyeonggi-do, South Korea

S 57 P04-08 Shear stress activates gap junction hemichannel current with subsequent P2X purinoceptor activation in rat atrial myocytes
Min-Jeong Son, Sun-Hee Woo
Lab of Physiology, College of Pharmacy, Chungnam National University, 99 Daehak-ro, Yuseong-gu, 34134 South Korea

S 57 P04-09

Cell cycle-dependent expression of TASK3 in human bladder cancer cell lines
Yangmi Kim[1]*, Kyung-Ah Kim[2]
[1]Department of Physiology, College of Medicine, Chungbuk National University, [2]Department of Biomedical Engineering, College of Medicine, Chungbuk National University

S 58 P04-10(YP-02)

Characteristics of mitochondrial calcium efflux regulation in permeabilized single ventricular myocytes
Jeong Hoon Lee, Duong Duc Pham, Ga Yul Kim, Ji Yeon Song, Ji Eun Kim, Chae Hun Leem
Department of Physiology, College of Medicine, Ulsan University, Seoul, Korea

S 58 P04-11

Charged amino acids in the cytoplasmic c-terminal of TREK-2 K^+ channel for the sensitivity to intracellular pH
Joohan Woo[1], Hyun Jong Kim[2], Dong Hoon Shin[3], Yin-Hua Zhang[1], Joo Hyun Nam[2], Sung Joon Kim[1]
[1]Department of Physiology, College of Medicine, Seoul National University, Korea; [2]Department of Physiology, College of Medicine, Dongguk University, Korea; [3]Division of Natural Medical Sciences, Chosun University, College of Health Science, Korea

S 58 P04-12

Hydrogen peroxide-induced up-regulation of TASK-5 two-pore domain K+ channel reduces death of breast cancer cells
Xiaoming Liu[1], Ji Hyeon Ryu[1,2], Jae-Young Nam[3], Eun-Jin Kim[1], Dong Keun Lee[1], Seong-Geun Hong[1], Jaehee Han[1], and Dawon Kang[1,2]*
[1]Department of Physiology, College of Medicine, Gyeongsang National University, Jinju 52727, South Korea; [2]Departments of Convergence Medical Science, Gyeongsang National University, Jinju 52727, South Korea; [3]Department of Premedicine, College of Medicine, Gyeongsang National University, Jinju 52727, South Korea

S 59 P04-13

A novel mechanism for activation of TRPC4 through modulation of positive electrostatic potential on the CIRB domain
Chansik Hong[1], Seok Choi[1], Jae-Yeoul Jun[1], Insuk So[2]
[1]Department of Physiology, Chosun University School of Medicine, Gwangju 61452, South Korea, [2]Department of Physiology, Seoul National University College of Medicine, Seoul 03080, South Korea

S 59 P04-14

Regulation of TRPC4, TRPC5 homotetrameric and TRPC1/4, C1/5 heterotetrameric channel activity by $PI(4,5)P_2$ hydrolysis
Juyeon Ko, Jongyun Myeong, Ju-hong Jeon, Insuk So
Department of Physiology, College of Medicine, Seoul 110-799, Republic of Korea

S 59 P04-15

Dual action of the Gαq-PLCβ-PI(4,5)P2 pathway on TRPC1/4 and TRPC1/5 heteromultimer
Jongyun Myeong[a], Juyeon Ko[a], Misun Kwak[a], Kodaji Ha[a], Chansik Hong[a], Dongki Yang[b], Hyun Jin Kim*[c], Ju-Hong Jeon[a] and Insuk So*[a]
[a]Department of Physiology, Seoul national University college of Medicine, Seoul, 110-799, Republic of Korea; [b]Department of Physiology; College of Medicine; Gachon University; Incheon, Republic of Korea; [c]Department of Physiology, Sungkyunkwan University School of medicine, Suwon 440-746, Republic of Korea

S 60 P04-16

The state-dependent inhibition of voltage-dependent K^+ channels by specific calmodulin inhibitor CGS 9343B in rabbit coronary arterial smooth muscle cells
Hongliang Li, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, 200-701, South Korea

S 60 P04-17

Shear stress induces transverse global Ca^{2+} waves via autocrine activation of P2X purinoceptors in rat atrial myocytes
Joon-Chul Kim, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Korea

S 60 P04-18

Shear stress enhances Ca^{2+} spark occurrence via mitochondrial ROS generation and sarcoplasmic reticulum Ca^{2+} increase in rat ventricular myocytes
Jun Wang, Joon-Chul Kim, Sun-Hee Woo
College of Pharmacy, Chungnam National University, 99 Daehak-ro, Daejeon 34134, South Korea

S 61 P04-19

Closed-state inhibition of voltage-dependent K+ channels by sertraline in rabbit coronary arterial smooth muscle cells
Han Sol Kim, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, 200-701, South Korea

S 61 P04-20

Regulation of calcium and rhythm in atrial cells via autocrine activation of $P2X_4$ purinoceptors during shear stress
Kyeong-Hee Kim, Joon-Chul Kim, Min-Jeong Son, Sun-Hee Woo
College of Pharmacy, Chungnam National University, 99 Daehakro, Yuseong-gu, Daejeon 305-764, South Korea

S 61 P04-21

Hydrogen peroxide-induced intracellular calcium accumulation through plasma membrane Ca^{2+}-ATPase inactivation in mouse parotid acinar cells
Min Jae Kim, Mi Na Yoon, Dong Kwan Kim, Se hoon Kim, and Hyung Seo Park
Department of Physiology, College of Medicine, Konyang University, Daejeon 35365, Korea

S 61 P04-22 Down-Regulation of THIK-1 Expression in Inflammatory Pain and Asthma
Marie Merci[1,2], Ji Hyeon Ryu[1,2], Eun-Jin Kim[2], Adrian S. Siregar[1,2], Jaehee Han[2], Dawon Kang[1,2]*
Departments of [1]Convergence Medical Science, [2]Physiology, Institute of Health Sciences, Gyeongsang National University, Jinju 52727, South Korea

S 62 P04-23 Intracellular calcium dependent regulation of sperm-specific calcium activated potassium channel, hSlo3 by BKCa activator LDD175
Tharaka Wijerathna[1], Jihyun Kim[1], Minji Kim[1], Dongki Yang[2], Kyu Pil Lee[1]*
Laboratory of Physiology, College of Veterinary Medicine, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 305-764, Republic of Korea [2]Department of Laboratory Medicine, Gachon University Gil Hospital, Incheon 21565, [2]Department of Physiology, College of Medicine, Gachon University, Incheon 21936, Korea

S 62 P04-24 The regulatory role of rolipram on isolated primary mouse submandibular gland cells
Dong Un Lee, Wanhee Suk, Jeong Hee Hong
Department of Physiology, College of Medicine, Gachon University, 191 Hambakmeoro, Yeonsu-gu, Incheon 406-799, Republic of Korea

S 62 P04-25 Gαi-mediated TRPC4 activation by polycystin-1 contributes to the endothelial function via STAT1 activation
Misun Kwak, Chansik Hong, Jongyun Myeong, Kotdaji Ha, Insuk So
Department of Physiology, Seoul National University College of Medicine, Seoul, Republic of Korea

S 63 P04-26(PO-01) TRPC channels are a novel culprit for hepatic stellate cell activation and hepatic fibrosis
Kyu-Hee Hwang[1,2], Ji-Hee Kim[1,2], Soo-Jin Kim[1,2], Seong-Woo Jeong[1,2,3], In Deok Kong[1,2,3], Kyu-Sang Park[1,2,3] and Seung-Kuy Cha[1,2,3]
Departments of [1]Physiology and [2]Global Medical Science, and [3]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, South Korea

S 63 P04-27(PO-02) Down-regulation of NKCC and AE2 transporters in salivary gland cells by dexmedetomidine
Minjeong Ji, Wanhee Suk, Jeong Hee Hong*
Department of Physiology, College of Medicine, Gachon University, Lee Gil Ya Cancer and Diabetes Institute, 155 Getbeolro, Yeonsu-gu, Incheon, 21999, South Korea

S 63 P04-28(YP-03) Mechanism of hERG current inhibition by 4-hydroxynonenal (4-HNE), a polyunsaturated fatty acid-derived electrophile
Seong Woo Choi[1], Hyang-Ae Lee[1,2], Yin-Hua Zhang[1], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, Korea, [2]Next-generation Pharmaceutical Research Center, Korea Institute of Toxicology, Korea

S 64 P04-29(YP-05) Ethanol inhibition of KCNQ2/3 channel is regulated by plasma membrane $PI(4,5)P_2$
Kwon-Woo Kim, Dongil Keum and Byung-Chang Suh*
Department of Brain & Cognitive Science, DGIST, Daegu, Korea

S 64 P04-30(YP-09) ATP release via gap junction hemichannels in rat atrial myocytes under shear stress
Joon-Chul Kim, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Korea

P05: Molecular Physiology

S 64 P05-01 The level of nitric oxide regulates lipocalin-2 expression under inflammatory condition in RINm5F beta-cells
Seo-Yoon Chang, Yang-Hyeok Jo, Myung-Jun Kim
Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, Korea

S 65 P05-02 Inhibition of neddylation facilitates cell migration through enhanced phosphorylation of caveolin-1 in PC3 and U373MG
Sung Yeon Park[1,3], Jong-Wan Park[1,2], Lan Li[2],
Yang-Sook Chun[1,2,3]
[1]Ischemic/Hypoxic Disease Institute, [2]Department of Biomedical Sciences and 3Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 65 P05-03 Necrox-5 attenuates ischemic heart injury via regulation of mitochondria biogenesis and inflammation response
Hyoung Kyu Kim, Yeon Hee Noh, Tae Hee Ko, Sung Ryul Lee, Jubert Marquez, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center, Department of Health Sciences and Technology, BK21 Project Team, Department of Physiology, Inje University, Busan, Korea

S 65 P05-04

Fetuin-B affects atherosclerotic plaque stability by regulating adipogenic differentiation of human mesenchymal stem cells
Seung Hyo Jung[1], Kang Pa Lee[1], Suji Baek[1], Donghyen Lee[1], Junghwan Kim[2], Hwan-Myung Lee[3], Kyung-Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, 322 Danwol-dong, Chungju, Korea; [2]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin, Korea; [3]Department of Cosmetic Science, College of Life and Health Sciences, Hoseo University, Asan, Korea

S 66 P05-05

Wnt, GSK-3β, JNK, and PKC pathway signaling regulates neural induction of human bone marrow-derived mesenchymal stem cells
Sujeong Jang[1], Hyong-Ho Cho[2], Jong-Seong Park[1], Han-Seong Jeong[1]
[1]Department of Physiology and [2]Department of Otolaryngology-Head and Neck Surgery, Chonnam National University Medical School, Gwangju, Korea

S 66 P05-06

Blockage of neddylation elevates c-Src stability and facilitates cancer cell migration
Gunwoo Lee, Sung Yeon Park, Yang-Sook Chun
Departments of Physiology and Biomedical Science, Seoul National University College of Medicine, Seoul, Korea

S 66 P05-07

Ethanol extract of *Brassica rapa* subsp. *Pekinensis* suppress TNF-α-induced inflammatory response in human umbilical vein endothelial cells
Eun Ok Lee, Sunga Choi, Hee Kyoung Joo, Yu Ran Lee, Myoung Soo Park, and Byeong Hwa Jeon
Infectious Signaling Network Research Center and Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, Daejeon, Korea

S 66 P05-08(PO-03)

A Jumonji C (JmjC) domain-containing protein negatively regulates RANKL-mediated osteoclastogenesis
Seon-Young Kim[1], Hye-Jin Kim[1], Jong-Wan Park[2], Yang-Sook Chun[1,2]
[1]Department of Physiology, and [2]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 67 P05-09

The role of 18-kDa translocator protein on protein kinase C-induced endothelial activation in HUVECs
Hee Kyoung Joo[1], Yu Ran Lee[1], Ki Mo Lee[1], Myoung Soo Park[2], Eun Ok Lee[1], Sunga Choi[1], Byeong Hwa Jeon[1]
[1]Research Institute for Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, Daejeon, Korea; [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Korea

S 67 P05-10

Pharmacologic activation of PGC-1α via AKT/mTOR signaling improves mitochondrial biogenesis in skeletal muscle cell mouse model
Jubert Marquez[1], Hyoung Kyu Kim[2], Joon Yong Noh[1], Jin Han[1,2]
[1]Department of Health Science and Technology, [2]Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, Korea

S 67 P05-11

Enhanced store-operated calcium entry induced by over-expression of G-protein subunit beta 5
Soonhong Park, Namju Kang, Dong Min Shin
Department of Oral Biology and BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea

S 68 P05-12

KSP inhibitor SB743921 induces death of multiple myeloma cells via inhibition of the NF-kB signaling pathway
In-Sung Song, Yu Jeong Jeong, Bayalagmaa Nyamaa, Seung Hun Jeong, Hyoung Kyu Kim, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, and Department of Physiology, College of Medicine, Inje University, Busan, Korea

S 68 P05-13

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

S 68 P05-14

Downregulation of HN1 is essential for the induction of autophagy in colorectal cancer cells
Yu Chuan Liu and Soo Mi Kim
Department of Physiology, and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 68 P05-15

Activation of hippo signaling by ursolic acid inhibits growth of human gastric cancer cells
Hua Jin and Soo Mi Kim
Department of Physiology, and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 69 P05-16

Anticancer effect of ursolic acid on esophageal cancer cells through ROS dependent autophagy
Navin Ray and Soo Mi Kim
Department of Physiology, and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 69 P05-17

Altered secretory activity of APE1/Ref-1 D148E variants identified in human patients with bladder cancer
Yu Ran Lee[1,2], Sunga Choi[1], Hee Kyoung Joo[1], Eun Ok Lee[1], Jae Sung Lim[3], Ju Hyun Shin[3], Byeong Hwa Jeon[1,2]
[1]Department of Physiology, Chungnam National University School of Medicine, Daejeon, Korea; [2]Department of Medical Science, Chungnam National University, Daejeon, Korea; [3]Department of Urology, Chungnam National University Hospital, Chungnam National University College of Medicine, Daejeon, Korea

S 69 P05-18

Suberoylanlide hydroxamic acid inhibits the growth of lung cancer cells via caspase-dependent apoptosis
Bo Ram Han, Hyun Kyung Park, Sung Kun Chun, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim and Woo Hyun Park*
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, Jeonju, Korea

S 70 P05-19

Gallic acid inhibits the growth of calf pulmonary arterial endothelial cells through cell death and glutathione depletion
Bo Ram Han, Hyun Kyung Park, Sung Kun Chun, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim and Woo Hyun Park
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, Jeonju, Korea

S 70 P05-20

Gallic acid induces HeLa cell death via increasing GSH depletion rather than ROS levels
Hyun Kyung Park, Bo Ram Han, Sung Kun Chun, Soo Mi Kim, Sung Zoo Kim, Suhn Hee Kim and Woo Hyun Park*
Department of Physiology, Medical School, Research Institute for Endocrine Sciences, Chonbuk National University, Jeonju, Korea

S 70 P05-21

Bitter taste receptors expression levels analysis of exocrine glands in mice
Su-Young Ki, Ki-Myung Chung, Young-Kyung Cho, and Kyung-Nyun Kim
Department of Physiology and Neuroscience, College of Dentistry and Research Institute of Oral Sciences, Gangneung-Wonju National University, Gangneung, Korea

S 71 P05-22

Activation of TGFß/ERK signaling by CTHRC1 promotes growth and metastasis in esophageal adenocarcinoma
Yulia Ga-eun Lee, and Soo Mi Kim
Department of Physiology, and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 71 P05-23(PO-06)

WNK1 promotes renal tumor progression by TRPC6-NFAT pathway via activating phosphatidylinositol 4-kinase IIIα
Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Minseob Eom[3],
Seong-Woo Jeong[1,4], In Deok Kong[1,2,4], Kyu-Sang Park[1,2,4] and Seung-Kuy Cha[1,2,4]
Departments of [1]Physiology, [2]Global Medical Science, [3]Pathology, and [4]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea

S 71 P05-24

Secreted APE1/Ref-1 inhibits TNF-α-stimulated endothelial inflammation via thiol-disulfide exchange in TNF receptor
Myoung Soo Park, Sunga Choi, Yu Ran Lee, Hee Kyoung Joo, Eun ok Lee, Byeong Hwa Jeon
Preclinical Research Center, Chungnam National University Hospital, Daejeon, Korea; Infectious Signaling Network Research Center and Research Institute for Medical Sciences, and Department of Physiology, School of Medicine, Chungnam National University, Daejeon, Korea.

S 71 P05-25

Role of oxidative stress via TGF-β-ERK-mTOR-NOX4 pathway in transdifferentiation of mouse hepatic stellate cells
Soo-Jin Kim[1], Ranjan Das[1], Kyu-Hee Hwang[1], Ji-Hee Kim[1], Seung-Kuy Cha[1,2], Seong-Woo Jeong[1,2], In Deok Kong[1,2], and Kyu-Sang Park[1,2]
[1]Departments of Physiology and Global Medical Science, and [2]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea

S 72 P05-26

VEGFR2 aptamer as tool of commitment for hPSC-derived endothelial progenitor cells
Jung Won Yoon[1], Jin Ju Park[1], Jae Ho Kim[1,2]
[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan, Korea; [2]Research Institute of Convergence Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, Korea

S 72 P05-27

Echinochrome A prevents vascular smooth muscle cell proliferation via mTOR/Akt-OPN signaling pathway in atherosclerosis
Kyo Won Seo, Jin Han, Nari Kim
NLRL for Innovation Cardiovascular Engineering, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

S 73 P05-28 Endothelin-1 and phenylephrine activate cAMP response element binding protein via IP3 receptor/Ca^{2+}-dependent PKC-CaMKII signaling in ventricular myocytes
Krishna P. Subedi, Min-Jeong Son, Bojjibabu Chidipi, Seong-Woo Kim, Jun Wang, Kyeong-Hee Kim, Joon-Chul Kim, Sun-Hee Woo
College of Pharmacy, Chungnam National University, Daejeon, Korea

S 73 P05-29 Intracellular Ca^{2+} channel TRPML3 regulates early autophagosome biogenesis by interaction with phosphoinositides
So Woon Kim[1], Mi Kyung Kim[1], Kyoung Sun Park[2], and Hyun Jin Kim[1]
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P06: Muscle, Heart, Vessel

S 73 P06-01 The effect of the fully lengthened immobilization on the change of the apoptotic and myosin heavy chain expression in soleus muscle in rats
Hye Rim Suh, Eui Ho Park, Sun Wook Moon and Hee Chul Han
Department of Physiology, College of Medicine and Neuroscience Research Institute, Korea University, Seoul 136-705, Korea

S 74 P06-02 Expression profile of mitochondrial voltage-dependent anion channel-1 influenced genes is associated with pulmonary hypertension
Young-Won Kim, Hyemi Bae, Donghee Lee, Jeongyoon Choi, Inja Lim, Hyoweon Bang, Jae-Hong Ko
Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

S 74 P06-03 Mitsugumin 53 regulates extracellular Ca^{2+} entry and intracellular Ca^{2+} release via Orai1 and RyR1 in skeletal muscle
Mi Kyoung Ahn[1], Keon Jin Lee[1], Chuanxi Cai[2], Mi Ri Oh[1], Mei Huang[1], Chung-Hyun Cho[3], Jianjie Ma[4,*], and Eun Hui Lee[1,*]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, [2]Center for Cardiovascular Sciences, Department of Molecular and Cellular Physiology, Albany Medical College, New York, USA, [3]Department of Pharmacology, College of Medicine, Seoul National University, Korea, [4]Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University, USA

S 74 P06-04(PO-10) The maintenance ability and Ca^{2+} availability of skeletal muscle are enhanced by sildenafil
Mei Huang[1], Keon Jin Lee[1], Kyung-Jin Kim[2], Mi Kyoung Ahn[1], Deok-Soo Han[1], Chung-Hyun Cho[2], Do Han Kim[3], and Eun Hui Lee[1,*]
[1]Department of Physiology, College of Medicine, The Catholic University, Korea, [2]Department of Pharmacology, Seoul National University College of Medicine, Korea, [3]School of Life Sciences and Systems Biology Research Center, Gwangju Institute of Science and Technology, Gwangju, Korea

S 74 P06-05 $K_{Ca}3.1$ upregulation preserves endothelium-dependent vasorelaxation during aging and oxidative stress
Shinkyu Choi, Ji Aee Kim, Hai-yan Li, Suk Hyo Suh
Department of Physiology, Medical School, Ewha Womans University, Seoul, South Korea

S 75 P06-06(YP-04) Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells
Tae Hee Ko, Hye Jin Heo, Hyoung Kyu Kim, Jae Boum Youm, Sung Woo Cho, In-Sung Song, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee and Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea.

S 75 P06-07 Mechanistic differences in calcium handling and contractility of the left and right ventricles of rat heart
Julius Ryan D. Pronto, Hyoung Kyu Kim, Jin Han, Nari Kim
NLRL for Innovative Cardiovascular Engineering, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University

S 76 P06-08 Functional analysis of colonic motility in response to Ang III, Ang IV, and Ang-(1-7) in rats
Byung Mun Park[1], Jong Hun Kim[2], Suhn Hee Kim[1]
Department of [1]Physiology and [2]Surgery, Chonbuk National University Medical School, Jeonju, Korea

S 76 P06-09 Insulin signaling targeting WNK1 kinase promotes GLUT4 trafficking in skeletal muscle
Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Seong-Woo Jeong[1,2,3], Kyu-Sang Park[1,2,3], Seung-Kuy Cha[1,2,3¶] and In Deok Kong[1,2,3¶]
Departments of [1]Physiology and [2]Global Medical Science, and [3]Institute of Lifestyle Medicine, Yonsei University Wonju College of Medicine, Wonju, Gangwon-do, Republic of Korea

S 76 P06-10 Sphingosylphosphorylcholine induces differentiation of human mesenchymal stem cells into smooth muscle cells by regulating ROS-mediated DJ-1 oxidation
Suji Baek[1], Kang Pa Lee[1], Seung Hyo Jung[1], Long Cui[1], Junghwan Kim[2], Hwan-Myung Lee[3], Kyung-Jong Won[1], Bokyung Kim[1]

[1]Department of Physiology, School of Medicine, Konkuk University, Chungju, Korea; [2]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin, Korea; [3]Department of Cosmetic Science, College of Life and Health Sciences, Hoseo University, Asan, Korea

S 76 P06-11 Nafamostate Mesilate improves endothelium-dependent vascular relaxation via the Akt-eNOS dependent pathway

Harsha Nagar[1]*, Su-jeong Choi[1]*, Shuyu Piao[1]*, Saet-byel Jung[2], Hee-Jung Song[4], Shin Kwang Kang[5], Min ho Shong[2], Dong Woon Kim[6], Kaikobad Irani[7],
Byeong Hwa Jeon[1], Gi Ryang Kweon[3], Cuk-Seong Kim[1]
[1]Department of Medical Science & physiology, [3]Department of Biochemistry, [5]Department of Thoracic and Cardiovascular Surgery, [6]Department of Anatomy, School of Medicine, Chungnam National University, Daejeon, Korea, [2]Department of Endocrinology, [4]Department of Neurology, Chungnam National University Hospital, Daejeon, Korea, [7]Division of Cardiovascular Medicine, Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA USA

S 77 P06-12 Repaglinide, an anti-diabetic drug, induces vasorelaxation by activation of PKA and PKG in aortic smooth muscle

Hye Won Kim, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, Korea

S 77 P06-13 Reversal of levosimendan-induced suppression of ANP secretion in the presence of PDE 4 inhibitor via PKA pathway

Lamei Yu, Kuichang Yuan, Byung Mun Park, Suhn Hee Kim
Department of Physiology, Research Institute for Endocrine Sciences, Chonbuk National University Medical School, Jeonju, Korea

S 77 P06-14 Scoparone, the bioactive component of chestnut inner shell ethanol extract, suppresses neointima formation by inhibiting migration via mitogen-activated protein kinase signaling pathway in vascular smooth muscle cells

Long Cui, Gyoung Beom Lee, Kang Pa Lee, Seung Hyo Jung, Suji Baek, Yun-kyoung Ryu, Kyung Jong Won, Bokyung Kim
Department of Physiology, School of Medicine, Konkuk University, Chungju, Korea

S 78 P06-15 Serum response factor regulates smooth muscle contractility via myotonic dystrophy protein kinases and L-type calcium channels

Moon Young Lee[2], Chanjae Park[1], Se Eun Ha[1], Paul J. Park[1], Robyn M. Berent[1], Robert D. Corrigan[1], Nathan Grainger[1], Peter J. Blair[1], Orazio J Slivano[3], Joseph M. Miano[3], Sean M. Ward[1], Terence K. Smith[1], Kenton M. Sanders[1], Seungil Ro[1]
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S 78 P06-16 Effects of high glucose and metabolic substrates on vascular reactivity in rat mesenteric and deep femoral arteries

Rany Vorn[1,2], Hae Jin Kim[3], Hae Young Yoo[1]
[1]Chung-Ang University Red Cross College of Nursing, Seoul 06974, Korea, [2]Chung-Ang University Graduate School, Seoul 06974, Korea, [3]Department of Physiology, Department of Biomedical Sciences and Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul 110-799, Korea

S 78 P06-17 The characteristics of morphology and respiration of the cryopreserved cardiac myocytes isolated from adult rat heart

Ga Yul Kim, Ji Yeon Song, Jeong Hoon Lee, Pham Duc Doung, Chae Hun Leem
Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

S 79 P06-18 Vascular sheet of hPSC-derived endothelial progenitor cells and smooth muscle cells using 3D culture

Jin Ju Park, Jung Won Yoon, Jae Ho Kim
Department of Physiology, School of Medicine, Pusan National University, Yangsan, Korea

S 79 P06-19(PO-07) Gross morphological properties of the primo-vascular system and its relations with the acupuncture meridian

Chae Jeong Lim, So Yeong Lee, Pan Dong Ryu
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S 79 P06-20(PO-04) IDH2 Inhibition impairs endothelium-dependent vasomotor function via mitochondrial function in endothelial cells

Sujeong Choi[1,7]*, Harsha Nagar[1,7]*, Shuyu Piao[1,7], Saet-byel Jung[2], Hyun-Woo Kim[1], Shin Kwang Kang[3], Jun Wan Lee[4], Jin Hyup Lee[5], Jeen-Woo Park[6], Byeong Hwa Jeon[1], Hee-Jung Song[7+], Cuk-Seong Kim[1,7+]
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700, Republic of Korea; [6]School of Life Sciences, College of Natural Science, Kyungbook National University, Taegu 702-701, Republic of Korea; [7]Department of Medical sciences, College of Natural Science, Chungnam National University Hospital, Daejeon 301-721, Republic of Korea

S 80 P06-21(YP-08) Impaired myogenic responses in the skeletal arteries from denervated hind limbs and their recovery by exercise training; underlying changes of ion channel currents in the arterial myocytes
Ming Zhe Yin[1,2], Eun Young Seo[1,2], Hae Jin Kim[1,2], Yin Hua Zhang[1,2], Sung Joon Kim[1,2]
[1]Department of Physiology, [2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul 110-799, Republic of Korea

S 80 P06-22(YP-10) Rapamycin ameliorates the portal hypertension, cardiovascular autonomic dysfunction, and alterations in the excitability of sympathetic and parasympathetic cardiac neurons in cirrhotic rats
Choong-Ku Lee & Seong-Woo Jeong
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 80 P06-23 Necrox-5 attenuates ischemic heart injury via regulation of mitochondria biogenesis and inflammation response
Tae Hee Ko, Hyoung Kyu Kim, Yeon Hee Noh, Sung Ryul Lee, Jubert Marquez, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center, Department of Health Sciences and Technology, BK21 Project Team, Department of Physiology, Inje University, Busan 47392, Korea

P07: Neurophysiology

S 81 P07-01 Regulation of voltage-gated Ca^{2+} channels by DREADD system
Yong-Seuk Kim, and Byung-Chang Suh
Department of Brain & Cognitive Sciences, DGIST, Daegu, 42988, Republic of Korea

S 81 P07-02 Endogenous progenitors on regeneration and repair after spinal cord injury: JAK3-dependent microgliosis
Sumit Barua, Jee-In Chung, A Young Kim, Soo-Yeon Lee, Eun Joo Baik
Department of Physiology, Ajou University School of Medicine, Suwon, Republic of Korea

S 81 P07-03 Adrenergic modulation of glial activity during noxious information processing in the cerebellum
Seung Ha Kim[1,2]*, Seung-Eon Roh[1], Sun Kwang Kim[3] and Sang Jeong Kim[1, 2]
[1]Department of Physiology and [2]Department of Biomedical Science, College of Medicine, Seoul National University, [3]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, South Korea * Presenter

S 81 P07-04 Dapoxetine induces neuroprotective effects against glutamate-induced neuronal cell death through inhibiting calcium signaling and mitochondrial depolarization in cultured rat hippocampal neurons
Imju Jeong[1], Sujeong Jeon[1], Ji Seon Yang[1], Yi Jae Hong[1], Hee Jung Kim[2], Sang June Hahn[1], Shin Hee Yoon[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 06591, [2]Department of Physiology, College of Medicine, Dankook University, Cheonan-si 31116, Republic of Korea

S 82 P07-05 Neonatal maternal separation enhances $GABA_A$ receptor-mediated inhibitory currents of ventral hippocampal dentate granule cells in adolescent
Sang Yep Shin[1], Seung Ho Han[1], Jae yong Yee[a] and Sun Seek Min[1]
Department of Physiology and Biophysics[1], School of Medicine, Eulji University, Daejeon, Republic of Korea

S 82 P07-06 Encoding strategy for sensory information processing with different stimuli features in Primary somatosensory cortex
Yoorim Kim[a], Chang-Eop Kim[a,c], Heera Yoon[b], Sun Kwang Kim[b]*, Sang Jeong Kim[a]*
[a]Department of Physiology, College of Medicine, Seoul National University, Seoul, 110-799, Korea , [b]Department of Physiology, College of Korean Medicine, Kyung Hee University, Seoul, 130-701, Korea, [c]Department of Physiology, College of Korean Medicine, Gachon University, Gyeonggi, 13120, Korea

S 82 P07-07 Progesterone metabolite suppresses visceral afferent signal transduction on medial nucleus tractus solitarius (mNTS) neurons
Sojin Kim, Eunhee Yang, Young-Ho Jin
Department of Physiology, School of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

S 83 P07-08 TRPC3 channels drive pacemaking and regulate tonic firing rate in nigral dopamine neurons
Ki Bum Um[1], Lutz Birnbaumer[2], Hyun Jin Kim[1], Myoung Kyu Park[1]
[1]Department of Physiology, Sungkyunkwan University School of Medicine, 2066, Seoburo, Jangangu, Suwon, KOREA, [2]IIB-INTECH, Univ Nacional de San Martin; Av 25 de Mayo y Francia, San Martin CP1650, Prov Buenos Aires, Argentina

S 83 P07-09 Differential density of IP_3 receptors confers dendritic domain-dependency on muscarinic modulation in layer 2/3 pyramidal neurons of the primary visual cortex
Kwang-Hyun Cho[1], Kayoung Joo[1], Hyun-Jong Jang[1,2] and Duck-Joo Rhie[1,2]

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

S 83 P07-10

Estrogen blocks the inhibitory-to-excitatory switch in GABAergic action in the vasopressin neurons of salt-dependent hypertension model rats
Young-Beom Kim, Woong Bin Kim, Xiangyan Jin, Won-Woo Jung, Hyung Kyung Kang and Yang In Kim
Department of Physiology, Neuroscience Research Institute, Korea University College of Medicine

S 84 P07-11

Suppression of the Sweat Gland Sensitivity to QSART in Tropical Africans Compared to Temperate Koreans
Jeong-Beom Lee[1]*, Young-Oh Shin[1], Young-Ki Min[1], Hyung-Seok Seo[2]
[1]Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan 330-090, Republic of Korea, [2]Department of Exercise Prescription, Konyang University, Nonsan-si, Chungnam, Republic of Korea

S 84 P07-12

Effects of acute ingestion of caffeine on the blood levels of dopamine and serotonin (5-HT) in exercising humans
Jeong-Beom Lee*, Young-Oh Shin
Department of Physiology, College of Medicine, Soonchunhyang University, Cheonan 330-090, Republic of Korea

S 84 P07-13

Bidirectional synaptic plasticity in late-spiking neurons of layer 2/3 in rat visual cortex
Kayoung Joo[1], Kwang-Hyun Cho[1], Hyun-Jong Jang[1,2], Duck-Joo Rhie[1,2]
[1]Department of Physiology, [2]Catholic Neuroscience Institute, College of Medicine, The Catholic University of Korea, Seoul 06591, Korea

S 85 P07-14

Thalamic miR-338-3p mediates auditory thalamocortical disruption and its late onset in 22q11.2 microdeletion models
Suk-hyun Yun, Jeong-mi Oh, Sungkun Chun
Department of Physiology, Chonbuk National University Medical School, Jeonju, South Korea

S 85 P07-15

Critical role of muscarinic acetylcholine receptors in synaptic depotentiation at the hippocampal SC-CA1 synapses
Woo Seok Song[1], Jin Hee Cha[1], Sang Ho Yoon[1], Kyeong-Yeol Park[1], Young-Soo Bae[1], Young Seon Cho[1], and Myoung-Hwan Kim[1,2,3,*]
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S 85 P07-16

Effects of acute hypoxic stimulus with inflammation and reperfusion on excitability and synaptic transmission of the hippocampal CA1 neurons
Yoon-Sil Yang, Sookjin Son, Jong-Cheol Rah
Laboratory of Neurophysiology, Korea Brain Research Institute

S 86 P07-17

Suppression of spinal IL-1β facilitates gap junction-mediated production of contralateral astrocyte D-serine leading to the early development of contralateral mechanical allodynia in carrageenan rats
Hoon-Seong Choi, Sheu-Ran Choi, Mi-Ji Lee, Ho-Jae Han, Jang-Hern Lee*
Department of Veterinary Physiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea

S 86 P07-18

The nociceptive effect of dephosphorylated astrocytic aromatase in mice formalin model is mediated by spinal sigma-1 receptor through calcineurin pathway
Mi Ji Lee[1], Hoon-Seong Choi[1], Alvin Beitz[2], Jang-Hern Lee[1]
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S 86 P07-19(YP-11)

A Study for Electrophysiological Characteristics of Neural Circuit between Ventral Tegmental Area and Medial Prefrontal Cortex in Rats
Yu Fan[1], Ho Koo[2], Sang Hu Han[2], Se Jin Moon[2], Jae Hyo Kim[1], Byung Rim Park[2], Min Sun Kim[2]
[1]Department of Meridian & Acupoint, College of Korean Medicine, Wonkwang University, Iksan, Korea, [2]Department of Physiology, Wonkwang University School of Medicine, Iksan, Korea

S 87 P07-20(YP-12)

Intrinsic plasticity amplifies long-term depression in parallel fibre input gain in cerebellar Purkinje cells
Hyun Geun Shim[1,2,4], Dong Cheol Jang[1,3,4], Sang Jeong Kim[1,2]
[1]Department of Physiology, [2]Department of Biomedical Science, Seoul National University College of Medicine, [3]Department of Brain and Cognitive Science, College of Science, Seoul National University, Seoul, Republic of Korea, [4]These authors contributed equally to this work

S 87 P07-21(YP-07) Analgesic effect of endogenous oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells
Rafael Taeho Han[1]*, Hanbyul Kim[2]*, Youngbum Kim[1], Kiyeon Park[2], Pareum Lee[2], Heung Sik Na[1], Seogbae Oh[2#]
[1]Neuroscience Research Institute and Department of Physiology, Korea University College of Medicine, Seoul, Korea
[2]National Research Laboratory for Pain, Dental Research Institute and Department of Physiology, School of Dentistry, Seoul National University, Seoul, Korea

S 87 P07-22(PO-08) Salt loading recruits Mg^{2+}-resistant extrasynaptic NMDA receptors in supraoptic nucleus neurons
Kyung-Ah Park, Chiranjivi Neupane, and Jin Bong Park
Department of Physiology and Biomedicine, School of Medicine, Chungnam National University, Republic of Korea

S 88 P07-23(PO-09) Low Intensity Ultrasound Decreases α-Synuclein Aggregation in PC12 Cells: Potential action on mitochondrial reactive oxygen species
Mrigendra Bir Karmacharya[1], Binika Hada[2], Byung Hyune Choi[2]*, and So Ra Park[1]*
[1]Department of Physiology and Biophysics, Inha University College of Medicine, Incheon, 22212, South Korea,
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p08: Physiom and Systems biology

S 88 P08-01(YP-13) Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan
Dang Van Cuong[1,#], Hyoung Kyu Kim[1,2,#], Yeon Hee Noh[1], Jubert Marquez[1], Nari Kim[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], and Jin Han[1]*
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S 88 P08-02(PO-05) Computational analysis of cerebrovascular flow reserve using patient-specific medical images
Ajin Ryu[1], Kyoung-Min Lee[2], Eun Bo Shim[1]
[1]Department of Mechanical and Biomedical Engineering Kangwon National University, [2]Department of Neurology, Seoul National University Hospital

S 89 P08-03 CT image-based computational fluid dynamic three-dimensional modeling in coronary artery atherosclerosis
Seonjoong Lee, Eunji Shin, Seonghoon Jeong, Jin Han, Nari Kim
NLRL for Innovative Cardiovascular Engineering, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University

S 89 P08-04 A computational fluid dynamics study on the image-based three-dimensional aorta
Eunji Shin, Seonjoong Lee, Seonghoon Jeong, Jin Han,
Nari Kim
NLRL for Innovative Cardiovascular Engineering, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University

S 89 P08-05 A comparison of extracellular matrix components produced by 2D and 3D cultured human conjunctival fibroblasts
Ju Hyun Lim, Hae-Rahn Bae
Department of Physiology, College of Medicine, Dong-A University, Busan 602-714, South Korea

S 90 P08-06 A comparison of cell morphology and behaviors between 2D and 3D cultured human conjunctival fibroblasts
Ju Hyun Lim, Hae-Rahn Bae
Department of Physiology, College of Medicine, Dong-A University, Busan 602-714, South Korea

S 90 P08-07 Computational Simulations of noninvasive Instant Flow Reserve by using Coronary Computed Tomographic Angiograms in non-hyperemia
Kyung Eun Lee[1], Eui Cheol Jung[1], Eun-Seok Shin[2] and Eun Bo Shim[1]
[1]Department of Mechanical and Biomedical Engineering, Kangwon national university, [2]Department of Cardiology, University of Ulsan College of Medicine, Ulsan, 44033, Republic of Korea

S 90 P08-08 Deep learning and neuroscience: Bridging the gap between artificial neural networks and natural neural networks
Chang-Eop Kim, Musun Park
Department of Physiology, Gachon University College of Korean Medicine

S 90 P08-09 Inflammatory regulation of spinal PPAR-gamma in contusive spinal thoracic injury
Jeonghwa Oh
Department of Physiology and Neuroscience Research Institute, Korea University College of Medicine, Seoul, Republic of Korea

YP-01

CR6-interacting factor 1 inhibition damages vascular function by inhibiting the Sirt1-eNOS pathway

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Mitochondrial dysfunction has emerged as a major contributing factor to endothelial dysfunction and vascular disease, but the key mechanisms underlying mitochondrial dysfunction-induced endothelial dysfunction remain to be elucidated. To determine whether mitochondrial dysfunction in endothelial cells play a key role in vascular disease, we examined the phenotype of endothelial-specific CR6-interacting factor 1 (CRIF1) knockout (CRIF1 KO) mice and *in vitro* pathophysiological mechanisms. CRIF1 KO mice exhibited lower body weight and cardiac hypertrophy. Downregulation of CRIF1 in vascular endothelial cells caused disturbances in the mitochondrial OXPHOS (oxidative phosphorylation) complexes, mitochondrial morphology, and function leading to enhanced mitochondrial reactive oxygen species (ROS) production and higher mitochondrial membrane potential (MMP). Downregulation of CRIF1 also caused decreased Sirt1 expression along with increased endothelial nitric oxide synthase (eNOS) acetylation leading to reduced nitric oxide (NO) production. Similar results were obtained in mice with CRIF1-deficient vascular endothelial cells. Endothelium-dependent vasorelaxation (EDR) of aortic rings from CRIF1 KO mouse was considerably less than in wild-type mice. Notably, EDR was partially recovered following in adSirt1 treated aortic rings from CRIF1 KO mice. Taken together, these findings indicate that CRIF1 plays an important role in the maintaining mitochondrial and endothelial function through its effects on the SIRT1-eNOS pathway.

Key Words: OXPHOS complex, CRIF1, SIRT1, eNOS, nitric oxide, mitochondrial dysfunction

YP-02

Characteristics of mitochondrial calcium efflux regulation in permeabilized single ventricular myocytes

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Regulation of mitochondrial Ca^{2+} is related to various physiological and pathophysiological phenomena. In a recent review, several Ca^{2+} transporting mechanisms were existed in mitochondria such as MCU, mRyR, RaM, NCX_{mito}, HCE_{mito}, etc. However, the role and the regulating mechanisms were not clear, yet. In this study, we would like to elucidate Ca^{2+} removing mechanisms. Ca^{2+} efflux was evoked by the removal of cytosolic Ca^{2+} after Ca^{2+} was loaded into mitochondria for 3 mins. We showed clearly that NCX_{mito} is a major Ca^{2+} efflux mechanism. However, in the absence of Na^+, Ca^{2+} efflux was still occurred, even though it was much slower. The proposed efflux route may be Ca^{2+} influx pathway such as MCU. The treatment of RU360 clearly delayed Ca^{2+} efflux. When 0.1 μM Ca^{2+} was applied continuously, $[Ca^{2+}]_m$ was initially increased, however, it was not maintained and continuously decreased even in the presence of $[Ca^{2+}]_c$. When 10 μM Ca^{2+} was applied continuously, initial $[Ca^{2+}]_m$ increase was faster and larger, however, $[Ca^{2+}]_m$ decrease was occurred in a much faster rate. During $[Ca^{2+}]_m$ decrease, the reduction of NADH and Ψ_m depolarization were accompanied. When we changed pHc from 6 to 8, Na^+ independent Ca^{2+} efflux was faster as pH became alkaline. We hypothesized the mechanism related to Ca^{2+}/Pi dependent Ψ_m depolarization may participate in Na^+-independent Ca^{2+} efflux (NICE). Since ATP and ADP could prevent Ca^{2+}/Pi dependent Ψ_m depolarization, it was identified whether ATP or ADP could also prevent the NICE. In the presence of ATP or ADP, NICE was clearly prevented while NADH and Ψ_m were maintained. From the above results, NICE pathway was existed. HCEmito may not work in rat ventricular myocytes since the effects of pHc were opposite and Ca^{2+} flux did not change mitochondrial pH. Ca^{2+} influx pathway could be Ca^{2+} efflux pathway because RU360 attenuated Ca^{2+} efflux but could not prevented. ATP or ADP could effectively prevented NICE, suggested that the similar mechanisms of Ca^{2+}/Pi-induced Ψ_m depolarization may be responsible for NICE. This work was supported by the grant (R0005739) from KIAT.

Key Words: Mitochondria, Calcium, ATP, ADP, RU360

YP-03

Mechanism of hERG current inhibition by 4-hydroxynonenal (4-HNE), a polyunsaturated fatty acid-derived electrophile

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Under oxidative stress, peroxidation of ω6-polyunsaturated fatty acids produces 4-Hydroxynonenal (4-HNE), a highly reactive electrophile forming 4-HNE-protein adducts. Here we investigate the effects of 4-HNE on cardiac hERG current that is a major target of arrhythmogenic conditions. Acute application of 4-HNE (30~100 μM) gradually decreased the tail current of hERG ($I_{hERG,tail}$), which was reversed by TCEP, a membrane impermeable reducing agent. The action potential duration (APD) of guinea-pig ventricular myocytes (GPVMs) was prolonged by 100 μM 4-HNE. Chronic incubation (1-3 h) with lower concentration of 4-HNE (10 μM) induced non-reversible, time-dependent suppression of $I_{hERG,tail}$ and prolongation of APD in GPCMs, which was not reversed by TCEP treatment. Western blot analysis revealed that mature glycosylated hERG (155 kDa) is reduced by chronic 4-HNE (10 μM, 1-12 h). In total cell preparations, the decrease of mature hERG was partly rescued by proteasome- and lysosome-dependent degradation inhibitors, bortezomib and bafilomycin, respectively. However, reduction of plasma membrane hERG proteins in the plasma membrane fraction and the chronic inhibition of IhERG,tail by 4-HNE were not restored by bortezomib and bafilomycin. Taken together, it is suggested that the electrophilic binding of 4-HNE with extracellular domain acutely inhibits hERG activity. Also, the sustained exposure to 4-HNE may accelerate the endocytosis and degradation of hERG proteins through proteasome- and lysosome-dependent process. The suppression of hERG by 4-HNE may participate in proarrhythmic effects of endogenous lipid peroxidants under pathological conditions.

Key Words: hERG channel, electrophile, 4-hydroxynonenal, protein degradation

YP-04

Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells

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Mitochondria are crucial for maintaining the properties of embryonic stem cells (ESCs) and for regulating their subsequent differentiation into diverse cell lineages, including cardiomyocytes. However, mitochondrial regulators that manage the rate of differentiation or cell fate have been rarely identified. This study aimed to determine the potential mitochondrial factor that controls the differentiation of ESCs into cardiac myocytes. We induced cardiomyocyte differentiation from mouse ESCs (mESCs) and performed microarray assays to assess messenger RNA (mRNA) expression changes at differentiation day 8 (D8) compared with undifferentiated mESCs (D0). Among the differentially expressed genes, Pdp1 expression was significantly decreased (27-fold) on D8 compared to D0, which was accompanied by suppressed mitochondrial indices, including ATP levels, membrane potential, ROS and mitochondrial Ca^{2+}. Notably, Pdp1 overexpression significantly enhanced the mitochondrial indices and pyruvate dehydrogenase activity and reduced the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate compared to a mock control. In confirmation of this, a knockdown of the Pdp1 gene promoted the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate. In conclusion, our results suggest that mitochondrial PDP1 is a potential regulator that controls cardiac differentiation at an early differentiation stage in ESCs.

Key Words: mitochondria, stem cells, ROS, differentiation, cardiomyocyte, pyruvate dehydrogenase phosphatase

YP-05

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

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KCNQ2/3 channel is known as M-type K^+ channel which is suppressed by muscarinic receptor stimulation. These channels broadly expressed in the central nerve systems including ventral tegmental area (VTA) dopamine neurons. Recently, it is reported that ethanol inhibits M-current in VTA neurons, and thus increases excitability of the cells and may reinforce the brain reward system. However, the molecular mechanism of the ethanol regulation of KCNQ2/3 channels and the effects on action potential firing in neurons have not been studied well. In this study, we investigated the mechanism by which alcohol modulate the KCNQ2/3 channel activity and neuronal excitability. We found that in superior cervical ganglia (SCG) neurons 200 mM ethanol inhibits M-current by ~20% and increases action potential firing about 1.5-fold. The alcohol inhibition was dependent upon carbon chain length and conformation of alcohols in tsA201 cell (200 mM methanol, ~18%; ethanol, ~22%; 2-propanol, 25%). The alcohol inhibition occurred rapidly (τ = < 4 s). We also found that the ethanol inhibition was decreased when the cells were co-transfected with PIPKIγ (~8%), suggesting that membrane phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is an important regulator of the alcohol regulation of M-channels. In addition, KCNQ2 and KCNQ3 homomeric channel showed different alcohol sensitivity (~40% and 10%, respectively, at 200 mM ethanol). Therefore, these results indicate that alcohols could play an important regulator of neuronal excitability. We expect that understanding the mechanism will give some evidences to develop alcohol-selective therapy for overcoming or preventing alcohol addiction.

Key Words: KCNQ2/3 channel, Ethanol, Neuron, $PI(4,5)P_2$

YP-06

Neurosteroids-mediated actions on GnRH neuron in letrozole-induced polycystic ovarian syndrome (PCOS) mouse model

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Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disorder in women on their reproductive age which is distinguished by accretion of small cystic follicles in the ovary. Ovarian function in all mammals is controlled by gonadotropin releasing hormone (GnRH) neurons, which are the central regulator of hypothalamic pituitary gonadal axis (HPG-axis). Till date, few is known the impact of letrozole-induced PCOS in GnRH neuronal development and its role in reproductive physiology at hypothalamic level is still unknown. In this study, we investigated the effects of various neurotransmitters including GABA, kainate, muscimol and baclofen and neurosteroids including THDOC and THIP on GnRH neurons from letrozole-treated mice (1 μg/g body weight; 21 successive days) exposed from PND 23. Neurotransmitters- and neurosteroids-mediated responses on GnRH neurons from letrozole-injected mice were smaller than those of control. We also found that letrozole-induced irregular estrous cycle, increased in body weight and anovulation in female mice. These results suggest that the PCOS is an endocrine disorder mostly common in females which may directly affect GnRH neuronal activity and adverse impact on reproductive physiology at hypothalamic level. (This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1C1A1A02036793))

Key Words: GnRH neurons, Letrozole, Polycystic ovarian syndrome, Patch clamp

YP-07

Analgesic effect of endogenous oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells

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Recent studies have provided several lines of evidence that peripheral administrations of oxytocin (OT) induced analgesia in human and rodents. However, the exact underlying mechanism of the analgesia still remains elusive. In the present study, we investigated to identify which receptor mediated analgesic effect of intraperitoneal (IP) injection of OT in thermal pain behavior, and to verify the expression patterns of mRNA of OT-related receptors in dorsal root ganglion (DRG) neurons. We interrogated whether OT affected capsaicin-induced intracellular calcium transients and the membrane excitability in peripheral sensory neurons. If so, we examined which receptor mediated these changes. For these aims, we examined whether the IP injection of specific antagonist for oxytocin and vasopressin (AVP) receptors reversed the OT-induced analgesia during the pain behavioral tests such as Hargreaves' in rats. We determined mRNA expression patterns of transient receptor potential vanilloid 1 (TRPV1) and the oxytocin-related receptors, such as OT, AVP1a, AVP1b, and AVP2 receptors, in the individual cells by single cell RT-PCR. We explored the effect of oxytocin and its antagonists on capsaicin-induced intracellular calcium transient using fluorescent microscopy in the dissociated DRG cells. In addition, we also investigated the effect of oxytocin and its antagonists on the membrane excitability using patch clamp. The behavioral results showed that the antagonist for AVP1a receptor reversed the oxytocin-induced analgesia. Single cell RT-PCR discovered predominnt co-expression of mRNA of TRPV1 and AVP1a receptor in the DRG cells. Also, the number of cell expressing mRNA of AVP1 receptor was more predominant than that of OT, AVP1b, and AVP2 receptors. The fluorescent imaging experiments demonstrated that oxytocin partially inhibited capsaicin-induced intracellular calcium transient through AVP1a receptor in the DRG cells. The electrophysiological study also demonstrated that OT hyperpolarized the membrane potential by increasing the permeability of potassium channel via AVP1a receptor in the DRG cells. Taken together, our findings possibly suggested that OT-induced membrane hyperpolarization of the DRG cells contributed to the analgesic effects of peripheral administration of OT through AVP1a receptor.

Key Words: analgesia, oxytocin, vasopressin, single cell rt pcr, electrophysiology, calcium imaging

YP-08

Impaired myogenic responses in the skeletal arteries from denervated hind limbs and their recovery by exercise training; underlying changes of ion channel currents in the arterial myocytes

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K^+ channel currents such as voltage-gated K^+ current (I_{Kv}) and inwardly rectifying K^+ current (I_{Kir}) set the negative membrane potential of arterial myocytes, inhibiting voltage-operated L-type Ca^{2+} currents ($I_{Ca,L}$). Previous studies demonstrated that both I_{Kv} and I_{Kir} in deep femoral arterial smooth muscle cells (DFASMCs) are significantly increased in the rats underwent endurance exercise training (ET). In this study, a unilateral sciatic nerve was severed to induce lower hindlimb paralysis and atrophy. In deep femoral arteries (DFAs) and the myocytes isolated from DFAs (SkASMCs), pressurized artery video-analysis and whole-cell patch clamp were applied, respectively. The myogenic response (MR) of DFA from paralyzed legs disappeared in 5 wk of denervation. In DFASMCs from the paralyzed legs, both $I_{Ca,L}$ and TRPC-like current induced by intracellular GTPγS ($I_{GTPγS}$) were decreased. In addition, I_{Kir} and I_{Kv} became decreased in the DFASMCs from atrophic legs. When combined with ET (4 weeks) after the sciatic nerve injury, the MR of DFAs showed almost complete recovery with associated increase of $I_{GTPγS}$ while not $I_{Ca,L}$ in DFASMCs. I_{Kir} and I_{Kv} also showed recovery in the ET-combined rats. The atrophy side-specific decreases of MR and ion channel currents might reflect the importance of blood flow-induced mechanical stimuli to the arterial smooth muscle function. The recovery of MR and ion channel currents by ET in skeletal arteries suggest beneficial effects on the blood pressure regulation as well as the regional blood flow control in motor nerve injury patients.

Key Words: denervation, atrophy, skeletal artery, myogenic response, smooth muscle, ion channel

YP-09

ATP release via gap junction hemichannels in rat atrial myocytes under shear stress

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We have previously shown that shear stress (~16 dyn/cm^2) induces longitudinal Ca^{2+} propagation wave with a 0.2- to 3-s delay in atrial myocytes through the activation of $P2Y_1$ purinergic signaling, and that the shear-mediated wave generation is inhibited by the blockade of gap junction hemichannels.[1)] In the present study, we further examined whether atrial cells release ATP via the gap junction hemichannels. ATP releases of cells were directly measured with 2-s intervals as chemiluminescence emitted (at 562 nm) during ATP-driven luciferin-luciferase reaction. Shear stress was applied to fields of cells in a laminar flow chamber attached to the flow regulator. ATP was released from atrial myocytes by the application of shear stress in the range of 1-16 dyn/cm^2 in a strength-dependent manner. The shear-induced ATP release occurred transiently with a maximal release (~1.3 moles/μm^2) at about 2 s and 90% decay at 4-6 s after the shear application (~16 dyn/cm^2 for 10 s). Removal of external Ca^{2+} to enhance the activity of the gap junction hemichannels increased the shear-mediated ATP release to ≅ 320% of control. In cells pre-treated with carbenoxolone (50 μM for 10 min) or La^{3+} (2 mM for 5 min), the hemichannel blockers, ATP release by shear application was suppressed by ~50% and ~90%, respectively. The shear-induced ATP release was not affected by the pre-treatment of 50 μM Gd^{3+} (5 min) or 1 mM 9-anthracenecarboxylic acid (10 mM), suggesting no role of maxi anion channel and Cl^- channels in shear-induced ATP release in these myocytes. Our data suggest that shear stress induces ATP release through the gap junction hemichannels in atrial myocytes.

Reference

1) JC Kim and SH Woo. Shear stress induces a longitudinal Ca^{2+} wave via autocrine activation of P2Y1 purinergic signalling in rat atrial myocytes. *J Physiol* 593(23):5091-5109 (2015).

Key Words: Atrial myocyte, Shear stress, ATP, Gap junction hemichannel, Chemiluminescence

YP-10

Rapamycin ameliorates the portal hypertension, cardiovascular autonomic dysfunction, and alterations in the excitability of sympathetic and parasympathetic cardiac neurons in cirrhotic rats

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Cardiovascular autonomic dysfunction (CAD), which is manifested by the decreased baroreflex sensitivity (BRS) and heart rate variability (HRV), is prevalent irrespective of etiology and contributes to the increased morbidity and mortality in patients with liver cirrhosis. Recently, we have observed that liver cirrhosis and portal hypertension-induced CAD is attributable to an imbalance of excitability between sympathetic and parasympathetic cardiac neurons. A previous study has shown that rapamycin improves liver function by limiting inflammation, fibrosis, and portal pressure in the early phase of cirrhotic portal hypertension. Thus, we examined whether rapamycin ameliorates development of the portal hypertension, the CAD, and the functional plasticity of the cardiac autonomic neurons in cirrhotic rats. Biliary cirrhotic rats were generated via common bile duct ligation (CBDL). From the first day of CBDL, rats orally received 0.5 mg/kg/day for 2 weeks. Picrosirius red and Masson staining showed that rapamycin inhibited heavy collagen deposition, as verified by broad fibrotic septa that surround abnormal nodules. In accordance with the histological examination, rapamycin significantly inhibited CBDL-induced increase in the transcripts that encode alpha-smooth muscle actin, TGF-beta, and type I collagen. Development of the portal hypertension was prevented in the rapamycin-treated CBDL rats. More importantly, rapamycin inhibited the CBDL-induced decreases in the BRS and HRV. Under the current clamp mode of the gramicidin-perforated patch-clamp technique, cell excitability was recorded in sympathetic stellate ganglion (STG) and parasympathetic intracardiac ganglion (ICG) neurons. Rapamycin inhibited the CBDL-induced increase and decrease in the frequency of action potential discharge in the STG and ICG neurons, respectively. Taken together, these results suggest that cirrhotic portal hypertension is associated with CAD and functional plasticity of the cardiac autonomic neurons. Importantly, anti-fibrotic agents might be considered as potential therapeutics for the CAD in cirrhotic patients. (This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2013424)).
Key Words: arterial baroreflex, autonomic, cardiovascular autonomic dysfunction, excitability, heart rate variability, liver cirrhosis, portal hypertension, rapamycin

YP-11

A Study for Electrophysiological Characteristics of Neural Circuit between Ventral Tegmental Area and Medial Prefrontal Cortex in Rats

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The ventral tegmental area (VTA) is a vital part of brain that involve in avoidance, fear conditioning, addiction and other neuropsychiatric illnesses. The medial prefrontal cortex (mPFC) is a key structure for executive functions of the brain. Some papers has been shown that mPFC takes part in the modulation of the firing pattern of VTA dopaminergic neurons. However, the functional connectivity between mPFC and VTA GABAergic neuronal activities is still unclear. The purpose of this study was to observe electrophysiological characteristics of neural circuit between the GABAergic neurons in the VTA and mPFC. Spontaneous activities of VTA GABAergic neurons induced by Electrical stimulation (200Hz, 10 pulses) of mPFC were recorded using extracellular recording technique in Sprague - Dawley rats. The electrical stimulation to mPFC resulted in 3 different patterns of spontaneous firings in the VTA GABAergic neurons, in which main effect of the electrical stimulation was increase in firing rate. In order to see effects of neurochemical modulations of mPFC on VTA GABAergic neuronal activities. PEPA, glutamate receptor agonist and a mixture of muscimol, $GABA_A$ receptor agonist and baclofen, $GABA_B$ receptor agonist were injected through a cannula inserted into the mPFC, respectively. After injecting the PEPA into the mPFC, the spontaneous activities of 80% VTA GABAergic neurons was gradually increased for more than one hour. The increasing rate of the spontaneous activity was 30.9% following PEPA injection. Oppositely, the injection of a GABA mixture resulted in the reduction of the spontaneous firings of VTA GABAergic neurons for more than one hour. According to these results, we suggest that excitatory synapse is major component in neural circuit between the mPFC and VTA GABAergic neurons.
Key Words: ventral tegmental area, medial prefrontal cortex, GABAergic neurons.

YP-12

Intrinsic plasticity amplifies long-term depression in parallel fibre input gain in cerebellar Purkinje cells

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Cerebellar Purkinje cells (PCs) are the sole output neuron in the cerebellar cortical area and endowed with the neural plasticity, most investigation accounting for cerebellum-dependent motor learning, however, have been intensively focused on the synaptic plasticity between parallel fibre and the PCs. Here, we describe how PCs recognise learned the pattern and generate their output signal during long-term depression at parallel fibre to PC synapses occur using patch-clamp whole cell recordings from mouse Purkinje cells. Both parallel fibre tetanisation and burst stimulation with climb fibre reliably induce synaptic depression accompanied by long-term depression of intrinsic excitability (LTD-IE). Interestingly, the dendritic temporal summation of EPSP shows no significant alteration although the synaptic gain is attenuated after LTD induction. In addition, the ratio of spike frequency adaptation is also not changed after the manifestation of LTD-IE. Collectively, our findings suggest that LTD-IE of PCs enable to amplify the modification of synaptic weight, rather the strategy for information processing when the novel pattern of the parallel fibre input is delivered after neuronal plasticity induction.
Key Words: Long-term depression, intrinsic plasticity, cerebellar Purkinje cells

YP-13

Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan

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Mast cells are primary mediators of allergic inflammation. Beta-1,3-glucan (BG) protects against infection and shock by activating immune cells. Activation of the BG receptor induces an increase in intracellular Ca^{2+}, which may induce exocytosis. However, little is known about the precise mechanisms underlying BG activation of immune cells and the possible role of mitochondria in this process. The present study examined whether BG induced mast cell degranulation, and evaluated the role of calcium transients during mast cell activation. Our investigation focused on the role of the mitochondrial calcium uniporter (MCU) in BG-induced degranulation. Black mouse (C57) bone marrow-derived mast cells were stimulated with 0.5 μg/ml BG, 100 μg/ml peptidoglycan (PGN), or 10 μM A23187 (calcium ionophore), and dynamic changes in cytosolic and mitochondrial calcium and membrane potential were monitored. BG-induced mast cell degranulation occurred in a time-dependent manner, and was significantly reduced under calcium-free conditions. Ruthenium red, a mitochondrial Ca^{2+} uniporter blocker, significantly reduced mast cell degranulation induced by BG, PGN, and A23187. These results suggest that the mitochondrial Ca^{2+} uniporter has an important regulatory role in BG-induced mast cell degranulation.

Key Words: Beta-1,3-glucan, Mast cell degranulation, Mitochondrial calcium uniporter

PO-01

TRPC channels are a novel culprit for hepatic stellate cell activation and hepatic fibrosis

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Deregulation of Ca^{2+} signaling has been postulated as pathological event in fibrosis. A primary culprit for hepatic fibrosis is hepatic stellate cell (HSC) activation. Aberrant Ca^{2+} influx mediates either directly or indirectly HSC activation and leads to increase the expression of fibrosis markers such as α-smooth muscle actin and/or profibrotic ligand TGFβ causing hepatic fibrosis. Thus, we firstly monitored Ca^{2+}-permeable channels in *in vivo* hepatic fibrosis animal models by bile duct ligation (BDL) and thioacetamide (TAA) administration. We found that TRPC1 and TRPC6 channels were overexpressed in cirrhotic models whereas expressions of voltage-gated and store-operated Ca^{2+} channels were not altered. Notably, we found that expression level of TRPC channels were strongly correlated with Laennec scoring system for stating fibrosis in human liver biopsy specimens. To uncover underlying mechanism for TRPC-mediated fibrogenesis, we monitored TRPC6 activation in *in vivo* animal models and *in vitro* HSC activation model using primary HSCs culture. Expression of TRPC6 was significantly increased in hepatic fibrosis animal models and primary HSC model. Transgenic overexpression of TRPC6 in mouse liver *in vivo* induced *de novo* expression of fibrosis markers in liver. Functionally, fibrotic changes and Ca^{2+} influx were ameliorated by suppressing TRPC6 in *in vivo* animal model and *in vitro* HSCs, respectively. Together, these data demonstrate that TRPC6-mediated Ca^{2+} influx causes hepatic fibrosis, at least in part, via HSC activation. These provide a new perspective on the pathogenesis of hepatic fibrosis and offer clues for therapeutic strategies for liver cirrhosis. [This study was supported by NRF-2015R1D1A1A01060454]

Key Words: TRPC6, Ca^{2+} signaling, Hepatic fibrosis, Hepatic stellate cell (HSC)

PO-02

Down-regulation of NKCC and AE2 transporters in salivary gland cells by dexmedetomidine

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Dexmedetomidine (Dex), a highly selective α2-adrenoceptor agonist, has been shown to attenuate inflammatory responses induced by lipopolysaccharide (LPS) and induce sedative and analgesic effects. Administration Dex has been shown to reduce salivary flow in human subjects and inhibit osmotic water permeability in the rat cortical collecting duct. However, little is known about the regulatory effects of Dex on salivary fluid secretion. To investigate fluid secretion upon treatment with Dex, we studied the effects of Dex on secretion in salivary glands by the regulation of ion transporters such as Na^+-K^+-$2Cl^-$ cotransporter (NKCC) and Cl^-/HCO_3^- exchanger (CBE) and downstream pro-inflammatory cytokine expression in isolated primary mouse submandibular glands (SMG). Dex increased CBE and NKCC activities

in SMG ducts and enhanced salivary fluid secretion in the sealed duct system. It showed differential effects on neurotransmitter inputs and inflammatory mediator-induced Ca^{2+} signaling in mouse SMG cells. Histamine and LPS-induced Ca^{2+} signals were inhibited but not carbachol-stimulated Ca^{2+} signals. Long-term Dex treatment for 2 hrs reduced CBE and NKCC activities in SMG and HSG cells. Moreover, when isolated SMG cells were stimulated Dex for 2 hrs, phosphodiesterase 4D (PDE4D) expression was enhanced. The obtained results confirm the anti-inflammatory properties of Dex against LPS-mediated signaling; Dex also inhibited interleukin-6 and NADPH oxidase 4 expression. α2-Adrenoceptor-mediated inhibition of inflammatory signaling was involved in reduced NADPH oxidase 4 expression. The present study showed that the role of α2-adrenoceptor activation by Dex on the hyposecretion of salivary glands could be related to the involvement of PDE4D and subsequently reduced cAMP level.
Key Words: NKCC, AE2, salivary gland, dexmedetomidine

PO-03

A Jumonji C (JmjC) domain-containing protein negatively regulates RANKL-mediated osteoclastogenesis

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The regulation of osteoclastogenesis is critical to maintain physiological bone homeostasis and prevent bone-destructive diseases. The nuclear factor of activated T-cells calcineurin-dependent 1 (NFATc1) plays an essential role in osteoclastogenesis, and its expression is induced during early osteoclastogenesis. On the other hand, the Jumonji C (JmjC) domain-containing protein (JHDM), a histone demethylase, catalyzes histone 3 lysine 9 and is involved in osteoblastic bone formation. However, the mechanism for regulation of the enzymatic activity of JHDM in osteoclastogenesis is not yet well known. Here, we show that JHDM is a key negative regulator during receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis. The expression level of JHDM gradually decreased during osteoclastogenesis in bone marrow macrophages (BMMs) treated with RANKL. Down-regulated expression of JHDM strongly facilitated osteoclast formation together with induction of several osteoclast-specific genes such as TRAP, Oscar and CathepsinK. NFATc1 proteins are ubiquitinated and rapidly degraded during late stage osteoclastogenesis. Interestingly, overexpression of JHDM induces NFATc1 degradation during late stage osteoclastogenesis. Taken together, the present study demonstrated that JHDM is a post-translational co-repressor for NFATc1 that attenuates osteoclastogenesis.
Key Words: Jumonji C (JmjC) domain-containing protein (JHDM), NFATc1, Stability, Osteoclastogenesis

PO-04

IDH2 Inhibition impairs endothelium-dependent vasomotor function via mitochondrial function in endothelial cells

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Mitochondrial NADP(+)-dependent isocitrate dehydrogenase (IDH2) plays an essential role protecting cells against oxidative stress-induced damage. A deficiency in IDH2 leads to mitochondrial dysfunction and the production of reactive oxygen species (ROS) in cardiomyocytes and cancer cells. In this study the effects of IDH2 deficiency on mitochondrial and vascular function were investigated in endothelial cells. IDH2 knockdown decreased the expression of mitochondrial oxidative phosphorylation (OXPHOS) complexes I, II and III, which lead to increased mitochondrial ROS (mtROS). In addition, the levels of fission and fusion proteins (Mfn-1, OPA-1, and Drp-1) were significantly altered and MnSOD expression also was decreased by IDH2 knockdown. Knockdown of IDH2 decreased eNOS phosphorylation and nitric oxide (NO) concentration in endothelial cells. Interestingly, treatment with Mito-TEMPO, a mitochondrial-specific superoxidescavenger, recovered mitochondrial fission- fusion imbalace and blunted mt superoxide production, and reduced the IDH2 knockdown-induced decrease in MnSOD expression, eNOS phosphorylation and NO production in endothelial cells. Endothelium-dependent vasorelaxation was impaired, and the concentration of bioavailable NO decreased in the aortic ring in IDH2 knockout mice. These findings suggest that IDH2 deficiency induces endothelial dysfunction through the induction of dynamic mitochondrial changes and impairment in vascular function.
Key Words: IDH2, mitochondrial dysfunction, ROS, eNOS, vascular function

PO-05

Computational analysis of cerebrovascular flow reserve using patient-specific medical images

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Here, we provide a novel method to evaluate the cerebrovascular flow reserve (CFR) by using a computer simulation model. Then, the hyperemic blood flows of the 25 patients with neurovascular stenotic diseases were simulated to test the efficacy of the present method as a non-invasive screening technique of stroke. We have implemented a novel computational method to compute the CFR by coupling computational fluid dynamics (CFD) with the lumped parameter model (LPM) of the neurovascular system. In the CFD model, we used a finite element method to solve blood flow in three dimensional (3D) geometry of patient-specific cerebral arteries that was obtained from patients' magnetic resonance angiography (MRA). In the LPM, autoregulation mechanism to maintain cerebral flow homeostasis was included. Computed blood pressure distribution showed an abrupt decrease through stenosed cerebral arteries. This trend was more remarkable in hyperemic cases than non-hyperemic ones. Increased blood flow in internal carotid arteries was induced by the hyperemic condition, but it was comparably reduced in case of stenosed cerebral

arteries. The CFRs of the patients were obtained and the physiological significances of their cerebrovascular stenotic lesions were analyzed. The present simulation method provided non-invasively the CFR values of the patients and showed the functional significances of their stenotic lesions, indicating its clinical efficacy.
Key Words: Computational analysis, cerebrovascular flow reserve, patient-specific method

PO-06

WNK1 promotes renal tumor progression by TRPC6-NFAT pathway via activating phosphatidylinositol 4-kinase IIIα

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Deregulation of Ca^{2+} signaling and altered phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) level have been postulated as a feature of cancer progression. It is well characterized that WNK1 (with-no-lysine (K)) is a major regulator of renal ion transport whose perturbation by *WNK1* mutation causes hypertension and hyperkalemia. However, if and/or how WNK1 signaling contributes renal cancer development beyond regulation of ion homeostasis remains poorly understood. Here, we show that WNK1 activates TRPC6 channel/NFATc1 pathway by stimulating phosphatidylinositol 4-kinase IIIα (PI4KIIIα) promoting $PI(4,5)P_2$ synthesis. Compared with that of adjacent non-neoplastic tissue, the expression levels of WNK1, PI4KIIIα and NFATc1 in tumor tissues were elevated. Notably, blockade of WNK1-mediated TRPC6 activation and its downstream substrate calcineurin attenuated NFATc1 activation, proliferation and migration of renal cell carcinoma cell confirming the pivotal role of WNK1 signaling cascades in aggravating tumor development. These findings reveal novel perspectives on WNK1-dependent signaling targeting TRPC6 channel/NFATc1 pathway involving tumor progression and offers an attractive targets for therapeutic intervention. [This study was supported by NRF-2015R1D1A1A01060454]
Key Words: TRPC6, WNK1, NFATc1, Renal cell carcinoma, Phosphatidylinositol 4-kinase IIIα

PO-07

Gross morphological properties of the primo-vascular system and its relations with the acupuncture meridian

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The primo-vascular system (PVS) is a novel vascular network that was first reported in the 1960s by Bong-Han Kim, who claimed that the tissue corresponded with the acupuncture meridians. The PVS has been identified in various sites in several animal species. However, the PVS in subcutaneous tissues, which was related the acupuncture meridians, was not well identified. Here, we examined the subcutaneous PVS (scPVS) on the surface of abdominal subcutaneous tissue in rats using Hemacolor staining. The scPVS consisted of vessels that connect the node parts and that the vessels were frequently sub-branched. In the Hemacolor-stained whole scPVS tissue in isolation, the cells of the scPVS were found to be composed of leucocytes and mast cell. The inner space-channel (20-60 μm) structure containing PVS cells along the inside of a vessel of the scPVS was identified. Electron microcopy revealed that the scPVS has a bundle structure of subducts and round cells on the surface and that these were not found in the lymphatic vessel. In addition, the distribution pattern of scPVS tissues in the ventral midline was similar to the route of the conception vessel meridian. In conclusion, our results newly revealed that the PVS is present in the abdominal subcutaneous tissue layer, and indicate that the scPVS tissues are closely correlated with acupuncture meridians. The scPVS has major features in common with the well-established organ-surface PVS tissue in terms of gross morphology and cellular/structural characteristics. These findings may help to identify other PVS tissues in the body and further elucidate their pathophysiological roles in healthy and disease states.
Key Words: subcutaneous tissue, hemacolor staining, mast cell, electron microscopy, conception vessel meridian

PO-08

Salt loading recruits Mg^{2+}-resistant extrasynaptic NMDA receptors in supraoptic nucleus neurons

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Supraoptic nucleus (SON) of the hypothalamus containing magnocellular neurosecretory neurons (MNC) consists of vasopressin (VP) and oxytocin (OT) neurons and plays fundamental roles in reproduction and fluid balance homeostasis. Accumulating evidences suggest that ambient glutamate of synaptic and non-synaptic origin activate extrasynaptic NMDA receptors (NMDAR) mediating a persistent tonic excitatory current (tonic I_{NMDA}) in the brain. However, the physiological role(s) of tonic I_{NMDA} have not been well established. In this study, we show that 7 day of salt loading (SL) recruited a Mg^{2+}-resistant tonic I_{NMDA} in SON NMCs. While AP5, a NMDARs antagonist, failed to affect holding current ($I_{holding}$) in normal aCSF at holding potential of -70 mV in SON NMCs from euhydarted (EU) animals, it uncovered tonic I_{NMDA} shown by an outward shift in $I_{holding}$ in ~45% of the SL SON MNCs. SL recruited the Mg^{2+}-resistant tonic I_{NMDA} in ~90% of VP neurons genetically labelled with eGFP, but none of OT neurons labeled with mRFP. Extrasynaptic NMDAR inhibitor, mematine and PPDA mimicked the AP5-induced outward shift in $I_{holding}$ of SL VP neurons (AP5, 14.5 ± 3.0 pA, n = 9; memantine, 13.7 ± 3.3, n = 4; 12.7± 1.6, n = 5). In low Mg^{2+} aCSF, AP5-sensitive I_{NMDA} were significantly larger in SL VP neurons (24.3 ± 3.1, n = 6) than in EU VP neurons (14.2 ± 1.6, n =7, $p < 0.05$). Sequential application of PPDA and PPDA+AP5 further suggested that SL recruited additional PPDA-sensitive NMDARs in SL VP neurons. While PPDA induced outward shift in $I_{holding}$ in SL (15.0 ± 2.4, n =7) but not in EU VP neurons, additional application of PPDA (PPDA+AP5) induced $I_{holding}$ shift in both neurons (EU, 13.2 ± 3.8, n = 8; SL, 8.9 ± 1.3, n=7). Finally, PPDA significantly inhibited the neuronal firing rates in SL VP neurons, but not in EU VP neurons. Taken together, our results suggest that altered expression and composition of extrasynaptic NMDARs generating Mg^{2+}-resistant tonic I_{NMDA} regulate VP neuronal activity and, in turn, the hormone secretion and fluid balance during osmotic challenges.
Key Words: extrasynaptic NMDA receptors, supraoptic nucleus, osmotic challenge, salt loading

PO-09

Low Intensity Ultrasound Decreases α-Synuclein Aggregation in PC12 Cells: Potential action on mitochondrial reactive oxygen species

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by the loss of nigrostriatal dopaminergic neurons. Several mechanisms have been proposed to explain the underlying pathogenic mechanism of PD that include increase of reactive oxygen species (ROS), mitochondrial dysfunction, α-synuclein deposition, and apoptosis. α-Synuclein aggregates are the major protein component of the Lewy bodies, the neuro-pathological hallmark of PD. α-Synuclein aggregation has been shown to occur after the increase in the oxidative stress. Here, we studied the effects of low intensity ultrasound (LIUS) stimulation on the mitochondrial ROS-dependent α-synuclein aggregation in neurotoxin-treated PC12 cells. To induce oxidative stress and subsequent α-synuclein aggregation, we treated PC12 cells with a neurotoxin 1-methyl-4-phenylpyridinium ion (MPP^+); and then we exposed the cells to LIUS stimulation. We found that LIUS stimulation decreased α-synuclein aggregates in intensity-dependent manner. We hypothesized that LIUS stimulation initially suppressed the mitochondrial ROS generation and inhibited the neurotoxin-induced exacerbation of mitochondrial complex I activity, thereby causing the attenuation of α-synuclein aggregates in PC12 cells. Consequently, LIUS stimulation resulted in a better cell survival than that of the neurotoxin-treated and non-LIUS-stimulated PC12 cells.

Key Words: Low intensity ultrasound (LIUS); 1-methyl-4-phenylpyridinium ion (MPP^+); α-Synuclein; Parkinson's disease (PD); mitochondrial dysfunction; and reactive oxygen species (ROS).

PO-10

The maintenance ability and Ca^{2+} availability of skeletal muscle are enhanced by sildenafil

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Sildenafil relaxes vascular smooth muscle cells, and is used to treat pulmonary artery hypertension as well as erectile dysfunction. However, the effectiveness of sildenafil on skeletal muscle and the benefit of its clinical use have been controversial, and most studies have focused primarily on tissues and organs from disease models, without cellular examinations. Here, the effects of sildenafil on skeletal muscle at the cellular level were examined using mouse primary skeletal myoblasts (the proliferative forms of skeletal muscle stem cells) and myotubes along with single- cell Ca^{2+} imaging experiments and cellular and biochemical examinations. The proliferation of the skeletal myoblasts was enhanced by sildenafil, without dose-dependency. In the skeletal myotubes, sildenafil enhances the activity of ryanodine receptor 1, an internal Ca^{2+} channel, and Ca^{2+} movements that promote skeletal muscle contraction, possibly due to an increase in the resting cytosolic Ca^{2+} level and a unique microscopic shape in the myotube membranes. Therefore, these results suggest that the maintenance ability of skeletal muscle mass and the contractility of skeletal muscle could be improved by sildenafil via enhancing the proliferation of skeletal myoblasts and increasing the Ca^{2+} availability of skeletal myotubes, respectively.

Key Words: skeletal muscle, myoblast, skeletal muscle stem cell, myotube, proliferation

Young Physiologist Award Lecture

Astrocytes-mediated synaptic rewiring in the primary somatosensory cortex: A novel mechanism for peripheral neuropathic pain

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Chronic neuropathic pain following peripheral nerve injury is characterized by mechanical allodynia, a painful response to innocuous tactile stimulation. Although this pain is known to be induced by glial activation and altered nociceptive transmission within the spinal cord, an effective treatment is still insufficient, indicating that novel therapeutic targets are critically needed. One such target may be the synaptic rewiring in the primary somatosensory (S1) cortex that is highly associated with the severity of neuropathic mechanical allodynia. However, the causal relationship of S1 synaptic rewiring to mechanical allodynia and its underlying cellular mechanisms remain unknown. Furthermore, whether glial cells contribute to S1 synaptic plasticity is still unclear. Using *in vivo* two-photon microscopy imaging with genetic and pharmacological manipulations, here we show that partial sciatic nerve ligation (PSL) injury induces an early re-emergence of immature metabotropic glutamate receptor 5 (mGluR5) signaling in S1 astrocytes, which elicits spontaneous somatic Ca^{2+} transients, thrombospondin-1 release and synapse formation. Such activation of S1 astrocytes was evident only during a critical period (~1w post-injury), correlating with the temporal changes in S1 extracellular glutamate levels and dendritic spine turnover following PSL injury. Blocking this astrocytic signaling pathway suppressed mechanical allodynia, while activating this pathway in the absence of injury induced long-lasting (>1 month) allodynia. Thus, these synaptogenic astrocytes are a key trigger for S1 synaptic circuit rewiring that mediates neuropathic pain mechanical allodynia.

Key Words: Astrocytes, Mechanical allodynia, Neuropathic pain, Peripheral nerve injury, Primary somatosensory cortex, Synaptic rewiring

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Plenary Lecture

Renal renin producing cells: motors of the renin-angiotensin system

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The proteolytic renin- angiotensin (RAS) cascade exerts multiple physiological and pathophysiological effects including control of sodium homeostasis, blood pressure regulation, inflammation and organ fibrosis. RAS activity is driven by renin, which in the adult organism is almost exclusively produced by the kidneys. Although there is accumulating evidence that in certain stress situations also cells of the distal tubule and collecting duct can express renin, the predominant and physiological relevant intrarenal site of renin production and secretion in the adult kidney are the socalled juxtaglomerular cells. The cells develop as pericytes from the metanephric stromal precursors. After kidney maturation renin expressing cells are restricted to the walls of afferent arterioles at the transition zone of afferent arterioles into the glomerular capillaries. During persisting challenges of the RAS by falls of blood pressure or sodium deficiency additional renin expressing cells in afferent arterioles can be recruited in a retrograde fashion resembling the fetal renin expression pattern. Renin cells form gap junctions which are constituted by connexin 40 and which connect the cells among each other but also with neighbored cells such as endothelial and extraglomerular mesangial cells. The renin gene transcript encodes for prorenin, which after glycosylation in the Golgi apparatus is transferred to big lysosome related vesicles. Therein prorenin is cleaved to renin. Renin is released from the cells by a mechanism resembling compound exocytosis triggered by the cAMP-PKA signaling pathway, which is activated by neurotransmitters from sympathetic nerves or by prostaglandins. Also a fall of the renal perfusion pressure leads to an increased release of renin. Conversely, an increase of renal perfusion pressure as well as number of vasoconstrictors including angiotensin II or endothelins inhibit the secretion of renin. The inhibitory mechanisms of renin secretion are still not well understood. Rather mysterious in this context is the unusual effect of extracellular calcium on renin secretion. It is of note that both the effect of extracellular calcium and the effect of perfusion pressure on renin secretion are strictly dependent on the function of connexin 40. These aspects will be covered in more detail in the lecture.

CURRICULUM VITAE

Name: Armin Kurtz, MD

Education

1974-1980	study of medicine at universities of Regensburg and Munich, Germany
1980	medical exam

Training

1982	MD thesis „Functional properties of isolated hemoglobin subunits" at University of Regensburg
1981-1984	Research fellow and Postdoc at the Institute of Physiology of University of Regensburg, Germany
1984-1987	Postdoc in the lab of Christian Bauer at the Institute of Physiology of University of Zurich, Swtzld
1988	Postdoc in the lab of Erwin Neher at the Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany
1988	Habilitation for Physiology at the University of Zürich

Career

1989-1991	Lecturer, Institute of Physiology University of Zürich, Swtzld
since 1991	Full Professor and Chair of Physiology, University of Regensburg, Germany
1999-2001	Dean of the Faculty of Biology and Preclinical Medicine, University of Regensburg
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Honors and merits

1982	Bavarian award for excellent MD thesis (EoN-Prize)
1987	Volhard- Prize of German Society of Nephrology
1988	research fellowship of Max-Planck-Society
2004-2009	President of German Society of Nephrology
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2006-2017	organizer and spokesman of the DFG funded collaborative research center "structural, functional and molecular determinants of the kidney"
2007-2016	Member of the DFG study section Physiology
2010	Franz Gross Prize of German Hypertension Society
2010-2011	President of German Physiological Society
2013	Franz Volhard Medal of German Society of Nephrology
since 2016	Senator of German Academy of Science

Editorial boards

European Journal of Physiology (since 2106 editor in chief)
American Journal of Physiology
Hypertension
Kidney and Blood Pressure Research
Nephron

Fields of research

Kidney function, blood pressure regulation, RAAS, erythropoietin
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S-I-1

Phospholipase Cγ1 abnormality for neuropsychiatric disorders

Joung Hun Kim

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Bipolar disorder (BD) is a heritable, but genetically heterogeneous mental illness characterized by alternating episodes of mania and depression. Several lines of evidence suggest an association between BD and the gene encoding phospholipase Cγ1 (*PLCG1*), but its etiological bases remain unknown. We here report that mice lacking PLCγ1 expression in forebrain pyramidal neurons (*Plcg1*$^{f/f}$*;CaMKII*) exhibit hyperactivity, decreased anxiety, hyperphagia, impaired learning and memory, and exaggerated startle responses. *Plcg1*-deficient CA1 pyramidal neurons display a significantly lower frequency of inhibitory postsynaptic potentials than wild-type neurons because of a reduced number of GABAergic boutons, which may result from impaired localization and/or stabilization of postsynaptic CaMKII at inhibitory synapses. Moreover, these mice display impaired brain-derived neurotrophic factor–TrkB-dependent synaptic plasticity, which could account for deficits of spatial memory. Lithium and valproate, the drugs currently used to treat BD, rescue the hyperactive phenotypes of *Plcg1*$^{f/f}$*;CaMKII* mice. These findings provide evidence that PLCγ1 is critical for synaptic functions and plasticity, and that loss of PLCγ1 in forebrain pyramidal neurons results in manic-like behavior.

S-I-2

Dysfunction of GABAergic transmission in the emotional disorder

Sun Seek Min

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Evidences indicate that dysfunction of GABAergic transmission is involved in the emotional disorders. Recently we have demonstrated that that adolescent mice show anxiety- and aggressive-like behavior and reduction of long-term potentiation (LTP) in mossy fiber-CA3 synapses after neonatal maternal separation (MS). The present study investigated the mechanism of the reduction of LTP in mossy fiber-CA3 synapses after neonatal MS. After MS procedure for 19 days, we measured excitatory postsynaptic current (EPSC) and inhibitory postsynaptic current (IPSC) in hippocampal granule cell. As results, EPSC and intrinsic membrane property did not significantly different between handling group and MS group. However, neonatal MS showed significantly elevated GABAA receptor-mediated currents. We also measured physiological roles of receptor protein tyrosine phosphatase receptor T (PTPRT). Both PTPRT null and mutant mice showed significantly increased depressive behavior. PTPRT mutant mice showed a decreased GABA level in the hippocampus. In addition the frequency and amplitude of IPSC in the hippocampal pyramidal cell were decreased in the PTPRT null and mutant mice. These observations suggest that dysfunction of GABAergic neurotransmission induces emotional disorders such as depression and aggression. This research was supported by Basic Science Research foundation of Korea (NRF) funded by the Ministry of Education (2012R1A1A2008796 and 2015R1D1A1A01061326).

Key Words: depression, maternal separation, aggression, synaptic plasticity, hippocampus

S-I-3

Epigenetic basis of Bdnf gene suppression by drugs of abuse in the ventral tegmental area

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Brain-derived neurotrophic factor (BDNF) plays a crucial role in modulating neural and behavioral plasticity to drugs of abuse. Here, we demonstrate a persistent down-regulation of exon-specific *Bdnf* expression in the ventral tegmental area (VTA) in response to chronic opiate exposure, which is mediated by specific epigenetic modifications at the corresponding *Bdnf* gene promoters. Exposure to chronic morphine increases stalling of RNA polymerase II at these *Bdnf* promoters in VTA and alters permissive and repressive histone modifications and occupancy of their regulatory proteins at the specific promoters. Furthermore, we show that morphine suppresses binding of phospho-CREB (cAMP response element binding protein) to *Bdnf* promoters in VTA, which results from enrichment of trimethylated H3K27 at the promoters. These studies reveal novel epigenetic mechanisms of morphine-induced molecular and behavioral neuroadaptations.

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Key Words: Epigenetics, BDNF, ventral tegmental area, opiate, histone modification

S-I-4

NMDA receptor dysfunction and autism spectrum disorders

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Various synaptic proteins contribute to molecular organization of synapse development and function. For instance, synaptic adhesion molecules contribute to the modulation of trans-synaptic adhesion and synapse development. In addition, synaptic scaffolding proteins interact with receptors and signaling molecules in order to couple receptor activation with downstream signaling events. A growing number of synaptic proteins have recently been associated with diverse neuropsychiatric disorders, including autism spectrum disorders (ASDs), schizophrenia, attention deficit hyperactivity disorder, and mood disorders. In this presentation, I will discuss how defects in some of the excitatory synaptic proteins are associated with NMDA receptor dysfunctions and autistic-like and other behavioral abnormalities in mice.

S-II-1

Hypothalamic SF-1 regulates energy homeostasis and psychiatric behaviors

Ann W. Kinyua, Dong Joo Yang, Dong-Whee Son, My Khanh Q. Huynh, Jae-Won Choi, Chang Mann Ko, Ki Woo Kim

Department of Pharmacology and Global Medical Science, Wonju College of Medicine, Yonsei University, Korea

The ventral medial nucleus of the hypothalamus (VMH), among several hypothalamic nuclei, emerged recently as an important site for mediating body weight homeostasis and psychiatric behaviors. This lecture will be focused on the emerging homeostatic roles of the VMH, particularly highlighting the control of energy metabolism and psychiatric behaviors in the SF-1 neurons of the VMH. Furthermore, we will illustrate functional roles of transcription factors including SF-1 and FoxO1 and circulating factors such as insulin and leptin in the VMH. Finally, we will discuss the underlying molecular mechanisms responsible for the regulation of energy homeostasis and psychiatric behaviors in the VMH. **Acknowledgement:** NRF grants (2014K1A3A1A19066980 and 2016R1C1B3012748)
Key Words: Ventromedial nucleus of hypothalamus (VMH), Steroidogenic factor 1, Energy homeostasis, Nutrient condition, Anxiety

S-II-2

Nutrient availability in astrocytes is linked to whole body energy metabolism

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Astrocytes are the most abundant cells in the central nervous system, yet they have often been relegated to a less than prominent role in the control of complex brain functions supported by neuronal circuits. The regulation of food intake and energy expenditure is tightly linked to the hypothalamic neural circuits. However, the direct contribution of astrocytes in this system is ill defined. Nutrients availability in the brain is crucial for neuronal functions in which astrocytes have also been deeply implicated. In this study, we identified active roles of hypothalamic astrocytes as a sensor of nutrient concentration and a primary modulator to maintain the whole body energy metabolism. We found that the impairment of nutrient shuttling from the blood vessel to the neurons led to the abnormality of energy intake and glucose production utilizing genetic mouse model in which peroxisome proliferator-activated receptor gamma (PPAR gamma) genes are time-specifically ablated in astrocytes. In line of these findings, we also confirmed the direct involvement of hypothalamic astrocytes in the pathogenesis of obesity. Collectively, we argue an important role of hypothalamic astrocytes in the regulation of the whole body energy homeostasis. **Acknowledgement:** NRF grant (2015R1C1A1A02037802)
Key Words: Energy metabolism, Astrocyte, PPAR gamma, Hypothalamus

S-II-3

Divergence of food intake regulation by brain serotonin circuit

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Coordinated control of food intake is essential for survival. Clearly, a better understanding of the complex regulation of feeding behavior is essential for the development of rational strategies to combat obesity and various eating disorders. One notable advance in this regard is the identification of a critical role for the serotonin 2C receptor (*Htr2c*) in regulating food intake and body weight. The therapeutic potential of this pathway was first highlighted by the demonstration that the anorexigenic action of d-Fenfluramine was mediated, at least in part, through *Htr2cs*. Subsequently, lorcaserin (Belviq^{TM}), an *Htr2c* specific agonist, recently became one of the first FDA-approved weight-loss drugs after a long span of 15 years. Given the potential popularity (and potential misuse) of this new anti-obesity drug, it is imperative to understand how selective *Htr2c* agonists act in the brain to suppress food intake and lower body weight. In this lecture, cellular mechanisms that underlie divergent effects of *Htr2c* agonists on distinct hypothalamic neuronal populations as well as their metabolic/physiological relevance will be discussed. **Acknowledgement:** NRF grants (2015M3A9E7029177 and 2016R1C1B2006614)
Key Words: Serotonin, Feeding, Brain, Divergence, Patch Clamp Technique

S-II-4

Interplay between glucose and leptin signalling determines the strength of GABAergic synapses at POMC neurons

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Regulation of GABAergic inhibitory inputs and alterations in POMC neuron activity by nutrients and adiposity signals regulate energy and glucose homeostasis. Thus, understanding how POMC neurons integrate these two signal molecules at the synaptic level is important. Here we show that leptin's action on GABA release to POMC neurons is influenced by glucose levels. Leptin stimulates the JAK2-PI3K pathway in both presynaptic GABAergic terminals and postsynaptic POMC neurons. Inhibition of AMPK activity in presynaptic terminals decreases GABA release at 10 mM glucose. However, postsynaptic TRPC channel opening by the PI3K-PLC signalling pathway in POMC neurons enhances spontaneous GABA release via activation of presynaptic MC3/4 and mGlu receptors at 2.5 mM glucose. High-fat feeding blunts AMPK-dependent presynaptic inhibition, whereas PLC-mediated GABAergic feedback inhibition remains responsive to leptin. Our data indicate that the interplay between glucose and leptin signalling in glutamatergic POMC neurons is critical for determining the strength of inhibitory tone towards POMC neurons.
Key Words: Hypothalamus, POMC, Leptin

S-III-1

A new aspect of SOCE in invertebrates

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Store-operated calcium (SOC) channels are the major routes of Ca^{2+} entry (SOCE) in literally all eukaryotic cells including vertebrates and invertebrates. STIM1 is an ER luminal Ca^{2+} sensor and an activator of SOCE. It translocates to ER-PM junctions and binds to Orai channel proteins known as SOC channel pore subunit. A sequence of events leads to SOC (also known as Orai1 or CRAC) channel activation. However, very little is known about how distinct cells coordinate the activity of SOCs (SOCE) to generate coherent calcium signals in different eukaryotic cells. Here, we will show a new and unexpected mechanism by which invertebrate SOCE is regulated and show many distinguishing features of SOC channels compared to that of vertebrate SOCs. STIM1, the main regulator of SOCE, binds to different cytosolic portion of Orai1, the main SOC pore subunit, through its CRAC activation domain (CAD). The binding domain within Orai1 establishes a molecular mechanism by which STIM1 regulates Orai1 in invertebrate which is distinct to that in vertebrate and provides an insight into invertebrate SOC complex will be useful for basic research and can be developed further to generate a new generation of anti-insect agents for use in the clinic.
Key Words: Calcium Signaling, invertebrate SOCE, STIM1, Orai1.

S-III-2

Does Ca^{2+} influx through CD20 determine the efficacy of anti-CD20 antibodies in B cell depletion?

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CD20 antibodies have been developed as a drug for non-hodgkin's lymphoma, almost 16 kinds of antibodies were used in clinic, at present. Recently, use of Rituxan to rheumatoid arthritis had been also approved by FDA. Considering the age-rising population, the usage of CD20 antibody can be dramatically increased in compare to the usage for non-hodgkin's lymphoma. In compare to the clinical study of CD20 antibodies, however, the basic molecular and interaction mechanisms between CD20 and its antibodies is not well known. Especially, CD20 is known to as a Ca^{2+} channel when expressed in heterologous expression system. Some reports demonstrated that Ca^{2+} influx through CD20 can be activated by binding of Rituxan, the most used anti-CD20 antibody. However, the correlation of CD20 pore function and various effects of Rituxan binding to B cell (raft localization of CD20, capping of CD20, and B cell deletion activity) were not fully investigated. Moreover, the newly improved CD20-antibodies, such as Obinutuzumab and Ofatumumab, have not been investigated in this view. In this talk, I will present the typical characteristics of CD20 as a Ca^{2+} pore. In addition, the Ca^{2+} influx of CD20 induced by three kinds of anti-CD20 antibodies will be compared. Above all, our core question--whether Ca^{2+} influx through CD20 can affect on the efficacy of B cell depleting activity of three anti-CD20 antibodies-- will be answered. This work was supported by a grant from the National Research Foundation of Korea (Project No. 2015R1D1A1A02062027)
Key Words: CD20, Ca^{2+} influx pore, Rituxan, Immune anti-cancer therapy, B cell

S-III-3

Interplay of β2e and Ca^{2+} promotes inactivation of CaV channels in a calmodulin-independent manner

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Voltage-gated calcium (Ca_v) channels, which are regulated by membrane potential, cytosolic Ca^{2+}, phosphorylation, and membrane phospholipids, govern Ca^{2+} entry into excitable cells. Ca_v channels contain a pore-forming α1 subunit, an auxiliary α2δ subunit, and a regulatory β subunit, each encoded by several genes in mammals. In addition to a domain that interacts with the α1 subunit, β2e and β2a also interact with the cytoplasmic face of the plasma membrane through an electrostatic interaction for β2e and posttranslational acylation for β2a. Here, we found that an increase in cytosolic Ca^{2+} promoted the release of β2e from the membrane without requiring substantial depletion of the anionic phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) from the plasma membrane. Experiments with liposomes indicated that Ca^{2+} disrupted the interaction of the β2e N-terminal peptide with membranes containing PIP2. Ca^{2+} binding to calmodulin (CaM) leads to CaM-mediated inactivation of Ca_v currents. Whereas $Ca_v2.2$ coexpressed with β2a required Ca^{2+}-dependent activation of CaM for Ca^{2+}-mediated reduction in channel activity, $Ca_v2.2$ coexpressed with β2e exhibited Ca^{2+}-dependent inactivation of the channel even in the presence of Ca^{2+}-insensitive CaM. Inducible depletion of PIP2 reduced $Ca_v2.2$ currents, and in cells coexpressing β2e, but not a form that lacking the polybasic region, increased intracellular Ca^{2+} further reduced $Ca_v2.2$ currents. Many hormone or neurotransmitter-activated receptors stimulate PIP2 hydrolysis and increase cytosolic Ca^{2+}, thus, our findings suggest that β2e may integrate such receptor-mediated signals to limit Ca_v activity.
Key Words: Voltage-gated calcium channel, β2e subunit, phosphatidylinositol 4, 5-bisphosphate, Calcium

S-III-4

Ca^{2+} signaling and pacemaking in midbrain dopamine neurons

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Midbrain dopamine (DA) neurons are slow intrinsic pacemakers that require the elaborate composition of many ion channels in the somatodendritic compartments. Understanding the pacemaker mechanisms of midbrain DA neurons is important, because the spontaneous firing rate determines the basal DA levels in target areas, including the striatum. In midbrain DA neurons Ca^{2+} is not only important for determining firing patterns, but also important for pacemaking. Since spontaneous firing and Ca^{2+} spikes occur synchronously at the soma and dendrites, the electrical coupling between the soma and dendritic compartments has been regarded as a key determinant for the pacemaking. However, it is not known how

the somatodendritic organization of pacemaker activity is functionally organized in DA neurons. In the rat substantia nigra pars compacta DA neurons, by measuring spontaneous Ca^{2+} oscillations and action potentials, we demonstrate that the soma is a stably-oscillating, large inertial compartment, but that the proximal dendritic compartments are a stochastically-fluctuating highly-excitable element. Nevertheless, the tight electrical-coupling with the inertial soma makes dendritic Ca^{2+} fluctuations synchronized with the stable somatic Ca^{2+} oscillations. The stochastic and energetic actions of proximal dendritic compartments are counteracted and balanced by the centrifugally connected inertial soma. Thus, the dynamic interaction between the stably-oscillating inertial soma and the stochastically-fluctuating excitable proximal dendritic compartments constitutes pacemaker activity of DA neurons This somatodendritic organization underlies firing fidelities and determines evoked firing patterns in midbrain DA neurons.
Key Words: Ca^{2+} signaling, midbrain dopamine neurons, pacemakers

S-IV-1

Tauroursodeoxycholic acid (TUDCA) attenuates pressure-overload induced cardiac remodeling by ameliorating endoplasmic reticulum stress (ERS)

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Prolonged pressure-overload in the heart induces pathological hypertrophy associated with malfunctions. Apoptosis and fibrosis initiated by ERS are known to contribute to those malfunctions. The aim of this study was to investigate whether reduction of ERS by a known chemical chaperone, TUDCA can attenuate pressure-overload cardiac remodeling in a mouse model of transverse aortic constriction (TAC). Oral administration of TUDCA at a dose of 300 mg/kg body weight (BW) in mouse TUDCA-TAC group reduced ERS markers (GRP78, p-PERK, p-elf2α), compared to Vehicle (Veh)-TAC group. TUDCA administration also diminished cardiac hypertrophy as shown by decreased heart weight (HW) to BW ratio, and increased expression of hypertrophic marker genes (ANF, BNP, α-SKA). Masson's trichrome staining showed that myocardial fibrosis and collagen deposition were also significantly reduced in TUDCA TAC group. We also found that TUDCA significantly decreased expression of TGFβ signaling proteins and collagen isoforms in TUDCA-TAC group. TUDCA also reduced cardiac apoptosis by affecting the signaling proteins in TUDCA TAC group. Microarray analysis followed by Gene Ontology (GO) and pathway analysis demonstrated that extracellular matrix genes and mitochondrial genes are mostly affected in Veh-TAC group, but the alterations were normalized in TUDCA-TAC group, suggesting that TUDCA is a potentially useful drug for curing pressure-overload related heart diseases.
Key Words: hypertrophy, cardiac remodeling, tauroursodeoxycholic acid

S-IV-2

The Matricellular Protein CCN5 Reverses Established Cardiac Fibrosis

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Cardiac fibrosis (CF) is associated with increased ventricular stiffness and diastolic dysfunction, and is an independent risk factor for heart failure. Previous studies in this laboratory have shown that the matricellular protein, CCN5, inhibits CF in a mouse model of heart failure. To further evaluate the effects of CCN5 on established CF, CF was induced with transverse aortic constriction for eight weeks, followed by adeno-associated virus-mediated transfer of CCN5. Established CF was reversed by CCN5 as revealed by the normalized trichrome staining and myofibroblast contents. Endothelial-mesenchymal transition (EndMT) and fibroblast-to-myofibroblast transdifferentiation were significantly inhibited by CCN5 both *in vivo* and *in vitro*. In addition, myofibroblast-specific apoptosis was induced by CCN5. Taken together, these data suggest that CCN5 reverses established CF through inhibition and clearance of myofibroblasts from the myocardium. Thus, CCN5 could be used for the development of novel anti-CF therapies.
Key Words: cardiac fibrosis, matricellular protein, CCN5

S-IV-3

Mechanisms of hypoxic vasoconstriction in rat femoral arteryProtein phosphatase 2A/heat shock protein 70 dynamically regulates phosphorylation of histone deacetylase 2 and its activity in cardiac hypertrophy

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Cardiac hypertrophy represents a form of global remodeling in response to increased hemodynamic demands. Histone deacetylase (HDAC) 2 plays a crucial role in the process. Recently, we demonstrated posttranslational modification-based regulation of HDAC2; however, the phosphatase for HDAC2 remained unclear. Here we identified protein phosphatase (PP) 2a and inducible heat shock protein (HSP) 70 as binding partners of Hdac2. Hypertrophic stresses led Ppp2ca to dissociate from Hdac2. Ppp2ca negatively regulated the hypertrophic response through Hdac2 S394 dephosphorylation and Hdac2 inactivation. Hsp70 was induced and bound to activated Hdac2, resulting in dissociation of the Ppp2ca-Hdac2 complex. Double transgenic mice expressing Ppp2ca and Hsp70 showed cardiac hypertrophy, implicating Hsp70 as an endogenous inhibitor of Ppp2ca. Our results suggest that basally HDAC2 forms a complex with PP2A and remains inactivated. Hypertrophic stresses result in PP2A detachment from HDAC2 for phosphorylation, which is maintained by binding of HSP70 during the development of cardiac hypertrophy.
Key Words: Cardiac hypertrophy, HDAC2, posttranslational modifications

S-IV-4

Function of junctional proteins in Ca^{2+} dynamics

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Orai1 and STIM1 mediate store-operated Ca^{2+} entry (SOCE) in immune cells. STIM1, an endoplasmic reticulum (ER) Ca^{2+} sensor detects store depletion and interacts with plasma membrane (PM)-resident Orai1 channels at the ER-PM junctions. However, the molecular composition of these junctions in T cells remains poorly understood. Here we show that junctophilin-4 (JP4), a member of junctional proteins in excitable cells, is expressed in T cells and localized at the ER-PM junctions to regulate Ca^{2+} signaling. Silencing or genetic manipulation of JP4 decreased ER Ca^{2+} content and SOCE in T cells, impaired activation of the NFAT (nuclear factor of activated T cells) and ERK (extracellular signaling-related kinase) signaling pathways, and diminished expression of activation markers and cytokines. Mechanistically, JP4 directly interacted with STIM1 via its cytoplasmic domain and facilitated its recruitment into the junctions. Accordingly, expression of this cytoplasmic fragment of JP4 inhibited SOCE. Furthermore, JP4 also formed a complex with junctate, a Ca^{2+}-sensing ER-resident protein, previously shown to mediate STIM1 recruitment into the junctions. We propose that junctate-JP4 complex located at the junctions cooperatively interacts with STIM1 to maintain ER Ca^{2+} homeostasis and mediate SOCE in T cells.
Key Words: Store-operated Ca^{2+} entry (SOCE), Endoplasmic reticulum-plasma membrane (ER-PM) junction, Junctophilin-4 (JP4)

S-V-1

Next generation optogenetics: tool development and applications

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My group has been investigating cellular processes in various cell types by combining conventional and novel bio-imaging technologies and optogenetic tools. Optogenetic strategies have brought significant changes the way in which signaling in living cells is studied in neurobiology and other disciplines. A familiar example is optogenetics based on channelrhodopsins, light-gated ion channels from *Chlamydomonas*, which have become the main means for controlling neural activity until recently. My group has been putting efforts in developing next generation optogenetic toolkits for controlling signaling proteins in neuronal cells and animals using plant photoreceptors. New optogenetic tools my group has established will be useful not only for imaging based researches in cell biology, but also for the studies in neuroscience. Our optogenetic toolkits combined with various bio-imaging technologies are capable of providing what channelrhodopsins could not offer previously, contributing in a disparate perspective of neuroscience. I will present in the talk our new technologies to the study of spatiotemporal roles of signaling proteins and second messengers in cell systems as well as in learning and memory in mouse models.
Key Words: bio-imaging, optogenetics, cell signaling, memory, cell migration

S-V-2

Application of optogenetics for treatments of urological disorders

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Lower urinary tract (LUT) function, regulated by a complex nervous system with muscular structures, is vulnerable to various disease and injuries. Current clinical approaches such as pharmacological and electrical treatments for LUT dysfunction lack spatial and temporal accuracy, resulting in suboptimal outcomes. In this study, we demonstrate that modulating the membrane potential of smooth muscle cells (SMCs) with optogenetics enabled us to control the contractile behaviors of the urinary bladder. We delivered optogenes to bladder SMCs using either the cre-loxp transgenic system or a viral transfection method. Using patch clamp and cystometry, we confirmed that depolarizing SMCs with Channelrhodopsin-2 (ChR2) induced bladder contraction, whereas hyperpolarizing SMCs with Halorhodopsin suppressed overactive contraction. The feasibility shown with a virally transfected ChR2-bladder model suggests the potential for future clinical applications in human patients. **Acknowledgements:** This research was supported by the National Agenda Project of the Korea National Research Council of Science & Technology (NAP-09-04-KIST to JKFS), the Institutional Grant from KIST (2E26220), and the National Research Foundation grant from the Korea government (MSIP) (NRF-2014R1A2A1A03074416 to TMK).
Key Words: channelrhodopsin, halorhodopsin, urinary bladder, smooth muscle, optogenetics

S-V-3

Optogenetic control of stress and mood disorders

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In response to chronic stress, some individuals develop maladaptive symptoms while others retain normal behavior. The functional hemispheric asymmetry of medial prefrontal cortex (mPFC) is known to be implicated in control of stress responses. Despite the potential importance of mPFC, the contribution of each hemisphere of mPFC in mediating stress resilience remains unclear. To elucidate the specific role of each hemispheres, we investigated the neural activity and gene expression profiles of mPFC in social defeat stress model. In the left mPFC, mice expressing social avoidance showed depressed neural activity, while resilient mice showed normal firing rates. The neural activity stimulation of left mPFC optogenically leads to social behavior change. The same photomodulation has no effect on the right mPFC. From the analysis of microarray data in each mPFC hemispheres, we discovered that the genetic changes in the left cortices determines the adaptive behavior upon social defeat stress. Evidenced together with the neural activity, we conclude that it is the activity of left mPFC plays critical role in behavioral expression of stressed mice.
Key Words: optogenetics, stress, mood disorder

S-V-4

Optogenetic dissection of neurons in the basal forebrain

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The basal forebrain (BF) is a final node of arousal pathway from brain stem to cortex. Understanding the function of the basal forebrain (BF) poses a challenge due to the intermingled presence of cholinergic, GABAergic, and glutamatergic neurons. All three BF neuronal subtypes project to the cortex and are implicated in cortical function and sleep-wake control. Optogenetic stimulation of parvalbumin (PV)-positive neurons in BF enhances cortical gamma band oscillations in the cortex. On the other hand optogenetic inhibition of BF PV neurons reduced GBO generated by auditory steady state response (ASSR). Therefore, we concluded that BF PV neurons control GBO. These results are surprising and novel in indicating that this presumptively inhibitory BF PV input controls cortical GBO, likely by synchronizing the activity of cortical PV interneurons. BF PV neurons may represent a previously unidentified therapeutic target to treat disorders involving abnormal GBO, such as schizophrenia. On the other hand, recent studies using optogenetics have shown that "selective" stimulation of BF cholinergic neurons increases transitions between NREM sleep and wakefulness, implicating cholinergic projections to cortex in wake promotion. However, the interpretation of these optogenetic experiments is complicated by interactions that may occur within the BF. For instance, a recent in vitro study from our group found that cholinergic neurons strongly excite neighboring PV/GABAergic neurons (Yang et al., 2014). Thus, the wake-promoting effect of optogenetic stimulation of BF cholinergic neurons could be mediated by local excitation of GABA/PV or other non-cholinergic BF neurons. Using a newly designed "opto-dialysis" probe to couple selective optical stimulation with simultaneous in vivo microdialysis, we demonstrated that optical stimulation of cholinergic neurons locally increased acetylcholine levels and increased wakefulness in mice. The wake-promoting effect caused by cholinergic stimulation was abolished by simultaneous reverse microdialysis of cholinergic receptor antagonists into BF. We found that cholinergic stimulation in the BF promotes wakefulness through local release of acetylcholine in BF and susquent activation of cortically projecting, non-cholinergic neurons, including the GABAergic/PV neurons.
Key Words: optogenetics, basal forebrain, opto-dialysis, wakefulness, cholinergic neuron

S-VI-1

Properties of waxing and waning in the mouse small intestine

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Small intestine exhibits a unique motility pattern called segmentation which promotes absorption of nutrients and water. Waxing and waning has been thought to be a spontaneous electrical activity behind segmental activity. Recently, it was proposed that waxing and waning is triggered by lower frequency rhythmic transient depolarizations generated by ICC associated with the deep muscular plexus (ICC-DMP). However, no cellular or multicellular rhythmic activity is observed in ICC-DMP. Therefore, mechanisms of the generation of waxing and waning have not been understood. We studied the electrical properties of circular smooth muscle cells (CSMC) and myenteric interstitial cells of Cajal (ICC-MY) with mucosa on or off preparations isolated from the mouse small intestine using intracellular recording. Waxing and waning was not detected in the preparations without mucosa. In contrast, waxing and waning was recorded spontaneously from both CSMC and ICC-MY in the preparations with mucosa on. Waxing and waning was inhibited by diclofenac, a non-selective COX inhibitor, or NS398, a COX2 inhibitor. In the presence of diclofenac, cloprostenol, an analog of PGF_2alpha, regenerated waxing and waning. Waxing and waning was also inhibited by NPPB, a Ca^{2+} - activated Cl^- channel inhibitor, and bumetanide, a Na^+ - K^+ - $2Cl^-$ co-transporter (NKCC1) inhibitor. Waxing and waning was not affected by TTX. These results suggest that prostanoids such as PGF_2alpha released from mucosa or submucosa may activate Ca^{2+} - activated Cl^- channels in ICC-MY, which results in the generation of waxing and waning in the mouse small intestine .

Key Words: Interstitial cells of Cajal; Prostanoid; Ca^{2+} - activated Cl^- channels; Small intestine

S-VI-2

Automaticity of interstitial cells of Cajal: A computational study

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The automaticity of interstitial cells of Cajal (ICCs) is believed to be a key mechanism responsible for the rhythmic contraction of gastro-intestinal tracts. The automaticity is generated and maintained by a coupled activity between the intracellular Ca^{2+} oscillation and Ca^{2+}-dependent activation of membrane ion channels in the ICCs. The periodic Ca^{2+} oscillation is mainly driven by stochastic opening of inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} channel and ensuing reuptake of Ca^{2+} by endoplasmic reticulum Ca^{2+} pump. Incorporation of the random opening and closing of individual IP_3-mediated Ca^{2+} channel into our mathematical model of ICCs successfully reproduced stochastic features of pacemaker potential such as random events of unitary potential-like depolarization and irregular length of plateau. As for the Ca^{2+}-dependent membrane ion channels, there is still a controversy on which channel plays the role of pacemaker current. Accumulating evidence indicates that the Ano1 is the most probable membrane ion channel which transduces Ca^{2+} oscillation into the pacemaker activity. As the Ano1 is Cl^--selective, persistent activation of Ano1 should result in Cl^- loss which subsequently disrupts Cl^- equilibrium and pacemaker activity. We hypothesized that the Na-K-Cl cotransporter (NKCC) recovers the Cl^- loss to maintain pacemaker activity. Indeed, the block of NKCC in ICCs not only disrupted the Cl^- equilibrium but also terminated the pacemaker activity. Time courses of deterioration in pacemaker activity in response to block of NKCC were similar between experiment and model simulation. In addition to ICCs, the fibroblast-like PDGFα+ cells were recently identified in the functional syncytium of gut. They are believed to mediate neural input signal from enteric nervous system to smooth muscle cells although they are rather inhibitory than excitatory on muscle contraction. A simple biophysical model of $PDGF\alpha^+$ cells are also presented.

Key Words: ICCs, $PDGF\alpha^+$ cells, NKCC, Ano1, pacemaker activity

S-VI-3

Hyperpolarization-activated cyclic nucleotide-gated channels are working as a pacemaker channels in colonic interstitial cells of Cajal

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The function and regulation of gastrointestinal (GI) motility is a complicated process involving communication of many cell types such as smooth muscle cells, interstitial cells of Cajal (ICCs), and enteric nerve cells. Recent research with advanced techniques made a better understanding of ICC function, as pacemaker cell, transduce inputs from enteric neurons and so on in GI tract. However, the exact mechanism of pacemaker activity is in dispute in ICCs. In here, we suggest hyperpolarization-activated cyclic nucleotide gate (HCN) channel can be a crucial element for generating electrical pacemaker activity in ICCs. To testify our suggestion, we took steps as following.

1. The existence of HCN channel in colonic ICCs

With immunohistochemistry technique and Anoctamin-1 (ANO1) and HCN antibodies, we could identify the existence of HCN1 and HCN3 channels in ANO1 positive cells within muscular and myenteric layers in colonic tissues.

2. The action of drugs related with HCN channel in colonic ICCs

With patch clamp and intracellular recording, we found that a HCN channel blockers (ivabradine, ZD7288 and zatebradine) and an adenylated cyclase blocker (SQ-22536) blocked the spontaneous electrical pacemaker activity in colonic cultured ICCs and inhibited the generation of slow waves in colon intact tissues. On the contrary to this, a cAMP-dependent phosphodiesterase inhibitor and cell permeable 8-bromo cAMP elevated them.

3. The reconfirm of HCN channel involvement in colonic ICCs

With siRNA about HCN1 and HCN3, we verified that the frequency or generation of electrical pacemaker activity was reduced after silencing HCN1 and HCN3 in colonic cultured ICCs.

Although many reports are advising ANO1 channel is an important for generating pacemaker activity of ICCs recently, our results also suggest HCN role on ICCs function. Further studies are needed to understand the interacting mechanism between HCN and ANO1 channel to clarify the mechanism of spontaneous pacemaker activity in ICCs.

Key Words: Interstitial cells of Cajal; Pacemaker activity; Hyperpolarization-activated cyclic nucleotide gate channel; Gastrointestinal motility

S-VI-4

The Role of Korean Medicines in GI motility

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From ancient to modern history, traditional plant-based medicines have played an important role in health care, especially gastrointestinal (GI) tract. Korean medicine may be an attractive alternative based on the perception of its 'natural' approach and low risk of side effects. However, while extensively studied and widely used in Asia, there is a paucity of data upon which physicians in other parts of the world may draw conclusions regarding the effectiveness of herbal medicine for gastrointestinal disorders. Also, the lack of standardization of drug components has limited the ability to perform rigorous clinical studies in Western countries. We investigated the effects of Korean medicines on murine intestinal interstitial cells of Cajal (ICC), pacemaker cells in GI tract *in vitro* and GI motility *in vivo*. Many Korean medicines modulated the pacemaker activities of the ICC and the GI motility. These results suggest that the ICC can be targets for Korean medicines and their interaction can affect intestinal motility. Also, Korean medicines in the field of GI disorders may have the potential to serve a valuable role in the management of patients with functional GI disorders.
Key Words: Interstitial cells of Cajal, Gastrointestinal tract, Korean medicine, Functional GI disorders.

S-VII-1

Cortical parvalbumin inhibitory interneurons and Homeostatic dysfunction in Schizophrenia model

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NMDA receptor (NMDAR) hypofunction in parvalbumin-positive inhibitory interneurons (PV-IN) may underlie the pathogenesis of psychiatric diseases such as autism and schizophrenia by excitation-inhibition (E/I) imbalance. However, cellular mechanism of cognitive dysfunction by NMDAR hypofunction in PV-IN is still unclear. It remains uncertain how chronic NMDAR hypofunction in PV-IN leads to E/I imbalance at basal as well as evoked synaptic strength. Here, we applied not only in vitro system with primary cortical culture pre-treated with the NMDAR antagonist, mk-801 but also in vivo mouse model with systemically repeated injection of mk-801 showing several cognitive dysfunctions including hyperlocomotion, impaired working memory, social deficit, and disrupted paired-pulse inhibition (PPI). In in vitro primary cortical culture system and medial prefrontal cortex (mPFC) in our in vivo mouse model, chronic NMDAR hypofunction in PV-INs induced AMPA receptor (AMPAR) downregulation, while NMDAR and AMPAR in pyramidal neurons (PY) were not affected. Moreover, alteration of synaptic strength in PV-IN is highly correlated with mPFC dependent cognitive function, working memory. The reduced synaptic strength of PV-IN caused disinhibition onto PY and increased E/I ratio and EPSP summation on PY, resulting in hyper-excitation in mPFC. This also disturbed synaptic plasticity of PY in mPFC. Furthermore, disruption of PV-IN synaptic strengths decreased not only parvalbumin but also phosphorylated protein kinase A (PKA) protein expression in PV-IN. This reduced AMPAR strength in PV-IN was recovered by microinjection of PKA activator, forskolin into mPFC, giving rise to improved working memory and PPI. Together, these results indicate that chronic NMDAR hypofunction in PV-IN is associated with downregulation of AMPAR through PKA inactivation, which leads to E/I imbalance in mPFC by disinhibition onto PY, conferring psychiatric symptoms.
Key Words: NMDAR hypofunction, Homeostatic synaptic plasticity, E/I balance, Parvalbumin-positive inhibitor interneuron, Medial prefrontal cortex, Psychiatric symptoms

S-VII-2

Regrowth of serotonin axons in the adult mouse brain following injury

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It is widely believed that damaged axons in the adult mammalian brain have little capacity to regrow, thereby impeding functional recovery after injury. Studies using fixed tissue have suggested that serotonin neurons might be a notable exception, but they remain inconclusive. To address these issues, we have employed *in vivo* two-photon microscopy to produce time-lapse images of serotonin axons in the neocortex of the adult mouse. Serotonin axons undergo massive retrograde degeneration following amphetamine treatment and the subsequent slow recovery of axonal density is dominated by new growth with little contribution from local sprouting. Similarly, a stab injury that transects serotonin axons running in the neocortex is followed by local regression of cut serotonin axons and followed by their regrowth into and across the stab rift zone, continuing for several months. Unlike regrowing axons in the peripheral nervous system, serotonin axons do not follow the pathways left by degenerated axons. The regrown axons release serotonin and their regrowth is correlated with recovery in behavioral tests. Thus, serotonin axons in the brain have an unusual capacity for regrowth and this regrowth is functional and approximates the pre-lesion state.
Key Words: Serotonin, 5HT, Axon Regrowth, Degeneration, CNS Injury

S-VII-3

Whole-cell recordings in freely moving rodents

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The brain controls all of our body's functions including mental processes such as learning and memory. Information processing within the brain is carried out by an enormous number of neurons that are wired together and communicate with each other by sending and receiving electrical signals. Within such a network, each neuron receives thousands of synaptic inputs from other neurons and processes these inputs through various computational mechanisms within the neuron to determine whether it generates outputs or not. Therefore, understanding information processing inside neurons in an awake, working brain is a fundamental step for linking cellular level computations to higher level brain functions. In this talk I will present recent advances in whole-cell intracellular recording techniques that can be applied to animals engaged in free behavior, thus allowing precise measurement and manipulation of electrical signals within a single neuron in the fully functioning brain. I will also present how these techniques have been applied to study the cellular mechanisms of the spatially tuned spiking activity of CA1 neurons ("place cells") in the hippocampus, a critical brain structure for spatial information processing and memory. This work is supported by the Institute for Basic Science and the Howard Hughes Medical Institute, US.
Key Words: Hippocampus, Place cell, Memory, Whole-cell recording

S-VII-4

Astrocytes increase the activity of synaptic GluN2B NMDA receptors

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Astrocytes regulate excitatory synapse formation and surface expression of glutamate AMPA receptors (AMPARs) during development. Less is known about glial modulation of glutamate NMDA receptors (NMDARs), which mediate synaptic plasticity and regulate neuronal survival in a subunit- and subcellular localization-dependent manner. Using primary hippocampal cultures with mature synapses, we found that the density of NMDA-evoked whole-cell currents was approximately twice as large in neurons cultured in the presence of glia compared to neurons cultured alone. The glial effect was mediated by (an) astrocyte-secreted soluble factor(s), was Mg2C and voltage independent, and could not be explained by a significant change in the synaptic density. Instead, we found that the peak amplitudes of total and NMDAR miniature excitatory postsynaptic currents (mEPSCs), but not AMPAR mEPSCs, were significantly larger in mixed than neuronal cultures, resulting in a decreased synaptic AMPAR/NMDAR ratio. Astrocytic modulation was restricted to synaptic NMDARs that contain the GluN2B subunit, did not involve an increase in the cell surface expression of NMDAR subunits, and was mediated by protein kinase C (PKC). Taken together, our findings indicate that astrocyte-secreted soluble factor(s) can fine-tune synaptic NMDAR activity through the PKC-mediated regulation of GluN2B NMDAR channels already localized at postsynaptic sites, presumably on a rapid time scale. Given that physiologic activation of synaptic NMDARs is neuroprotective and that an increase in the synaptic GluN2B current is associated with improved learning and memory, the astrocyte-induced potentiation of synaptic GluN2B receptor activity is likely to enhance cognitive function while simultaneously strengthening neuroprotective signaling pathways.
Key Words: NMDA receptor, GluN2B subunit, astrocytes, synapses, protein kinase C

P01-01

Rg3-enriched Korean Red Ginseng improves stability of blood pressure in spontaneously hypertensive rats

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Korean Red Ginseng (*Panax ginseng*) has been shown to exert anti-hypertensive effect. In particular, ginsenoside Rg3 is thought to be a potent modulator of vascular function. In this study, we examined the anti-hypertensive efficacy of Korean Red Ginseng (KRG) extract and Rg3-enriched Korean Red Ginseng (REKRG) extract. REKRG treatment significantly decreased systolic blood pressure (SBP) and diastolic blood pressure (DBP) 3 hours post-treatment in spontaneously hypertensive rats (SHR) compared to SHR control. However, SBP and DBP were not significantly different in KRG treated SHR compared to SHR control. Interestingly, REKRG treatment did not significantly change SBP and DBP 3 hours post-treatment in Wistar Kyoto Rats (WKY) compared to WKY control. Similarly, there was no difference with KRG treatment in SBP and DBP in WKY and WKY control. Both KRG and REKRG increased eNOS phosphorylation levels in aorta and the increase of eNOS phosphorylation levels by REKRG treatment is higher than TKRG treatment. Similarly, nitric oxide (NO) production in plasma from WKY and SHR also was increased in both of KRG and REKRG. Taken together, these results suggest that REKRG has more beneficial effect on blood pressure control than KRG in SHR. Therefore, REKRG could be critical for stabilizing blood pressure.
Key Words: Rg3-enriched Korean Red Ginseng, eNOS, SHR, Nitric oxide, blood pressure

P01-02

Prunella vulgaris attenuates diabetic nephropathy by suppressing renal fibrosis and inflammation

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Prunella vulgaris, well-known traditional medicinal plant, is used for the cure of abscess, scrofula, hypertension and urinary diseases.The present study was designed to investigate the beneficial effects of aqueous extract of *Prunellae vulgaris* (APV) on high glucose (HG)-promoted human mesangial cell and STZ-induced diabetic rats.Pretreatment of APV (1-50 μg/ml) attenuated 25mMHG-induced inflammatory marker such as intracellular cell adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1). Additionally, APV suppressed transforming growth factor β1 (TGF-β1) and Smad-2/4 expression, whereas increased Smad-7 expression level. Connective tissue growth factor (CTGF) and collagen IV, fibrosis biomarkers, were significantly decreased byAPV. Moreover, APV inhibited activation and translocation of nuclear factor kappa B (NF-κB) in HG-stimulated mesangial cell. Sprague-Dawley rats were divided into five groups: control, STZ and STZ were fed on APV (100 or 300 mg/kg/day) for 8 weeks respectively. The renal tissue in the APV group exhibited clear signs of inflammation compared with the control group. Additionally, APV increased nephrin expression in STZ-induced diabetic kidney. Glomerulus TGF-β1expression in immunohistochemical staining was significantly decreased by APV.These findings suggest that APV have protective effect against renal injury including inflammation and fibrosis through disturbing TGF-β/Smad signaling.
Key Words: *Prunellavulgaris*; Diabetic nephropathy; renal inflammation; glomerular fibrosis

P01-03

Ligustilide attenuates vascular inflammation and activates Nrf2/HO-1 induction, NO synthesis in HUVECs

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(Z)-ligustilideis a bioactive phthalide derivative isolated from Cnidii Rhizoma(*Cnidiumofficinale*, rhizome) and Angelicae Gigantis Radix (*Angelica gigas* Nakai, root). Vascular endothelium is a central spot in developing cardiovascular diseases and chronic vascular inflammation results in atherosclerosis development. We investigated anti-inflammatory effects of ligustilide against TNF-α, and whether ligustilide can activate Nrf2/HO-1 induction, intracellular NO synthesis in endothelial cells. Expression of VCAM-1, ICAM-1, E-selectin, p-IκB-α, HO-1 and translocation of NF-κB, Nrf2 were determined by western blot. Nuclear translocation of NF-κB, Nrf2 was visualized by immunofluorescence and DNA binding activity of NF-κB was measured with EMSA. ROS production, HL-60 monocyte adhesion, and intracellular NO synthesis were also measured using a fluorescent indicator. Ligustilide significantly suppressed adhesion of HL-60 monocyte to HUVECs via suppressing expression of VCAM-1, ICAM-1, and E-selectin against TNF-α. Also, ligustilide significantly inhibited ROS production and IκB-α phosphorylation, and it leaded to suppression of NF-κB activation. Furthermore, ligustilide up-regulated induction of HO-1 via Nrf2 nuclear translocation and synthesis of intracellular NO in HUVECs. Present study demonstrates that ligustilde has vascular protective potential andit might prevents developing cardiovascular complications such as thrombosis or atherosclerosis.
Key Words: (Z)-ligustilide, Vascular inflammation, Atherosclerosis, HUVECs, Nrf2/HO-1

P01-04

The effect of dietary fatty acid composition on food intake and hypothalamus gene expressions in mice

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The previous studies suggested that differently composed diets may induce satiety of the brain in different ways. However, the direct

effects of these diets on satiety in the hypothalamus are not clear. Therefore, in this study, gene expressions associated with food intake regulation of the hypothalamus were analyzed using real time PCR to consider whether the types of the purified fatty acid dissolved in DMSO administrated into stomach affect hypothalamic gene expressions. The objective of this study was to assess whether the fat composition change in the diet differently affects hypothalamic gene expressions, when compared with high-carbohydrates diets. The POMC mRNA expressions of the hypothalamus after 2 hours after administration of various types of fatty acids dissolved in DMSO into stomach in the groups of the SFA and n-3-PUFA were significantly increased to 238% and 151%, respectively. However, no difference was seen in n-6-PUFA mice compared with the control group. The n-6-PUFA group gained significantly lower gene expression compared with SFA and n-3 PUFA group. The NPY mRNA expression in the SFA group was not found to be significantly different from that of the control, whereas the gene expressions decreased to 59% and 67%, respectively in n-3-PUFA and n-6-PUFA groups. The gene expression in the n-3-PUFA group was lower than in the SFA group. AGRP mRNA expression in the SFA group was not found to be significantly different from the control, whereas gene expressions decreased to 63% and 62%, respectively in n-3-PUFA and n-6-PUFA groups. The gene expression in the n-3-PUFA group was lower than in the SFA group. In the diet experiment, POMC mRNA expression was highest in the group of SU and then in SFA and n-3-PUFA groups. There was no significant difference in the POMC mRNA expression between n-3-PUFA and ST diet groups. NPY mRNA expression in the SFA and the n-3-PUFA groups were higher compared with SU and ST. There were no significant differences in the NPY mRNA expression between ST and SU diet groups, and in the AGRP mRNA expression among groups. In conclusion, high-saturated fat diet may affect neuronal pathway via anorexigenic POMC neuron rather than orexigenic NPY neuron, and vice versa in high-n-3 PUFA diet. Thus, these findings indicate that fat composition change in a diet is an important determinant of food intake regulation in the hypothalamus

P01-05

Effects of the methanolic extract of *Schisandra chinensis* fruit and γ-schisandrin on transient receptor potential vanilloid 3

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Transient receptor potential vanilloid 3 (TRPV3) is a non-selective cation channel with modest permeability to calcium ions. It is involved in intracellular calcium signaling and is therefore important in processes such as thermal sensation, skin barrier formation, and wound healing. TRPV3 was initially proposed as a warm temperature sensor. It is activated by synthetic small-molecule chemicals and plant-derived natural compounds such as camphor and eugenol. *Schisandra chinensis* (*Turcz*.) *Baill* (*SC*) has diverse pharmacological properties including antiallergic, antiinflammatory, and wound healing activities. It is extensively used as an oriental herbal medicine for the treatment of various diseases. In this study, we investigated whether SC fruits extract and seed oil, as well as five compounds isolated from the fruit can activate the TRPV3 channel. By performing whole-cell patch clamp recording in HEK293T cells overexpressing TRPV3, we found that the methanolic extract of SC fruits has an agonistic effect on the TRPV3 channel. Furthermore, electrophysiological analysis revealed that γ-schisandrin, one of the isolated compounds, activated TRPV3 at a concentration of 30 μM. In addition, γ-schisandrin (~100 μM) increased cytoplasmic Ca^{2+} concentrations by approximately 20% in response to TRPV3 activation. This is the first report to indicate that SC extract and γ-schisandrin can modulate the TRPV3 channel. This report also suggests a mechanism by which γ-schisandrin acts as a therapeutic agent against TRPV3-related diseases. **Acknowledgement:** This research was supported bythe Convergence of Conventional Medicine and Traditional Korean Medicine R&D program funded by the Ministry of Health & Welfare (Korea) through the Korean Health Industry Development Institute (KHIDI: HI15C0256).
Key Words: *Schisandrachinensis*; TRPV3; γ-schisandrin; Calcium channel; 2-aminoethyl diphenyl borate

P01-06

Agrimonia pilosa leaf extract accelerates skin barrier restoration by activation of transient receptor potential vanilloid 3 (TRPV3)

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The formation and maintenance of the skin barrier are controlled by proliferation and differentiation of epidermal keratinocytes, and impairment of skin barrier homeostasis due to abnormal keratinocyte function causes many skin disorders, including atopic dermatitis and psoriasis. Keratinocyte differentiation is regulated by intra- and extracellular calcium ion concentrations. Recently, it was reported that the transient receptor potential vanilloid 3 (TRPV3) ion channel is functionally expressed in human keratinocytes. Activation of TRPV3 increased intracellular calcium signaling, which in turn increased transglutaminase 1, 3 activities as well as the subsequent formation of cornified cell envelopes in the epidermis. It was also reported that activation of TRPV3 promotes epithelial cell proliferation and wound healing in oral epithelia. These studies suggest that TRPV3 activation could be a potential therapeutic target for skin barrier recovery in various dermatological diseases. The leaves of *Agimonia pilosa Ledeb* (Rosaceae, AP), which have traditionally been used in East Asia to treat conditions including sore throat, parasitic infection, and eczema, have been reported to possess anti-inflammatory and anti-allergic effects in lipopolysaccharide- and ovalbumin-induced mouse models. We performed whole-cell patch-clamp and tape stripping tests to determine whether AP extract enhances barrier recovery via TRPV3 activation. As a result, we found that AP_{H2O} potently activates human TRPV3 and subsequently activates TGase activity *in vitro*. We also confirmed that topical application of AP_{H2O} improves recovery of skin barrier disruption induced by tape stripping in mice. APH2O could be useful for the prevention and treatment of skin barrier disorders, including inflammatory skin diseases such as atopic dermatitis and psoriasis. **Acknowledgement:** This research was supported by the Convergence of Conventional Medicine and Traditional Korean Medicine R&D program funded by the Ministry of Health & Welfare (Korea) through the Korean Health Industry Development Institute (KHIDI: HI15C0256).
Key Words: *Agrimonia pilosa* leaf, TRPV3, Skin barrier, TEWL

P01-07

Foeniculum vulgare extract and its constituent, *trans*-anethole, inhibit UV-induced melanogenesis *via* ORAI1 channel inhibition

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Ultraviolet radiation exposure is the most important cause of extrinsic skin aging (photoaging), which causes skin wrinkling and hyperpigmentation. Although many factors are involved in the photoaging process, calcium release-activated calcium channel protein 1 (ORAI1) has been reported to be involved in UV-induced melanogenesis. The aim of the present study was to find inhibitory effects of the extract of *Foeniculum vulgare* (fennel) fruits on ORAI1 ion channels and UV-induced melanogenesis in melanoma cells and to identify its active constituents. Active compounds were isolated and quantitatively analyzed. An electrophysiological assay was performed by using the whole-cell patch-clamp technique. Intracellular free calcium concentration was measured by Fura-2. Tyrosinase activity was evaluated by levodopa colorimetry. Effects of the most active compound on cell viability of murine B16F10 melanoma cells and inhibition of melanin content after UVB irradiation were determined. *F. vulgare* fruits extract and its hexane fraction strongly blocked ORAI1 currents and tyrosinase activity and significantly inhibited UV-induced melanogenesis. Of the 13 compounds isolated from the hexane fraction, *trans*-anethole (TA) exhibited inhibitory effects on ORAI1 (IC_{50} = 8.954 ± 1.36 μM) and increased cytoplasmic Ca^{2+} concentrations in response. TA inhibited UV-induced melanogenesis without affecting tyrosinase activity. Our findings suggest that the fruits extract of *F. vulgare* and its active constituent, TA, provide a possible novel approach for treating and preventing UV-induced melanogenesis. **Acknowledgement:** This study was funded by a grant from the Korean Health Technology R&D Project, Korean Ministry of Health & Welfare, Republic of Korea (Grant No. HN12C0057).
Key Words: Ultraviolet; Melanogenesis; *Foeniculum vulgare*; *trans*-Anethole; ORAI1 channel; Tyrosinase

P01-08

Skin protective effect of guava leaves against UV-induced melanogenesis *via* inhibition of ORAI1 channel and tyrosinase activity

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Ultraviolet (UV) irradiation is a major environmental factor affecting photoaging, which is characterized by skin wrinkle formation and hyperpigmentation. Although many factors are involved in the photoaging process, UV irradiation is thought to play a major role in melanogenesis. Tyrosinase is the key enzyme in melanin synthesis; therefore, many whitening agents target tyrosinase through various mechanisms, such as direct interference of tyrosinase catalytic activity or inhibition of tyrosinase mRNA expression. Furthermore, the highly selective calcium channel ORAI1 has been shown to be associated with UV-induced melanogenesis. Thus, ORAI1 antagonists may have applications in the prevention of melanogenesis. Here, we aimed to identify the anti-melanogenesis agents from methanolic extract of guava leaves (*Psidium guajava*) that can inhibit tyrosinase and ORAI1 channel. The *n*-butanol (47.47% ± 7.503% inhibition at 10 μg/mL) and hexane (57.88% ± 7.09% inhibition at 10 μg/mL) fractions were found to inhibit ORAI1 channel activity. In addition, both fractions showed effective tyrosinase inhibitory activity (68.3% ± 0.50% and 56.9% ± 1.53% inhibition, respectively). We also confirmed that the hexane fraction decreased the melanin content induced by UVB irradiation and the ET-1 induced melanogenesis in murine B16F10 melanoma cells. These results suggest that the leaves of *P. guajava* can be used to protect against direct and indirect UV-induced melanogenesis. (supported by the Convergence of Conventional Medicine and Traditional Korean Medicine R&D program funded by the Ministry of Health & Welfare (Korea) through the Korean Health Industry Development Institute (KHIDI: HI15C0256))
Key Words: *Psidium guajava*; B16F10 melanoma cells;anti-melanogenesis; ORAI1 channel; tyrosinase

P01-09

Anti-allergic effect of Oleamide isolated from *Arctium lappa L.*

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Arctium lappa L. is known as burdock and is a popular edible vegetable cultivated in Asia and Europe. The anti-allergic potential of Arctium lappa L. was investigated in Sprague-Dawley rats, ICR mice, and RBL-2H3 cells. Ethanol extract (90%) of A. lappa (ALE, 100 μg/ml) inhibited the degranulation rate by 52.9%, determined by the level of β-hexosaminidase. ALE suppressed passive cutaneous anaphylaxis (PCA) in rats and attenuated anaphylaxis and histamine release in mice. To identify the active compound of ALE, we subsequently fractionated and determined the level of β-hexosaminidase in all sub-fractions. Oleamide was identified as an active compound of ALE, which attenuated the secretion of histamine and the production of tumor necrosis factor (TNF)-α and interleukin-4 (IL-4) in cells treated with compound 48/80 or A23187/PMA. Oleamide suppressed FcεRI-tyrosine kinase Lyn-mediated pathway, c-Jun N-terminal kinases (JNK/SAPK), and p38 mitogen-activated protein kinases (p38-MAPKs). These results showed that ALE and oleamide attenuated allergic reactions and should serve as a platform to search for compounds with anti-allergic activity.
Key Words: Arctium lappa L, oleamide, anti-allergic, Lyn, RBL-2H3 cell

P01-10

Crinum asiaticum var. japonicum Baker extract inhibits adipocyte differentiation and adipogenesis

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Obesity is a medical condition characterized by excessive body weight due to accumulation of inordinate amounts of body fat. The anti-adipogenesis effects of *Crinum asiaticum var. japonicum Baker extract (CAE)* and its potential use as an anti-obesity aid have not yet been evaluated. In this study, the inhibitory effects of CAE on adipocyte differentiation and adipogenesis were determined using differentiation induction medium in 3T3-L1 cells. To get an insight into underlying molecular actions of CAE, we investigated the changes in the expression levels of genes involved in lipogenesis by CAE treatment using qRT-PCR. CAE strongly suppressed adipocyte differentiation through downregulation of PPARγ, C/EBPα, C/EBP β, and aP2. CAE treatment could also suppress the expression levels of ACC, FAS, LPL and HMGCR gene in 3T3-L1 cells. Male C57BL/6 strain and C57BL/6J-ob/ob strain mice were fed with high fat diet (HFD) containing 60% fat and normal diet in the presence or absence of 25, 50, and 100 mg/kg CAE for 7 weeks. CAE supplementation in HFD and monogenic mouse models of obesity significantly decreased body weight and epididymal fat and improved lipid profile. This anti-obesity effect of CAE may be associated with attenuation of adipocyte differentiation and adipogenesis through inhibition of the C/EBPα and PPARγ pathway and JNK.

Key Words: Crinum asiaticum var. japonicum Baker, Obesity, Adipocyte differentiation, Lipogenesis, High fat diet, Monogenic obese mice

P01-11

Cynanchum wilfordii extract attenuates an atherogenic diet with fructose-induced liver damage

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Excessive consumption of fat and fructose augments the pathological progression of nonalcoholic fatty liver disease through hepatic fibrosis, inflammation, and hepatic de novo lipogenesis. *Cynanchum wilfordii (Maxim.) Hemsl*, part of the family Asclepiadaceae, is named to Paeksuo or Paekhasuo in Korea and Baishouwu in China. In this study we investigated whether *Cynanchum wilfordii* extract (CWE) supplementation would decrease fat accumulation and damage in the liver. The beneficial effect of CWE was evaluated in a murine model of nonalcoholic fatty liver disease. Mice were fed either a normal diet or an atherogenic diet with fructose (ATHFR) in the presence or absence of CWE (50, 100, or 200 mg/kg; n = 6/group). Treatment with ATHFR induced a hepatosplenomegaly-like condition (increased liver and spleen weight); this pathological change was attenuated in the presence of CWE. The ATHFR group exhibited impaired liver function, as evidenced by increased blood levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, fat accumulation in the liver, and lipid profiles. Supplementation of CWE (100 and 200mg/kg, $P < .05$) ameliorated these impaired liver functions. Atherogenic diet with fructose increased the protein levels of COX-2 and p38 MAPK, as well as the nuclear translocation of NF-κB. These signaling pathways, which are associated with the inflammatory response, were markedly suppressed after CWE treatment (100 and 200 mg/kg). In summary, CWE supplementation reduced high-fat and high-fructose diet–induced fat accumulation and damage in the liver by suppressing COX-2, NF-κB, and p38 MAPK.

Key Words: Cynanchumwilfordii, Cyclooxygenase-2, Hepatic inflammation, Nonalcoholic fatty liver disease, p38 MAPK, Nuclear factor κB

P01-12

Bee venom and bee venom derived phospholipase A_2: Their analgesic effects in oxaliplatin-induced neuropathic pain in mice

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Colorectal cancer (CRC) was the third most common cancer in both men and women, and it caused about 608,000 deaths in 2008 worldwide, making it the fourth most common cause of death from cancer. Oxaliplatin is an important and widely used chemotherapy drug for the treatment of patients with metastatic CRC. However, it induces peripheral neuropathy characterized by dysesthesias of the hands and feet, which is a major dose-limiting side effect. Even a single administration of oxaliplatin can evoke this abnormal sensation, aggravated by cold and mechanical stimuli, to about 90% of treated patients. Bee venom (BV) has been traditionally used in Korea to alleviate pain, and a recent clinical trial has suggested that BV may be effective for chemotherapy-induced peripheral neuropathy. Here, we investigated the anti-allodynic effect of BV and further examined whether a co-administration of BV with morphine could have an additive effect on oxaliplatin-induced neuropathic pain in mice, as BV is known to be mediated by spinal noradrenergic and serotonergic receptors, whereas morphine by opioidergic receptors. The cold and mechanical allodynia signs were evaluated using acetone and von Frey hair test on the hind paw, respectively. The most significant allodynic sign was observed at three days after the injection of oxaliplatin (6 mg/kg, i.p.). BV (0.25, 1, and 2.5 mg/kg, s.c.) or morphine (0.5, 2, and 5 mg/kg, i.p.) alone showed dose-dependent anti-allodynic effects. The combination of BV and morphine at intermediate doses showed a greater and longer effect than either BV or morphine alone at the highest dose. Intrathecal pretreatment with the opioidergic (naloxone, 20 μg) or 5-HT_3 (MDL-72222, 15 μg) receptor antagonist, but not with α2-adrenergic (idazoxan, 10 μg) receptor antagonist, blocked this additive effect. Furthermore, we assessed the preventive and curative effects of BV derived phospholipase A_2 (bvPLA$_2$) on oxaliplatin-induced neuropathic pain in mice. Daily treatment with bvPLA$_2$ (0.2 mg/kg, i.p.) for five consecutive days prior to the oxaliplatin injection markedly inhibited the development of cold and mechanical allodynia, and suppressed the infiltration of macrophages and the increase of IL-1β level in the DRG. Such preventive effects of bvPLA$_2$ were completely blocked by depleting regulatory T cells with CD25 antibody pre-treatments. Moreover, daily administration of bvPLA$_2$ (0.2 mg/kg, i.p.), after an oxaliplatin injection, for five consecutive days markedly attenuated cold and mechanical allodynia, which was more potent than the effect of BV (1 mg/kg, i.p.). In sum, these results suggest that BV can effectively attenuate cold and mechanical allodynia induced by an oxaliplatin injection, and that morphine can reinforce this effect. Also, bvPLA$_2$ may play an important role in this analgesic effect of BV, as it has shown a preventive and curative effect on oxaliplatin-induced neuropathic pain. (supported by a grant from the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (NRF-2013R1A1A1012403), Korea Health Technology R & D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI14 C0738))

Key Words: Bee venom; Chemotherapy-induced neuropathic pain; Morphine; Oxaliplatin; Phospholipase A_2

P01-13

Effect of *Imperatae Rhizomaon* on the mechanism of nitric oxide-mediated vasorelaxation

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The purpose of the present study was to investigate the underlying cellular mechanisms of the nitric oxide (NO)-mediated property of the aqueous extract of *Imperatae Rhizoma* (AIR), pronounced anti-inflammatory herb, in vascular endothelium. The roles of the NO signaling in the AIR-induced effects were tested in human umbilical vein endothelial cells (HUVECs) and aortic ring.HUVEC treated with AIR produced higher amount of NO compared to control. AIR increased in the expression and phosphorylation levels of eNOS and Akt in HUVECs, which were attenuated by L-NAME, a NOS inhibitor, and wortmannin, a PI3K inhibitor. mRNA or protein levels of guanosine triphosphate cyclohydrolase I (GTPCH) was upregulated in HUVECs treated with AIR, suggesting a role of eNOS coupling. In addition, AIR-induced dose-dependent relaxation of phenylephrine-precontracted aorta was abolished by removal of functional endothelium. Pretreatment with L-NAME, and ODQ inhibited the AIR-induced vasorelaxation. Wortmannin and LY-294002 an upstream signaling molecule of eNOS, attenuated the AIR-induced vasorelaxation. Incubation of endothelium-intact aortic rings with AIR increased the production of cGMP, however, which were attenuated by L-NAME and ODQ. Two-kidney-one-clip (2K1C) hypertensiveratswere established to investigate the hypotensive effect of AIR in renovascular hypertension. 2K1C rats were treated with AIR at dose of 100 mg/kg/day orally for 3 weeks. AIR significantly lowered blood pressure. Interestingly, AIR ameliorated both endothelium-dependent and independent vascular relaxation in the phenylephrine-precontracted thoracic aorta. 2K1C-induced hypertension model increased plasma renin activity, however, AIR attenuated those activities. Taken together, the present study suggests that AIR ameliorates vascular dysfunctionvia the regulation of renin-angiotensin system and endothelium-dependent activation of through the PI3K/Akt-mediated NO-cGMP-PKG signaling.
Key Words: *Imperatae Rhizoma*; vasorelaxation; HUVECs; renovascular hypertension; 2-kidney and 1-clip;NO-cGMP-PKG signaling

P01-14

Protective effect of betulinic acid on early atherosclerosis in diabetic apolipoprotein-E knockout mice

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Atherosclerosis, a chronic and progressive disease, is a leading cause of endothelial dysfunction, diabetes mellitus, hypertension, and hypercholesterolemia. Betulinic acid (BA), a pentacyclictriterpene, has been reported to have a variety of biological effects, such as anti-inflammatory and immunomodulatory properties.This study was designed to determine whether BA may prevent atherosclerosis in diabetic apolipoprotein-E gene knockout (ApoE KO) mice. The mice were treated with BA for 12 weeks to examine the beneficial effects on atherosclerosisin ApoE KO mice. Male ApoE KO mice and age-matched control group mice (C57BL/6Jms) were used as experimental systems and were test on systolic blood pressure, insulin resistance and vascular inflammation.BA-treated ApoE KO mice lowered systolic blood pressure. Metabolic parameter showed that BA decreased BUN, triglyceride and total-cholesterol levels. Blood glucose, insulin, glucose tolerance test, and HOMA-IR index were better in BA treated ApopE KO mice than untreated ApopE KO mice. Consistent with the change in lipid profile, oil red O and H&E staining revealed that treatment with BA reduced atherosclerotic lesion such as roughened endothelial layers. BA restored the reduction of endothelial nitric oxide synthase (eNOS) expression, leading to the inhibition of intracellular adhesion molecule-1 (ICAM-1) and endothelin-1 (ET-1) expression. These results suggest that BA may be useful in the treatment and prevention of early atherosclerosis via attenuation of the endothelial dysfunction in diabetic ApoE KO mice.
Key Words: Betulinic acid; ApoE KO mice; Atherosclerosis; Vascular dysfunction; Insulin resistance

P02-01

Angiotensin-(4-8), an active mediator of renin-angiotensin system, suppresses ANP secretion via angiotensin type 1 receptor

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Angiotensin-(2-8) (Ang III), Ang-(3-8) (Ang IV), and Ang-(4-8) are metabolites of Ang-(1-8) (Ang II) catalyzed from amine terminal by aminopeptidase A or N. Ang-(1-9), Ang-(1-7), and Ang-(1-5) are metabolites of Ang-(1-8) (Ang II) catalyzed from carboxyl terminal by Ang converting enzyme (ACE). It has been reported that Ang II inhibits the secretion of atrial natriuretic peptide (ANP) Ang II type 1 receptor (AT1R), whereas Ang III and Ang IV stimulate the ANP secretion via AT2R and AT4R, respectively. Ang-(1-9), Ang-(1-7), and Ang-(1-5) also stimulate the ANP secretion via AT2R, MasR, and AT2R, respectively. However, it still remains unknown whether Ang-(4-8) is an active peptide of RAS and has some function on ANP secretion. Therefore, we investigated the effect of Ang-(4-8) on ANP secretion and if so, defined its signaling pathway using isolated perfused beating rat atria. The volume load was achieved by elevating the height of outflow catheter connected with isolated atria from 5 cmH_2O to 7.5 cmH_2O. Atrial stretch by volume load caused increases in atrial contractility by 60% and in ANP secretion by 100%. Ang-(4-8) (0.01, 0.1, 1 μM) suppressed high stretch-induced ANP secretion in a dose-dependent manner. The potency of Ang-(4-8) to inhibit ANP secretion was similar to Ang II. Ang-(4-8)-induced suppression of ANP secretion (0.1 μM) was attenuated by the pretreatment with an antagonist of AT1R (losartan) but not by an antagonist of AT2R (PD123,319), AT4R, or Mas R (A-779). Therefore, we suggest that Ang-(4-8) suppresses stretch-induced ANP secretion through the AT1R. More studies related to the signaling pathway of Ang-(4-8)-induced suppression of ANP secretion are ongoing. (supported by NRF grant funded by the Korea government, No 2008-0062279).
Key Words: Angiotensin, Angiotensin receptor, ANP, secretion

P02-02

Anthropometry-based estimation of the body heat capacity for individuals aged 7-69 y: the Size Korea Survey 2010

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In previous study, we developed an anthropometric-based calculation of heat capacity (HC) for adult. Although the equation appeared to be precise and valid, its application to children and adolescent may induce bias. In the present study, we employed a large dataset from the Size Korea survey, a national anthropometric survey conducted in 2010, to re-validate our previous HC equation and to develop an equation of HC for children and adolescent. 12766 participants aged 7 to 69 years with body composition measured by multi-frequency bioelectrical impedance analysis were employed. Age strongly determined HC and its related factors including body weight (BW) and body surface area (BSA) in immature individuals, but not in adults. Linear regression was appropriate to describe the relation between HC and BSA in adults, whereas this regression in children and adolescent was quadratic. The predictive equation of HC developed in our previous study revealed a high reliability and predictive power in above-20 age group. The model composed of gender, BW, BSA, and BSA square was appropriate to predict HC in immature individuals aged 7 to 19 years. Percent of fat slightly influences the HC prediction in all age groups. In conclusion, anthropometric-based modeling is a simple, reliable, and useful method for calculation of HC in the general Korean population. This work was supported by the grant (R0005739)from KIAT.
Key Words: Heat capacity, anthropometry, gender, weight, height, equation, body composition

P02-03

Exposure to Bisphenol-A affects neurotransmitter-mediated responses on GnRH neurons in mice

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Bisphenol-A (BPA), a monomer of polycarbonate plastic is an endocrine disruptor and is a potential hazard for reproductive physiology. In this study, using patch clamp technique we examined the agonists of various neurotransmitter receptors on GnRH neurons from the pre- and post- natal BPA exposed mice. In the first set of experiment, neonatal offsprings from the BPA-exposed pregnant mice were examined. Interestingly, pups from BPA-exposed mothers showed increased response to GABA (100 μM), kainate (10 μM), NMDA (30μM) and AMPA (10 μM) mediated responses than their respective controls. In contrary, GnRH neurons from BPA-injected juvenile mice showed decreased $GABA_A$, NMDA and kainate receptor mediated responses than their control counterparts. Further, in another set of experiment using gramicidin perforated mode was used to examine the effect of BPA exposure on various neurotransmitter receptor-mediated responses on adult GnRH neurons. There was a decreased kainite- and AMPA-mediated responses on GnRH neurons in BPA exposed mice than in control counterparts. These results suggest that BPA exposure may directly affect GnRH neurons which are the central regulator of hypothalamic pituitary gonadal axis. This research was supported by Basic Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241)
Key Words: Bisphenol-A, GnRH neurons, patch-clamp

P02-04(YP-01)

CR6-interacting factor 1 inhibition damages vascular function by inhibiting the Sirt1-eNOS pathway

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Mitochondrial dysfunction has emerged as a major contributing factor to endothelial dysfunction and vascular disease, but the key mechanisms underlying mitochondrial dysfunction-induced endothelial dysfunction remain to be elucidated. To determine whether mitochondrial dysfunction in endothelial cells play a key role in vascular disease, we examined the phenotype of endothelial-specific CR6-interacting factor 1 (CRIF1) knockout (CRIF1 KO) mice and *in vitro* pathophysiological mechanisms. CRIF1 KO mice exhibited lower body weight and cardiac hypertrophy. Downregulation of CRIF1 in vascular endothelial cells caused disturbances in the mitochondrial OXPHOS (oxidative phosphorylation) complexes, mitochondrial morphology, and function leading to enhanced mitochondrial reactive oxygen species (ROS) production and higher mitochondrial membrane potential (MMP). Downregulation of CRIF1 also caused decreased Sirt1 expression along with increased endothelial nitric oxide synthase (eNOS) acetylation leading to reduced nitric oxide (NO) production. Similar results were obtained in mice with CRIF1-deficient vascular endothelial cells. Endothelium-dependent vasorelaxation (EDR) of aortic rings from CRIF1 KO mouse was considerably less than in wild-type mice. Notably, EDR was partially recovered following in adSirt1 treated aortic rings from CRIF1 KO mice. Taken together, these findings indicate that CRIF1 plays an important role in the maintaining mitochondrial and endothelial function through its effects on the SIRT1-eNOS pathway.
Key Words: OXPHOS complex, CRIF1, SIRT1, eNOS, nitric oxide, mitochondrial dysfunction

P02-05

Effect of fibroblast growth factor 23 on osteoblastic differentiation and mineralization of D1 mesenchymal stem cells

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Although fibroblast growth factor 23 (FGF23) is exclusively produced in osteoblasts and osteocytes, its main target is the kidney, where it decreases phosphate reabsorption by suppressing Na-phosphate cotransporters. Independently of its action on phosphate homeostasis, FGF23 also inhibits bone formation *in vivo*. In a calvarial osteoblastic cell model, FGF23 has also been shown to negatively affect extracelluar matrix mineralization. This study investigated whether FGF23 has similar effects on osteoblast maturation, including differentiation and mineralization of bone marrow-derived mesenchymal stem cells (MSCs). D1 MSCs were cultured in osteogenic medium containing β-glycerophosphate, ascorbic acid and dexamethazone. Osteoblastic differentiation was evaluated by alkaline phosphatase (Alp) staining and matrix mineralization was evaluated by alizarin red staining and calcium deposition. Expression of differentiation-stimulating genes *Runx2*, *Alp* and *osteocalcin* and mineralization-inhibiting genes *Enpp1* and *Ank* was analyzed using semiquantitative RT-PCR. Supraphysiological doses of FGF23 did not stimulate proliferation or osteoblastic differentiation of MSCs. Matrix mineralization 1, 2, and 3 weeks after FGF23 treatment did not vary between control and FGF23 groups, although time-dependent enhancement of mineralization was obvious. Calcium deposition was also unchanged after FGF23 treatment. mRNA expression levels of differentiation- and mineralization-related genes were also similar between groups. Despite these negative findings, FGF23 signaling through FGF receptors seemed to function normally, with phosphorylation of Erk protein more evident in the FGF23 group than in controls. These findings suggest that unlike calvarial osteoblasts, FGF23 is not likely to affect osteoblastic differentiation and mineralization of MSCs.
Key Words: Fibroblast growth factor 23, Mesenchymal stem cell, Differentiation, Mineralization

P02-06

Direct effects of neurosteroid on gonadotropin releasing hormone neurons

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Neurosteroids influence neuronal activities via conventional phasic postsynaptic currents (PSCs, I_{phasic}) via type A gamma-amino butyric acid ($GABA_A$) receptors. Further, $GABA_A$ receptors mediate a sustained tonic current (I_{tonic}) as well as I_{phasic} on GnRH neurons. In addition, steroidal modulation of I_{tonic} on GnRH neuron is still unclear. In this study, we examined the influence of neurosteroids on GnRH neurons and tried to figure out the subunit configuration of $GABA_A$ receptors ($GABA_AR$) mediating neurosteroid sensitivity of I_{tonic} on GnRH neurons. 3α,5α-THDOC increased inward currents on GnRH neurons and the inward currents were persisted in the presence of gabazine, which blocks the synaptically mediated PSCs. In addition, 3α,5α-THDOC-mediated currents were persisted in the presence of amino acid blocking cocktail (AP-5, CNQX, strychnine: and TTX) suggesting that the tonic currents are mainly postsynaptic events. Further 3α,5α-THP also induced inward currents on GnRH neurons and the currents were remained in the presence of gabazine and were partially blocked by L-655708 (10 μM), a $GABA_A$-α5 inverse agonist. In addition, $GABA_A$-α5 mRNA transcripts were detected on GnRH neurons. These results suggest that there exists an α5 and/or δ $GABA_A$ receptor-mediated tonic conductance via prognane neurosteroids on GnRH neurons and these tonic currents may affect the hypothalamus-pituitary-gonadal axis and reproductive physiology. This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1C1A1A02036793).
Key Words: Gonadotropin releasing hormone neuron, tonic $GABA_A$ current, Patch clamp

P02-07(YP-06)

Neurosteroids-mediated actions on GnRH neuron in letrozole-induced polycystic ovarian syndrome (PCOS) mouse model

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Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disorder in women on their reproductive age which is distinguished by accretion of small cystic follicles in the ovary. Ovarian function in all mammals is controlled by gonadotropin releasing hormone (GnRH) neurons, which are the central regulator of hypothalamic pituitary gonadal axis (HPG-axis). Till date, few is known the impact of letrozole-induced PCOS in GnRH neuronal development and its role in reproductive physiology at hypothalamic level is still unknown. In this study, we investigated the effects of various neurotransmitters including GABA, kainate, muscimol and baclofen and neurosteroids including THDOC and THIP on GnRH neurons from letrozole-treated mice (1 μg/g body weight; 21 successive days) exposed from PND 23. Neurotransmitters- and neurosteroids-mediated responses on GnRH neurons from letrozole-injected mice were smaller than those of control. We also found that letrozole-induced irregular estrous cycle, increased in body weight and anovulation in female mice. These results suggest that the PCOS is an endocrine disorder mostly common in females which may directly affect GnRH neuronal activity and adverse impact on reproductive physiology at hypothalamic level. (This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241) and (2015R1C1A1A02036793))
Key Words: GnRH neurons, Letrozole, Polycystic ovarian syndrome, Patch clamp

P02-08

Postnatal exposure of TCDD affects gonadotropin releasing hormone neuronal activities in mice.

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TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is the most potent toxic environmental pollutant which has adverse effects on reproductive and brain development. To date, the direct impact of TCDD on gonadotropin releasing hormone (GnRH) neuronal activities and its role in reproductive physiology at hypothalamic level are still unknown. Thus, in this study, we investigated the effect of various neurotransmitters on GnRH neurons from mice exposed to TCDD at prenatal and at juvenile stage. $GABA_A$, receptor- and NMDA receptor-mediated responses on

juvenile GnRH neurons exposed to TCDD at juvenile stage were smaller than those of control. Otherwise, kainite-mediated responses on juvenile GnRH neurons exposed to TCDD at juvenile stage were bigger than those of control. $GABA_A$ receptor or kainate receptor-mediated response on juvenile GnRH neurons exposed to TCDD at prenatal stage showed no difference compared to control. Furthermore, $GABA_A$ receptor mediated neurotransmission on adult GnRH neurons exposed to TCDD at juvenile stage showed no significant difference compared to control over the estrous cycle. Whereas, there was the increase in kainate receptor-mediated neurotransmission in diestrus, proestrus and estrus phase of estrous cycle in adult GnRH neuron when exposed at juvenile stage. Furthermore, neurosteroid THDOC-mediated inward current was also decreased in juvenile GnRH neurons when exposed to TCDD on juvenile stage. These results suggest that TCDD is a potent environmental pollutant which may directly affect GnRH neuronal activity and reproductive physiology at hypothalamic level. (This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2014R1A1A2054241))
Key Words: GnRH neurons, TCDD, Patch clamp, Neurotransmitter.

P02-09

Alkalinization by Phosphate Uptake via PiT-1/2 Participates in High Phosphate-induced Oxidative Stress and Defective Insulin Secretion

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Elevated plasma level of inorganic phosphate (P_i) is harmful, causing among other complications vascular calcification and defective insulin secretion. The underlying molecular mechanisms of these complications remain poorly understood. Here, we demonstrate the role of P_i transport across the plasmalemma on P_i toxicity in insulin-secreting cells. To investigate the role of sodium-phosphate cotransporter (NaP_i), we performed knockdown of NaP_i in INS-1E cells as well as dispersed rat pancreatic islet cells. Electrophysiology and fluorescence imaging were used to measure cytosolic and mitochondrial changes by high P_i application. PiT-1 and -2, isotypes of type III NaP_i, are the predominant P_i transporters expressed in insulin-secreting cells. Transcript and protein levels of PiT-1 and -2 were upregulated by high P_i incubation. In patch clamp experiments, extracellular P_i elicited a sodium-dependent inwardly rectifying current, which was markedly reduced under acidic extracellular conditions. Cellular uptake of P_i elicited cytosolic alkalinization and, intriguingly, this pH change facilitated P_i transport into the mitochondrial matrix. Increased mitochondrial P_i uptake accelerated superoxide generation, mitochondrial permeability transition and ER stress-mediated translational attenuation, leading to reduced insulin content and impaired glucose-stimulated insulin secretion. Silencing of PiT-1 and -2 prevented all the P_i-induced pathogenic alterations including restorations of insulin secretion and content. P_i transport across plasma membrane and consequent cytosolic alkalinization could be a therapeutic target for protection from P_i toxicity in insulin secreting cells but also other cell types.
Key Words: inorganic phosphate, pancreatic beta cells, mitochondrial permeability transition, endoplasmic reticulum stress, superoxide

P02-10

VEGF-A expressing adipose tissue shows rapid beiging, enhanced survival after transplantation and confers metabolic improvements on the host in an IL4-independent manner

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Adipocyte-derived VEGF-A plays a crucial role in angiogenesis, but also contributes to adipocyte function and systemic metabolism, such as insulin resistance, chronic inflammation and beigeing of subcutaneous adipose tissue. By utilizing a doxycycline (Dox)-inducible adipocyte-specific VEGF-A overexpressing mouse model, we investigated the dynamics of local VEGF-A effects on tissue beiging adipose tissue transplants. As we reported, VEGF-A overexpression in adipocytes triggers angiogenesis. Here, we also observe a rapid appearance of beige fat cells in subcutaneous white adipose tissue (sWATs) within as little as 2 days post induction of VEGF-A. In contrast to conventional cold-induced beiging, VEGF-A induced beiging is independent of IL-4. Adipocytes and adipose-derived stem cells (ASCs) are common substrates for plastic- and regenerative surgery. We subjected metabolically healthy VEGF-A overexpressing adipose tissue to autologous transplantation. Transfer of subcutaneous adipose tissues taken from VEGF-A overexpressing mice into diet-induced obese mice resulted in systemic metabolic benefits, associated with improved survival of adipocytes and a concomitant reduced inflammatory response. These effects of VEGF-A are tissue autonomous, inducing WAT beigeing and angiogenesis within the transplanted tissue. Our findings indicate that manipulation of adipocyte functions with a *bona fide* angiogenic factor, such as VEGF-A, significantly improves the survival and volume retention of fat grafts and can conveys metabolically favorable properties on the recipient. (supported by the National Institute of Health (R01-DK55758, R01-DK099110 and P01-DK088761) as well as a grant from Priority Research Centers Program through the NRF, funded by the Ministry of Education, Science and Technology (2010-0020224))
Key Words: VEGF, adipose tissue, fibrosis

P03-01

Relative muscular strength is associated with reduced estimated glomerular filtration rate (eGFR) in community-dwelling young and middle-aged adults

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A lower level of physical fitness is an independent risk factor of chronic diseases and dysfunction of numerous organ systems. Patients with chronic renal failure have shown the apparent decline in physical fitness, especially in the end-stage. However, it is unclear whether the level of physical fitness is associated with subclinical kidney

dysfunction. Nine hundred fifty-two Koreans (female, 75.3%) aged 19 to 64 years were included in this cross-sectional study. All participants were interviewed face-to-face and received measure of anthropometry, body composition and serum biomarkers of metabolic diseases. eGFR (ml/min/1.73m^2) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The following health-related fitness tests were performed: multi-stage 20m shuttle run, sit-up, handgrip strength (absolute and relative values), standing long jump, and sit-and-reach. Each level of fitness components was divided into either unfit or fit based on the 25th percentile of fitness parameters for the gender and the age strata. Of the subjects, the prevalence of 45≤ eGFR <90 mL/min/1.73m^2 was 58.9%. The subjects with eGFR ≥90 mL/min/1.73m^2 showed significantly better fitness levels for aerobic capacity, muscular endurance, strength and anaerobic power than those with 45≤ eGFR <90 mL/min/1.73m^2. Binominal logistic regression analysis showed the significant association between relative handgrip strength and mild to moderate kidney dysfunction even after adjustment for potential confounders. Taken together, relative muscular strength is associated with even mildly reduced eGFR in the adult population. This finding suggests that relative muscular strength may be a contributory fitness factor to preserve renal function.
Key Words: estimated glomerular filtration rate, health-related physical fitness, relative handgrip strength

P03-02

Exercise training normalizes excitability of hypothalamic paraventricular neurons (PVN) in the rats with heart failure

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Sympathetic hyper-activation is a hallmark of heart failure (HF). Recent studies showed that exercise training (ExT) decreases sympathetic nerve activity in HF rats, which may explain the beneficial effects of Ext on HF patients. However, underlying mechanisms of the effects of ExT on the sympathetic nerve activity are not well understood. In this study, we investigated the effects of ExT on heart rate variability (HRV) and excitability of the neurons in the paraventricular nucleus projecting to the rostral ventrolateral medulla (PVN-RVLM) in the HF rats. HF rats were induced by ligation of left descending coronary artery. 3-4 weeks after HF ligation surgery or sham surgery, the rats were exercised on a motor-driven treadmill for 3 week period. Electrocardiogram data were collected for 24 h at 8thweek following the surgery using DSI Dataquest A.R.T.TM system and analyzed by Kubios HRV software. The PVN neuronsprojecting to the rostral ventrolateral medulla (PVN-RVLM) were identified by etrograde dye (FluoSphere-Red). The spontaneous firing, post-synaptic currents and action potential were recorded in the cell-attached or whole-cell mode with Axopatch 200B. Data were sampled at 20 KHz using pClamp 8 data-acquisition and analysis software (Axon Instruments). In the telemetry experiments, ExT increased the differences in heart rate and sympatho-vagal balance (measured as 'LF/HF ratio') between light and dark phases in the HF rats. In patch clamp experiments, ExT selectively decreased the firing rate of PVN-RVLM neurons (Sham 9.0% vs. HF 46.8%), and preferentially increased the variability of inter-spike intervals in HF rats (Sham 1.7 folds vs. HF 3.17 folds). ExT preferentially increased the frequency of spontaneous IPSC in PVN-RVLM neurons in HF rats (Sham, 2.33 folds vs. HF 3.32 folds) with little effects on the resting membrane potential. Bath application of GABA-A receptor blocker (bicuculline 20 μM) increased the firing rate in HF-ExT, but not in HF rats. Collectively, the results indicate that ExT normalizes the blunted diurnal variation of sympatho-vagal balance in HF rats by decreasing the excitability of the hypothalamic pre-sympathetic neurons. Our findings provide a brain mechanism of the beneficial effects of ExT in HF patients.
Key Words: Exercise training; HRV; PVN-RVLM neurons; Patch clamp

P03-03

Ursolic acid supplement attenuates exercise-induced cardiac damage biomarkers in resistance-trained men

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Ursolic acid (UA) plays an important role in resistance training (RT) on skeletal muscle function; however, whether UA inhibits biomarkers of cardiac damage in resistance-trained men is unknown. We examined the effects of UA during RT on cardiac damage markers including cortisol, B-type natriuretic peptide (BNP), myoglobin, creatine kinase (CK), creatine kinase-myocardial band (CK-MB), and lactate dehydrogenase (LDH). Sixteen healthy men were assigned to UA + RT or placebo + RT (n = 8 per group). Participants were trained to RT program with 26 exercise types (60~80% of 1 repetition, 6 times/week) and UA or placebo were administered as capsules (3 capsules/day) for 8 weeks. Post-intervention, the RT + placebo group showed no significant changes in body composition or markers of cardiac damage, whereas in the RT + UA group, body weight and body percentage were slightly decreased and lean body mass was slightly increased without statistical significance. UA supplementation significantly decreased the levels of circulating BNP, CK, CK-MB, and LDH ($P < 0.05$) comparing to baseline of RT+UA, and the changes in all cardiac damage markers were significantly higher than that of control. In conclusion, UA supplementation during short-term resistance training effectively suppressed biomarkers of cardiac damage in resistance-trained men.
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Key Words: ursolic acid, cardiac damage markers, resistance training

P03-04

The Effect of Bio-active Materials Coated Fabric on Mitochondria

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The bio-active materials coated fabric (BMCF) was coated with bio-active materials containing over 30 kinds of minerals. The clothing made of this fabric has layer of bio-active energy which reacts with far infrared rays from human body and it is penetration into human body. To observe the effects, the fabric (10 and 30%) was worn to old-aged rat and then the oxygen consumption efficiency and copy numbers of mitochondria, and mRNA expression of apoptosis- and mitophagy-related genes were verified. By wearing the BMCF, the oxidative respiration significantly increased when using the 30% materials coated fabric. The mitochondrial DNA copy number significantly decreased and subsequently recovered in a dose-dependent manner. The respiratory control ratio to mitochondrial DNA copy number showed a dose-dependent increment. As times passed, Bax, caspase 9, PGC-1α and β-actin increased, and Bcl-2 decreased in a dose-dependent manner on mRNA expressions. However, the BMCF can be seen to have had no effect on Fas receptor. PINK1 expression did not change considerably and was inclined to decrease in control group, but the expression was down-regulated then subsequently increased with the use of the BMCF in a dose-dependent manner. Caspase 3 increased and subsequently decreased in a dose-dependent manner. These results suggest that the BMCF invigorates mitophagy and improves mitochondrial oxidative respiration in skeletal muscle and in early stage of apoptosis induced by the BMCF is not related to extrinsic death-receptor mediated but mitochondria-mediated signaling pathway.

Key Words: functional fabric, alternate exercise effect, mitochondria, far infrared radiation, membrane potential

P04-01

Mitochondrial substrate dependent changes of mitochondrial function

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Mitochondria are essential organelles in the energy metabolism of cells. The production of ATP from the mitochondria is based on their metabolites, however, their functional changes depending on mitochondrial substrates has not been clearly described. In this study, NADH, mitochondrial membrane potential (ψ_m) and oxygen consumption were monitored in different set of mitochondrial metabolites such as malate, pyruvate, glutamate and succinate in the presence or absence of inorganic phosphate. Any single metabolite could not maintain mitochondrial function, except succinate, which could consume the oxygen, however, ψ_m was not effectively formed. In combinations of two metabolites, only pyruvate/succinate and pyruvate/malate combination could maintain mitochondrial functions. When the combinations of three metabolites were tested, generally mitochondrial functions were maintained except malate/glutamate/succinate combinations. When all metabolites were included, the mitochondrial functions were maintained. However, there were clear differences in NADH production, ψ_m formation, and oxygen consumption. The highest value was achieved in conditions of pyruvate/glutamate/succinate. In general, the addition of inorganic phosphate increased the generation of ψ_m up to threefold and the oxygen consumption, however, the maximal NADH was decreased. In conclusion, mitochondrial function was not maintained with only pyruvate or other single metabolite. This study suggests a suitable metabolite combination for production of mitochondrial energy and indicates an effective energy metabolism through its combination. This work was supported by the grant (R0005739) from KIAT.

Key Words: NADH, mitochondrial membrane potential (ψ_m), mitochondrial substrate, oxygen consumption

P04-02

Identification of PKD2L1 phosphorylation sites and the regulation of PKD2L1 channel activation by cAMP signaling pathway

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Polycystic kidney disease 2-like-1 (PKD2L1), or TRPP2 or Polycystin-L (PCL) is a transient receptor potential (TRP) superfamily member. It is a Ca^{2+}-permeable non-selective cation channel that regulates intracellular calcium concentration and thereby calcium signaling. PKD2L1 has been reported to be involved in hedgehog signaling in cilia, intestinal development, and sour tasting. However, in a recent study, PKD2L1 is reported to have a crucial role in hippocampal and thalamocortical hyperexcitability and it is found to be a novel ion channel to localize to neuronal cilia in mammals. PKD2L1 interacts and co-localizes with β2-adrenergic receptor (β2AR) on the neuronal primary cilia. The disruption of PKD2L1 leads to the loss of β2AR on the neuronal cilia and reduction in the concentration of intracellular cAMP, which is known to inhibit neuronal excitability. Together with the another preceding report that cAMP promotes growth and secretion in human polycystic kidney epithelial cells, we further investigated on the mechanism of cAMP regulation in relation to the role of PKD2L1. Since the activation of PKA is subjected to the regulation of cAMP, we considered the possibility of direct phosphorylation of PKD2L1 channel and consequently, regulation of the channel function and intracellular calcium concentration. We first observed PKD2L1 ion channels on the primary cilia of mouse renal inner medullary collecting duct (mIMCD-3) cells. We selected candidate phosphorylation sites of PKD2L1, S281, S603, S795 and each site was mutated to alanine. With patch clamp technique, PKD2L1 (WT) channel function was evaluated and compared with the mutants treated with calmidazolium (CMZ), the activator of PKD2L1 channel, and cyclic adenosine monophosphate (cAMP). Compared to WT PKD2L1 channel activation by cAMP, S603A and S795A mutants showed relatively less increase in channel currents. We identified S603 and S795 to be phosphorylation sites of PKD2L1 ion channel and found them significant in the activation of the channel through cAMP signaling pathway.

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Key Words: PKD2L1, TRPP2, PCL, calcium ion channel, phosphorylation, cAMP

P04-03

The changes of voltage-dependent K^+ channel activity during early phase of diabetes in the mesenteric arterial smooth muscle

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This study investigated the alteration of voltage-dependent K^+ (Kv) channels in mesenteric arterial smooth muscle cells from control (LETO) and diabetic (OLETF) rats during the early and chronic phases of diabetes. In the early phase of diabetes, the amplitude of mesenteric Kv currents induced by depolarizing pulses was greater in OLETF rats than in LETO rats. The contractile response of the mesenteric artery induced by the Kv inhibitor, 4-aminopyridine (4-AP), was also greater in OLETF rats. The expression levels of most Kv subtypes were increased in mesenteric arterial smooth muscle from OLETF rats compared with LETO rats. However, in the chronic phase of diabetes, the Kv current amplitude did not differ between LETO and OLETF rats. In addition, the 4-AP-induced contractile response of the mesenteric artery and the expression of Kv subtypes did not differ between the two groups. In summary, the increased Kv current amplitude and Kv channel-related contractile response were attributable to the increase in Kv channel expression during the early phase of diabetes. The increased Kv current amplitude and Kv channel-related contractile response were reversed during the chronic phase of diabetes.

Key Words: Type 2 diabetes; Voltage-dependent K^+ channel; Mesenteric artery

P04-04

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

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

Key Words: TRP, TRPC4, cGMP, PDE, BPH

P04-05

Maxi-K channel (BKCa) activity veils the myogenic response of mesenteric artery in rats

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Arteriolar and small arterial smooth muscles change their tone in response to transmural pressure changes, called myogenic response (MR). In comparison to the small arteries such as cerebral arteries (CA) showing prominent MR, mesenteric arteries (MA) usually show smaller MR. Here we aimed to analyze the electrophysiological differences responsible for the weaker MR in MA (MR_{MA}) than MR in CA (CA_{MR}). Whole-cell patch clamp study revealed higher levels of voltage-operated Ca^{2+} channel current and cationic background current in the MA smooth muscle cells (MASMCs) than CA smooth muscle cells (CASMCs). Although voltage-gated K^+ channel current (I_{Kv}) was also higher in MASMCs, treatment with Kv inhibitor (4-aminopyridine) did not affect MR_{MA} that was analyzed using video analysis of pressurized small arteries. Interestingly, big-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) current and spontaneous transient outward currents (STOCs) were consistently higher in MASMCs than CASMCs. Inside-out patch clamp showed that both Ca^{2+}- and voltage-sensitivities of BK_{Ca} are higher in MASMCs than CASMCs. Iberiotoxin, a selective BK_{Ca} inhibitor, augmented MR_{MA} by a larger extent than MR_{CA}. However, real-time PCR analysis did not reveal a significant difference of mRNAs for the alpha and beta subunits of BK_{Ca}. Above results indicate that the higher activities of BK_{Ca} in MASMCs veils the potentially strong MR_{MA}.

Key Words: mesenteric artery, myogenic response, smooth muscle cell, K^+ channel, BK_{Ca}

P04-06

Differential modulation of TASK-2 and TREK channel activity by pyrazole compounds

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Pyrazole derivatives were originally suggested as selective blockers of transient receptor potential cation (TRPC)3 and ORAI1 channel. In particular, pyr3 and 10 selectively inhibit TRPC3, whereas pyr2 (BTP2) and 6 inhibit ORAI1. However, their effects on background K^+ channel activity have not been elucidated. In this study, the effects of BTP2, pyr3, 6, and 10 were studied on cloned human TREKs and TASK-2 K^+ channels, which modulate Ca^{2+} signaling via control of membrane potential, in HEK293T-overexpressing cells via a whole-cell patch clamp technique. Pyr3 potently inhibited TREK-1 (I_{TREK1}), TREK-2 (I_{TREK2}), and TASK2 current (I_{TASK-2}) with a half-maximal inhibitory concentration (IC_{50}) of 0.89 ± 0.27, 1.95 ± 1.44 μM, and 2.42 ± 0.39 μM, respectively. BTP2 showed slight inhibitory effects on I_{TASK-2} (80.3 ± 2.5% inhibition at 100 μM). In contrast, pyr6 at 100 μM potentiated I_{TREK1} and I_{TREK2} about 2.6- and 3.6-fold compared to control and inhibited I_{TASK2} (38.7 ± 9.2% inhibition). Pyr10 showed a subtype-specific inhibitory effect on I_{TREK1} but not on I_{TREK2}. It also inhibited I_{TASK2} (70.9 ± 3.1% inhibition at 100 μM). To our knowledge, this study is the first to describe the differential modulation

of TREKs and TASK2 channels by pyrazole derivatives, which had been previously used as inhibitors for TRPC3 and ORAI1. Therefore, studies using these drugs should take into account their modulatory effects on other channels, such as TREK and TASK-2. **Acknowledgement:** This research was supported by the Convergence of Conventional Medicine and Traditional Korean Medicine R&D program funded by the Ministry of Health & Welfare (Korea) through the Korean Health Industry Development Institute (KHIDI: HI15C0256).
Key Words: pyrazole derivatives; TREK-1; TREK-2; TASK-2; calcium signaling; potassium channels

P04-07

Paliperidone, the active metabolite of risperidone, inhibits cloned hERG potassium channels

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Paliperidone, the main active metabolite of risperidone, is a potent serotonin-2A and dopamine-2 receptor antagonist, used in the treatment of schizophrenia and related disorders. Whole-cell patch-clamp technique and Western blot analysis were used to investigate the effects of paliperidone on the hERG (Kv11.1-1A). Paliperidone and risperidone blocked the tail current of hERG in a concentration-dependent manner with IC_{50} values of 0.57 μM and 0.16 μM, respectively. The block of the hERG by paliperidone was voltage-dependent with a steep increase across the voltage range of channel activation. Paliperidone induced use-dependent block during repetitive pulsing. Paliperidone did not exhibit notable shift in the steady-state inactivation curve. A fast drug perfusion system demonstrated that paliperidone interacts with both the open and inactivated states of the hERG. The fast application of paliperidone during the tail currents blocked the open state of the hERG in a concentration-dependent manner with an IC_{50} of 1.27 μM. In western blot analysis, paliperidone and risperidone did not inhibit the trafficking of hERG protein to the cell membrane. In addition, HEK293 cells transiently transfected with plasmid carrying Kv11.1-3.1, the primate-specific brain voltage-gated potassium channel isoform, also showed similar results to the trafficking of hERG protein with paliperidone and risperidone. These results suggest that paliperidone blocked the hERG at a supratherapeutic concentration and that it did so by preferentially binding to both the open and inactivated states of the channels with no effect on the generation and trafficking of hERG protein by paliperidone.
Key Words: hERG. paliperidone. risperidone. schizophrenia. channel trafficking

P04-08

Shear stress activates gap junction hemichannel current with subsequent P2X purinoceptor activation in rat atrial myocytes

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Atrial myocytes are subjected to shear stress under physiological and pathological conditions during cardiac cycle. We recently showed evidence that atrial Ca^{2+} waves, immediately activated by shear stress, is mediated by P2Y purinergic signaling activated by ATP released through the gap junction hemichannels. In this study, we further examined whether shear stress activates gap junction hemichannels by measuring ionic current under whole cell patch clamp in rat atrial myocytes. Shear stress of ~16 dyne/cm^2 was shortly applied (for 1 s) to single cells using automatic micro fluid-jet apparatus. At this strength of shear stress, a transient inward current was recorded in symmetrical CsCl-rich solutions at -70 mV. This current was enhanced by removal of external Ca^{2+} or quinin that increases gap junction hemichannel activity, but abolished by the inhibition of gap junction hemichannels using carbenoxolone (50 μM) or La^{3+} (2 mM). We next tested the possibility that ATP release through the gap junction hemichannels under shear stress may also activate P2X purinoceptors, contributing to inward current at resting conditions. Pre-treatment of suramin (10 μM), the general P2 purinoceptor inhibitor, suppressed shear-activated inward current by 70-80%. Blockade of P2X receptors using iso-PPADS (100 μM) suppressed this current by about 50%. When the transient receptor potential melastatin subfamily 4 (TRPM4) that has been shown to be activated by prolonged shear, was inhibited, 20-30% of the immediate shear-activated current was removed. When symmetrical $NMDG^+$ was used instead of Cs^+, the magnitude of shear-activated current at -70 mV was reduced to 20% of the control Cs^+ current. This $NMDG^+$ current under shear application was significantly increased by quinin, eliminated by La^{3+}, and showed linear current-voltage relationship with a reversal potential of ~0 mV, suggesting a fairly pure role of the hemichannels in this current. Our data suggest that shear stress may induce Na^+ influx at resting conditions via P2X purinoceptors through activation of gap junction hemichannels in rat atrial myocytes.
Key Words: shear stress; atrial myocytes; gap junction hemichannel; P2X purinoceptor; patch clamp

P04-09

Cell cycle-dependent expression of TASK3 in human bladder cancer cell lines

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The incidence of bladder cancer increases with age and the cancer was known to be the seventh most common cancer in Korean men. Potassium channels have been suggested to be important in cell cycle progression. Among them, two-pore domain potassium (K2P) channels, such as TASK3 and TREK1, have recently been shown to be overexpressed in breast and prostate cancer cells, respectively. Cell cycle progression in bladder cancer cells might be related with K2P channels. The present study was designed to investigate the relevance of K2P channels and cell cycle progression in human bladder cancer cell line, 253J. Real-time PCR analysis showed the expression of TWIK1, TASK1, TASK3, TREK1 and TREK2 in 253J cells. To observe the influences of K2P channels on the cell cycle in 253J cells, we used TREK2 and TASK3 small interfering RNAs (siRNA), siRNA-mediated TREK2 and TASK3 knockdown induced cell cycle arrest in 253J cells, whereas application of TASK3/TREK2 siRNA did not induce arrest of cell cycle. Also S-phase arrest by double thymidine block was associated with an increase in expressions of TASK3 mRNA. From the results, K2P channels may contribute to cell cycle progression in bladder cancer cell line, 253J cell.
Key Words: TASK3, TREK2, cell cycle, bladder cancer, K2P channel

P04-10(YP-02)

Characteristics of mitochondrial calcium efflux regulation in permeabilized single ventricular myocytes

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Regulation of mitochondrial Ca^{2+} is related to various physiological and pathophysiological phenomena. In a recent review, several Ca^{2+} transporting mechanisms were existed in mitochondria such as MCU, mRyR, RaM, NCX_{mito}, HCE_{mito}, etc. However, the role and the regulating mechanisms were not clear, yet. In this study, we would like to elucidate Ca^{2+} removing mechanisms. Ca^{2+} efflux was evoked by the removal of cytosolic Ca^{2+} after Ca^{2+} was loaded into mitochondria for 3 mins. We showed clearly that NCX_{mito} is a major Ca^{2+} efflux mechanism. However, in the absence of Na^+, Ca^{2+} efflux was still occurred, even though it was much slower. The proposed efflux route may be Ca^{2+} influx pathway such as MCU. The treatment of RU360 clearly delayed Ca^{2+} efflux. When 0.1 μM Ca^{2+} was applied continuously, $[Ca^{2+}]_m$ was initially increased, however, it was not maintained and continuously decreased even in the presence of $[Ca^{2+}]_c$. When 10 μM Ca^{2+} was applied continuously, initial $[Ca^{2+}]_m$ increase was faster and larger, however, $[Ca^{2+}]_m$ decrease was occurred in a much faster rate. During $[Ca^{2+}]_m$ decrease, the reduction of NADH and Ψ_m depolarization were accompanied. When we changed pHc from 6 to 8, Na^+ independent Ca^{2+} efflux was faster as pH became alkaline. We hypothesized the mechanism related to Ca^{2+}/Pi dependent Ψ_m depolarization may participate in Na^+-independent Ca^{2+} efflux (NICE). Since ATP and ADP could prevent Ca^{2+}/Pi dependent Ψ_m depolarization, it was identified whether ATP or ADP could also prevent the NICE. In the presence of ATP or ADP, NICE was clearly prevented while NADH and Ψ_m were maintained. From the above results, NICE pathway was existed. HCEmito may not work in rat ventricular myocytes since the effects of pHc were opposite and Ca^{2+} flux did not change mitochondrial pH. Ca^{2+} influx pathway could be Ca^{2+} efflux pathway because RU360 attenuated Ca^{2+} efflux but could not prevented. ATP or ADP could effectively prevented NICE, suggested that the similar mechanisms of Ca^{2+}/Pi-induced Ψ_m depolarization may be responsible for NICE. This work was supported by the grant (R0005739) from KIAT.

Key Words: Mitochondria, Calcium, ATP, ADP, RU360

P04-11

Charged amino acids in the cytoplasmic c-terminal of TREK-2 K^+ channel for the sensitivity to intracellular pH

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TREK-2, a member of two-pore domain K^+ channel (K2P) family, are activated by various physicochemical stimuli including acidic intracellular pH (pH_i), for which proximal region of cytoplasmic c-terminal (Ct) is critical. Despite high homology with TREK-1, previous studies of the pH_i-sensing amino acids showed partly inconsistent results. Here we analyze the pH_i sensitivity of human TREK-2 (hTREK-2) with point mutations introducing neutralization of Glu and Lys residues in the proximal Ct. In inside-out (i-o) patch clamp conditions, pH_i 5.0-induced activation was absent in E332A, which is consistent with the reported E306A of rat TREK-1. Surprisingly, neutralization of a cationic Lys (K330A) also eliminated the pHi-sensitivity of hTREK-2, and the corresponding mutant in hTREK-1 (K315A) was also insensitive to acidic pH_i. Interestingly, $PI(4,5)P_2$ generation-mediated inhibition of hTREK-2 by intracellular ATP was also disappeared in both E332A and K330A of hTREK-2. In addition, 2-aminoethoxy diphenyl borate (2-APB, 100 uM)-induced activation of hTREK-2 was also absent in E332A and K330A. These results suggest that both Glu332 and Lys330 of hTREK-2 are critical for the pH_i-sensitivity, ATP-dependent inhibition and the pharmacological activation by 2-APB. The two amino acids might cooperatively interact with other charged amino acids or with $PI(4,5)P_2$, which can be affected by pH_i changes, leading to the conformational changes of TREK-2 activation.

Key Words: two-pore K^+ channel, TREK-2, intracellular pH, gating mechanism

P04-12

Hydrogen peroxide-induced up-regulation of TASK-5 two-pore domain K+ channel reduces death of breast cancer cells

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TWIK-related acid-sensitive K^+ (TASK)-5, a member of the two-pore domain K^+ (K_{2P}) channel family, is a silent channel at the plasma membrane. Online cancer microarray database shows that TASK-5 is highly expressed in breast cancer. This study was performed to identify the role of TASK-5 in human breast cancer cells. TASK-5 is dominantly expressed at the mitochondrial region. Among TASK channels (TASK-1, TASK-3, and TASK-5), TASK-5 showed the highest mRNA expression level in breast cancer cells (MCF-7 and MDA-MB-231). Its expression levels were similar between MCF-7 and MDA-MB-231. However, the TASK-5 protein levels in MDA-MB-231 were higher than those in MCF-7 cells. In addition, the protein expression levels in MCF-10A, a non-tumorigenic epithelial cell line, were high compared to those in MCF-7 cells. The TASK-5 mRNA expression was increased by treatment with hydrogen peroxide (H_2O_2) in a time and dose dependent manner. Treatment with H_2O_2 induced cell death, but the pattern among cells was different. Compared to MCF-10A, MDA-MB-231 cells were less sensitive to H_2O_2, indicating that cancer cells show hyposensitivity to reactive oxygen species (ROS) compared to normal cells. Interestingly, MCF-7 cells showed hypersensitivity in response to ROS, despite they are cancer cells. MCF-7 and MDA-MB-231 cells overexpressed with TASK-5 significantly proliferated and migrated compared to vector-transfected cells. These results show that TASK-5 expression levels are affected by H_2O_2 and the H_2O_2-induced up-regulation increases proliferation and migration. These results suggest that TASK-5 might act as an oncogene.

Key Words: Background potassium channel, Breast neoplasms, Cell proliferation, Reactive oxygen species

P04-13

A novel mechanism for activation of TRPC4 through modulation of positive electrostatic potential on the CIRB domain

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The activity of transient receptor potential (TRP) channel complexes is regulated via interactions with various binding partners. Ubiquitously expressed classical TRP (TRPC) 4 shows low basal activity and it is governed by many binding partners including $G\alpha_i$ and CaM which bind to the C-terminus domain of TRPC4. However, the functional architecture and activation mechanism of TRPC4 by these partners on the C-terminus domain still remain controversial. Here, we investigate the role of highly conserved CIRB (CaM- and IP_3R-binding) domain and report a structural mechanism of TRPC4 activation by electrostatic surface potential. We identified the critical residues for channel activity and further probed the primary mechanism of TRPC4 activation by Gαi proteins. Conserved Arg711, Lys715 and Arg716 residues on the CIRB domain face the same direction on the alpha-helix and give rise to an intensely positive surface electrostatic potential. Neutralization (attenuation) of the positive potential on these residues increased the intrinsic activity of TRPC4 channel. In addition, the decreased charge potential on CIRB with Gαi synergically activates TRPC4 channel. Furthermore, charge modulation by substitution with histidine residues supports our observation, revealing that the Gαi masks surface potential of positive cluster of R711/K715/R716 resulting in attenuated positive potential. Collectively, these findings suggest a novel mechanism of TRPC4 channels can be modulated by surface potential via protein/ligand interaction (Gαi, phosphoinositides, etc.) on the helical CIRB. It provides better understanding of TRPC family that district intrinsic activity and its character in different manners. **Acknowledgement:** This study was supported by grants from the National Research Foundation of Korea, which is funded by the Ministry of Science, ICT (Information & Communication Technology) and Future Planning (MSIP) (2015R1A2A1A05001756 to I. So) and the Ministry of Education (2015R1A6A3A04058395 to C. Hong) of the Korean government.
Key Words: TRP, Ca^{2+}, CaM, G protein, charge masking, deprotonation

P04-14

Regulation of TRPC4, TRPC5 homotetrameric and TRPC1/4, C1/5 heterotetrameric channel activity by $PI(4,5)P_2$ hydrolysis

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Transient receptor potential canonical (TRPC)4 and TRPC5 are subfamily of TRPC channels, known to be modulated by Gq-PLC pathway. Since phosphatidylinositide 4,5-biphosphate ($PI(4,5)P_2$) maintains TRPC4 and TRPC5 channels, Gq-PLC pathway inhibits channel activities through the hydrolysis of $PI(4,5)P_2$. Although many researches showed the effects of $PI(4,5)P_2$ on TRPC4, C5 homotetrameric channel, the effects of $PI(4,5)P_2$ on heterotetrameric channel of TRPC4, TRPC5 with TRPC1 were not known. Thus we investigated the difference in $PI(4,5)P_2$ sensitivity not only between the channel types but also between homomer and heteromer. First, by using a voltage-sensing phosphatase(DrVSP), we show that $PI(4,5)P_2$ dephosphorylation robustly inhibited not only TRPC4α, C4β, C5 homotetramer but also TRPC1/4α, C1/C4β, C1/C5 heterotetramer currents which were induced by Englerin A(-) (EA). Secondly, we used step pulse to observe the sensitivity of $PI(4,5)P_2$ dephosphorylation. Applying depolarizing step pulses increased currents when only channel is overexpressed but decreased currents after activation when channels and VSP were co-expressed. The channel inhibition was defined by subtracting the increasing current value of channel only from the decreasing current value of VSP and channels. The sensitivity to $PI(4,5)P_2$ dephosphorylation was similar between TRPC4β homomer(61.0 ± 2.6 mV) and TRPC1/C4β heteromer(58.7 ± 1.5 mV). On the other hand, there was sensitivity difference between TRPC5 homomer(48.0 ± 3.0 mV) and TRPC1/5 heteromer(86.6 ± 3.0 mV) or TRPC4α homomer(73.2 ± 1.8 mV) and TRPC1/C4α heteromer(87.54 ± 10.2 mV). The inhibited currents of TRPC4α homomer and TRPC1/4α heteromer were recovered faster than that of the other channels. Thirdly, we compared the effect of DrVSP on $PI(4,5)P_2$ dephosphorylation at high voltage (like +100 mV) with that at low voltage (-60 mV). The current inhibitions between homotetrameric and heterotetrameric channels were same at the high voltages during the step pulses and low voltage (-60 mV) after step pulses. Finally, we tested whether the metabolite of $PI(4,5)P_2$, PI(4)P, is involved in the current inhibition by DrVSP. Overexpressing PIP5-kinase with channels and VSP reduced the degree of current inhibition, suggesting that current inhibition is due to $PI(4,5)P_2$ depletion not the synthesis of PI(4)P. These results indicate a fundamental role for $PI(4,5)P_2$ in regulating TRPC1/4 and C1/5 heterotetramer activity as well as TRPC4, C5 homotetramer.
Key Words: TRPC, Gq-PLC pathway, $PI(4,5)P_2$, DrVSP

P04-15

Dual action of the Gαq-PLCβ-PI(4,5)P2 pathway on TRPC1/4 and TRPC1/5 heteromultimer

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The transient receptor potential canonical (TRPC) 1 channel is widely distributed in mammalian cells and is involved in many physiological functions, but its molecular function remains highly controversial. TRPC1 is primarily considered a regulatory subunit that forms heterotetrameric TRPC1/4 and TRPC1/5 channels to modify the pore properties of TRPC4 and TRPC5 and their activation by Gαq-coupled receptors. However, the molecular mechanism by which Gαq-PLC coupled receptors activate TRPC1/4 and TRPC1/5 and the role of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) in channel gating are unknown. Here, we reveal the self-limiting regulation of the heterotetramers by the Gαq-PLC pathway is dynamically mediated by PI(4,5)P2. We recorded channel activity and plasma membrane PI(4,5)P2, while manipulating Gαq activity and PI(4,5)P2 levels to conclude that following GPCR activation, Gαq directly binds to TRPC1/4 and TRPC1/5 channels, resulting in channel gating. Simultaneously, Gαq-coupled PLC activation results in the breakdown of PI(4,5)P2. Dissociation of PI(4,5)P2 from the channels inhibits the activated currents. The subsequent increase in cytoplasmic Ca2+ due to Ca2+ release from the ER and Ca^{2+} influx through the heterotetrameric channels and activation of PKC resulted in a second phase of channel inhibition. These findings provide

a molecular mechanism for how TRPC1 regulates the function of TRPC4 and TRPC5 that explains the physiological function of these channels.
Key Words: TRPC, GPCR, Gq, PIP2, Calcium

P04-16

The state-dependent inhibition of voltage-dependent K^+ channels by specific calmodulin inhibitor CGS 9343B in rabbit coronary arterial smooth muscle cells

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We investigated the effects of the calmodulin inhibitor CGS 9343B on voltage-dependent K^+ (Kv) channels using whole-cell patch clamp technique in freshly isolated rabbit coronary arterial smooth muscle cells. CGS 9343B inhibited Kv currents in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC_{50}) value of 0.81 μM. The decay rate of Kv channel inactivation was accelerated by CGS 9343B. The rate constants of association and dissociation for CGS 9343B were 2.77 ± 0.04 $\mu M^{-1}s^{-1}$ and 2.55 ± 1.50 s^{-1}, respectively. CGS 9343B did not affect the steady-state activation curve, but shifted the inactivation curve toward to a more negative potential. Train pulses (1 or 2 Hz) application progressively increased the CGS 9343B-induced Kv channel inhibition. In addition, the inactivation recovery time constant was increased in the presence of CGS 9343B, suggesting that CGS 9343B-induced inhibition of Kv channel was use-dependent. Another calmodulin inhibitor, W-13, did not affect Kv currents, and did not change the inhibitory effect of CGS 9343B on Kv current. Our results demonstrated that CGS 9343B inhibited Kv currents in a state-, time-, and use-dependent manner, independent of calmodulin inhibition.
Key Words: CGS 9343B, Calmodulin, Coronary artery, Voltage-dependent K^+ channel

P04-17

Shear stress induces transverse global Ca^{2+} waves via autocrine activation of P2X purinoceptors in rat atrial myocytes

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Atrial myocytes are exposed to high shear stress during blood regurgitation and high intra-atrial pressure due to valve diseases and heart failure, since such disturbances disrupt endocardium. We have previously reported that shear stress induces two types of global Ca^{2+} waves in atrial myocytes, longitudinal and transverse Ca^{2+} waves (T-waves),[1] and that the longitudinal wave is triggered by Ca^{2+} release via $P2Y_1$ purinoceptor-inositol 1,4,5-trisphosphate receptor signaling.[2] Here, we investigated cellular mechanism for the generation of T-wave in atrial cells under shear stress. Shear stress of ~16 dyn/cm^2 was applied onto single myocytes using micro fluid-jet, and two-dimensional confocal Ca^{2+} imaging was performed. Shear stress-induced T-waves were observed repetitively under 3-4 min intervals between the stimuli, and occurred at ~1 event per 10 s. They were eliminated by inhibition of the voltage-gated Na^+ or Ca^{2+} channels, or ryanodine receptors, suggesting that the T-wave is mediated by action potential-triggered Ca^{2+} release. Blockades of key stretch signaling molecules, stretch-activated channel, Na^+–Ca^{2+} exchange, and NADPH oxidase, did not suppress T-wave generation by shear. However, shear-induced T-wave generation was abolished by pre-incubation of cells with external ATP-metabolizing enzyme apyrase, the gap junction blocker carbenoxolone, or with P2X purinoceptor antagonist iso-PPADS. Inhibition of $P2Y_1$ purinergic signaling that mediates the longitudinal Ca^{2+} wave under shear did not attenuate the occurrence of T-waves. Our data suggest that shear stress induces activation of P2X purinoceptors via gap junction-mediated ATP release, thereby triggering action potential with subsequent T-wave in atrial myocytes.

References

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Key Words: Atrial myocyte, Shear stress, Gap junction hemichannel, P2X, Transverse Ca^{2+} waves

P04-18

Shear stress enhances Ca^{2+} spark occurrence via mitochondrial ROS generation and sarcoplasmic reticulum Ca^{2+} increase in rat ventricular myocytes

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It has been reported that shear stress enhances Ca^{2+}-induced Ca^{2+} release during depolarization in ventricular myocytes. To know molecular basis for the increase in global Ca^{2+} releases we assessed the effects of shear stress (~16 dyn/cm2) on the frequency of Ca^{2+} sparks and underlying mechanism for the shear-mediated Ca^{2+} spark regulation using confocal Ca^{2+} imaging in rat ventricular myocytes. The frequency of Ca^{2+} sparks was immediately (within 1 s) increased by shear stress by ~80%, and further increased by ~150% by prolonged (20 s) shear exposure. Inhibition of nitric oxide synthase (NOS) and interference of cytoskeletal integrity using L-NAME and colchicine, respectively, partially attenuated the prolonged shear-mediated enhancement in spark frequency. Blockade of Na^+–Ca^{2+} exchanger and L-type Ca^{2+} channel did not alter the shear effect on spark occurrence. Pretreatment of reducing agent significantly reduced the shear-mediated spark enhancement. Inhibitor of NADPH oxidase (NOX) diphenyleneiodonium and mitochondrially targeted antioxidant mito-TEMPO suppressed shear-mediated spark enhancements. Measurement of intracellular reactive oxygen species (ROS) revealed increase in ROS level by shear stress. Sarcoplasmic reticulum (SR) Ca^{2+} content was not altered immediately after shear application, but significantly increased after 20-s long shear exposure. Our data suggest that shear stress enhances the frequency of Ca^{2+} sparks partly by producing ROS via mitochondrial NOX, and that prolonged enhancement in spark frequency by shear stress may be also mediated by increase in the SR Ca^{2+} loading and by NOS. These mechanisms may explain the shear-mediated enhancement in Ca^{2+} transient in ventricular myocytes.
Key Words: Shear stress, Ca^{2+} spark, Reactive oxygen species, Sarcoplasmic reticulum, Ventricular myocytes

P04-19

Closed-state inhibition of voltage-dependent K+ channels by sertraline in rabbit coronary arterial smooth muscle cells

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We examined the effects of the selective serotonin reuptake inhibitor (SSRI) sertraline on voltage-dependent K^+ (Kv) channels in native coronary arterial smooth muscle cells using the voltage-clamp technique. Sertraline decreased the Kv channel current in a dose-dependent manner, with an IC_{50} value of 0.18 μM and a slope value (Hill coefficient) of 0.61. Although the application of 1 μM sertraline did not affect the steady-state activation curves, sertraline caused a significant, negative shift in the inactivation curves. Pretreatment with another SSRI, paroxetine, had no significant effect on Kv currents and did not alter the inhibitory effects of sertraline on Kv currents. From these results, we concluded that sertraline dose-dependently inhibited Kv currents independently of serotonin reuptake inhibition by shifting inactivation curves to a more negative potential.
Key Words: Sertraline; Coronary artery; Voltage-dependent K^+ channel; Serotonin reuptake inhibition

P04-20

Regulation of calcium and rhythm in atrial cells via autocrine activation of $P2X_4$ purinoceptors during shear stress

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Atrial myocytes are exposed to high shear stress during pressure overload caused by heart failure, valve diseases, and hypertension. It has been previously shown that shear stress induces spontaneous transverse Ca^{2+} waves in isolated atrial myocytes, and that such waves are sensitive to gap junction hemichannel blockade and P2X receptor antagonist. These observations suggest that P2X receptors may be activated by shear-induced ATP release in atrial cells, thereby inducing arrhythmogenesis. This possibility has been tested in this thesis using autorhythmic adult atrial cell line HL-1 and genetic knock down (KD) of the most prominent P2X receptor subtype in atrial cells. $P2X_4$ receptors were found to be the most abundant P2X receptor subtype expressed in both rat atrial myocytes ($P2X_4 > P2X_5 > P2X_{2,3,6,7}$) and HL-1 cells, while $P2X_7$ receptors were only expressed in HL-1 cells ($P2X_7 > P2X_4 > P2X_{5,3}$). Chemiluminescence measurement for extracellular ATP revealed that shear stress (~16 dyn/cm^2) induced transient release of ATP from HL-1 cells. To isolate P2X receptor-mediated response, the $P2Y_1$ receptor that mediates inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} wave during shear, was inhibited by MRS 2179 (200 nM). Under these conditions, the effects of shear stress of ~16 dyn/cm^2 on autorhythmic Ca^{2+} signaling were assessed in wild-type (WT) and $P2X_4$ knock-down (KD) HL-1 cells using two-dimensional confocal Ca^{2+} imaging. Cycle length and magnitude of Ca^{2+} transients were not different between WT and $P2X_4$ KD cells under control conditions. Shear stimulus in the presence of MRS2179 induced an increase in the diastolic Ca^{2+} level with reduction in the Ca^{2+} transients in both WT and KD cells. Interestingly, cycle length of Ca^{2+} transients was decreased by shear exposure in WT, but not in $P2X_4$ KD cells. The connexin 43 KD using specific siRNA almost completely suppressed ATP release of HL-1 cells under shear stress. These results suggest that ATP release through the connexin 43 channels may activate $P2X_4$ receptors, functionally expressed in HL-1 cells, and that $P2X_4$ receptors may be responsible for the shear-induced tachyarrhythmic Ca^{2+} cycling in atrium.
Key Words: shear stress, $P2X_4$ purinoceptors, connexin 43 channels, rhythmic Ca^{2+} transient, HL-1 cells

P04-21

Hydrogen peroxide-induced intracellular calcium accumulation through plasma membrane Ca^{2+}-ATPase inactivation in mouse parotid acinar cells

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Intracellular calcium mobilization is intimately involved in initiating salivary secretion in parotid acinar cells. Reactive oxygen species are known to be related to a variety of oxidative stress-induced cellular disorders and believed to be involved in salivary impairments. In this study, we investigated the mechanism of hydrogen peroxide on intracellular calcium accumulation in mouse parotid acinar cells. The perfusion of hydrogen peroxide (0.1-10 mM) resulted in a dose-dependent elevation of intracellular calcium levels in the presence of normal extracellular calcium. In a calcium-free medium, hydrogen peroxide still enhanced the intracellular calcium level. Inositol 1,4,5-trisphosphate-induced calcium release from the intracellular calcium store was not affected by hydrogen peroxide perfusion in the permeabilized cells. Calcium extrusion through plasma membrane calcium ATPase (PMCA) was remarkably blocked by hydrogen peroxide in thapsigargin-treated intact acinar cells. The perfusion of antioxidants, either catalase or dithiothreitol, completely restored hydrogen peroxide-induced calcium accumulation through PMCA inactivation. These results provide evidence that excessive generation of hydrogen peroxide in pathological conditions could attenuate calcium extrusion through PMCA, rather than mobilize Ca^{2+} ions from extracellular mediums or intracellular stores in mouse parotid acinar cells.
Key Words: reactive oxygen species, hydrogen peroxide, calcium, plasma membrane calcium ATPase, parotid acinar cells

P04-22

Down-Regulation of THIK-1 Expression in Inflammatory Pain and Asthma

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The overall knowledge about the biophysical, pharmacological and physiological properties of THIK-1, a small conductance two-pore domain K^+ (K_{2P}) channel, remains far behind those of other K_{2P} channels. Here, we studied the relationship between THIK-1 and inflammation. RT-PCR and Western blot analyses showed that THIK-1 was expressed in macrophage and lung. The THIK-1 mRNA expression levels were

decreased in thioglycollate-elicited peritoneal macrophage and lung obtained from ovalbumin (OVA)-induced allergic asthma model. Treatment with pro-inflammatory mediators decreased THIK-1 mRNA in macrophages. In addition, overexpression of THIK-1 reduced pain behavior in formalin-induced inflammatory pain model. These results show that THIK-1 is regulated by inflammatory signals. We suggest that THIK-1 might act as a target for inflammation-dependent injury and pain.

Key Words: asthma, background K^+ channel, inflammation, pain

P04-23

Intracellular calcium dependent regulation of sperm-specific calcium activated potassium channel, hSlo3 by BKCa activator LDD175

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Plasm membrane hyperpolarization associated with calcium activated potassium channel activation plays an important role in sperm capacitation for fertilization. However its molecular identity is still controversial in human sperm. In this study, classical BK_{Ca} activators, NS1619 and LDD175 were tested on human Slo3, which known a Ksper molecular identity in mouse spermatozoa. Human Slo3 and its functional interacting γ2 subunit (hLRRC52) were heterologously transfected in HEK293 cell. As reported, iberiotoxin and 4-AP had not affected Slo3 K^+ current while 20mM TEA inhibited 50% of it. Extracellular alkalization still potentiated Slo3 K^+ current and internal alkalization and calcium can shift its activation voltage. NS1619, which modulate gating of Slo1 intracellularly, attenuated Slo3 K^+ current while LDD175 increased Slo3 K^+ current and hyperpolarized membrane potential. The potentiation by LDD175 was not associated with altering of half activating voltage in different intracellular pH, pH7.3 and pH8.0. In contrast, elevation of intracellular calcium dramatically enhanced LDD175 induced left shifting of half activation potential of hSlo3. Therefore, the mechanism of action won't modulate pH dependent gating of hSlo3, instead, LDD175 may modulate calcium dependent activation of hSlo3. Thus, LDD175 potentially activate native Ksper and may induce membrane hyperpolarization associated hyperactivation in human sperm. **Acknowledgements:** This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Minstry of Education (NRF-2015R1D1A1A01059590)

Key Words: Calcium activated potassium channel, Sperm motility, Membrane hyperpolarization, Channel modulator

P04-24

The regulatory role of rolipram on isolated primary mouse submandibular gland cells

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Exposure to bacterial lipopolysaccharides (LPS) induces inflammatory signals in salivary glands. We investigated the regulatory role of phosphodiesterase 4 (PDE4) inhibitor rolipramon inflammatory mediators and cholinergic/adrenergic stimulation-induced intracellular Ca^{2+} signaling in salivary acinar and ductal cells. Submandibular gland (SMG) expressed PDE4A through 4D mRNA and PDE4 was localized in the luminal membrane of SMG. LPS induced Ca^{2+} signaling and ROS production in SMG. Treatment with rolipram blocked LPS-induced Ca^{2+} increase and ROS production. The application of histamine evoked Ca^{2+} signals and ROS production, which were attenuated by rolipram in SMG cells. Moreover, LPS-induced NLRP3 inflammasome and cleaved caspase-1 were inhibited by rolipram. The inhibitory role of rolipram in ROS-induced Ca^{2+} signaling was mainly observed in acinar cells and not in ductal cells. Rolipram also protected SMG acinar but not ductal cells from LPS-induced cell membrane damage. In the case of cholinergic/adrenergic stimulation, carbachol/isoproterenol-induced Ca^{2+} signals were upregulated by the treatment of rolipram in SMG. In the case of cAMP-dependent ductal bicarbonate secretion by rolipram, no effect was observed on the modulation of ductal chloride/bicarbonate exchange activity. Rolipram could suppress the inflammatory signals and could be a potential therapeutic strategy against LPS-induced inflammation to protect the salivary gland cells.

Key Words: Rolipram, submandibular gland, calcium signal, LPS

P04-25

Gαi-mediated TRPC4 activation by polycystin-1 contributes to the endothelial function via STAT1 activation

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Polycystin-1 (PC1) regulates a number of cellular processes (ex. heterotrimeric G protein, transcription factor etc.) through the formation of complexes with the polycystin-2 (PC2) ion channel or with other signal transduction proteins. *PKD1* and *PKD2* (encoding PC1 and PC2) are the most commonly mutated genes in autosomal dominant polycystic kidney disease (ADPKD). These consist of cyst formation in other ductal organs and vascular abnormalities including aneurysms in the cerebral and coronary blood vessels. Although Ca^{2+} modulation by polycystins has been reported between transient receptor potential (TRP) channels, the function with TRPC subfamily regulated by G-protein signaling has remained elusive. We have previously reported that TRPC4 channel can be activated by $G\alpha_i$-coupled receptors. We assumed that PC1 might act as a Gαi-coupled receptor. Here, we identified that PC1 dominantly interacts with $G\alpha_{i3}$ using co-IP. Thus we recorded the activity of TRPC4β co-expressed with PC1 in HEK293 cells. PC1 activated TRPC4β channel (4 ± 1 → 41 ± 14 pA/pF) by modulating G-protein signaling. Intracellular 0.2 mM GTPγS-induced TRPC4β activation was not significantly different in the presence or absence of PC1. C-terminal fragment (CTF) of PC1 did not affect TRPC4β activity due to loss of N-terminus containing G-protein coupled receptor proteolytic site (GPS). Dominant negative $G\alpha_{i3}$ (G202T) mutant inhibited PC1-activated TRPC4β current. We investigated whether TRPC4β induces activation of STATs (signal transduction and transcription), leading to cell proliferation or death. We observed that STAT1 and STAT3, but not STAT6 activation by PC1 is independent on Src kinase cascades. When PC1 co-expressed with TRPC4β, STAT1 activation was further increased compared to each sole expression. To determine the role of PC1 with TRPC4 activation in endothelial cell migration, we performed a loss of function screening assay and a wound-

healing assay in HUVECs (human umbilical vein endothelial cells). The downregulation of PC1 and TRPC4 activity by the PC1 knockdown or TRPC4 antagonist inhibited the migration of HUVECs. Also the knockdown of STAT1 reduces endothelial cell migration, but not STAT3. The results of this study strongly suggest that the altered TRPC4 activity by polycystin-1 mutation contributes to endothelial dysfunctions. Our findings indicated an important function between PC1 and TRPC4β in modulation of intracellular Ca^{2+} signaling and provided a new potential therapeutic approach targeting TRPC4β channel in vascular diseases.
Key Words: Polycystin-1, TRPC4, endothelial cell, aneurysm, ADPKD

P04-26(PO-01)

TRPC channels are a novel culprit for hepatic stellate cell activation and hepatic fibrosis

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Deregulation of Ca^{2+} signaling has been postulated as pathological event in fibrosis. A primary culprit for hepatic fibrosis is hepatic stellate cell (HSC) activation. Aberrant Ca^{2+} influx mediates either directly or indirectly HSC activation and leads to increase the expression of fibrosis markers such as α-smooth muscle actin and/or profibrotic ligand TGFβ causing hepatic fibrosis. Thus, we firstly monitored Ca^{2+}-permeable channels in *in vivo* hepatic fibrosis animal models by bile duct ligation (BDL) and thioacetamide (TAA) administration. We found that TRPC1 and TRPC6 channels were overexpressed in cirrhotic models whereas expressions of voltage-gated and store-operated Ca^{2+} channels were not altered. Notably, we found that expression level of TRPC channels were strongly correlated with Laennec scoring system for stating fibrosis in human liver biopsy specimens. To uncover underlying mechanism for TRPC-mediated fibrogenesis, we monitored TRPC6 activation in *in vivo* animal models and *in vitro* HSC activation model using primary HSCs culture. Expression of TRPC6 was significantly increased in hepatic fibrosis animal models and primary HSC model. Transgenic overexpression of TRPC6 in mouse liver *in vivo* induced *de novo* expression of fibrosis markers in liver. Functionally, fibrotic changes and Ca^{2+} influx were ameliorated by suppressing TRPC6 in *in vivo* animal model and *in vitro* HSCs, respectively. Together, these data demonstrate that TRPC6-mediated Ca^{2+} influx causes hepatic fibrosis, at least in part, via HSC activation. These provide a new perspective on the pathogenesis of hepatic fibrosis and offer clues for therapeutic strategies for liver cirrhosis. [This study was supported by NRF-2015R1D 1A1A01060454]
Key Words: TRPC6, Ca^{2+} signaling, Hepatic fibrosis, Hepatic stellate cell (HSC)

P04-27(PO-02)

Down-regulation of NKCC and AE2 transporters in salivary gland cells by dexmedetomidine

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Dexmedetomidine (Dex), a highly selective α2-adrenoceptor agonist, has been shown to attenuate inflammatory responses induced by lipopolysaccharide (LPS) and induce sedative and analgesic effects. Administration Dex has been shown to reduce salivary flow in human subjects and inhibit osmotic water permeability in the rat cortical collecting duct. However, little is known about the regulatory effects of Dex on salivary fluid secretion. To investigate fluid secretion upon treatment with Dex, we studied the effects of Dex on secretion in salivary glands by the regulation of ion transporters such as Na^+-K^+-$2Cl^-$ cotransporter (NKCC) and Cl^-/HCO_3^- exchanger (CBE) and downstream pro-inflammatory cytokine expression in isolated primary mouse submandibular glands (SMG). Dex increased CBE and NKCC activities in SMG ducts and enhanced salivary fluid secretion in the sealed duct system. It showed differential effects on neurotransmitter inputs and inflammatory mediator-induced Ca^{2+} signaling in mouse SMG cells. Histamine and LPS-induced Ca^{2+} signals were inhibited but not carbachol-stimulated Ca^{2+} signals. Long-term Dex treatment for 2 hrs reduced CBE and NKCC activities in SMG and HSG cells. Moreover, when isolated SMG cells were stimulated Dex for 2 hrs, phosphodiesterase 4D (PDE4D) expression was enhanced. The obtained results confirm the anti-inflammatory properties of Dex against LPS-mediated signaling; Dex also inhibited interleukin-6 and NADPH oxidase 4 expression. α2-Adrenoceptor-mediated inhibition of inflammatory signaling was involved in reduced NADPH oxidase 4 expression. The present study showed that the role of α2-adrenoceptor activation by Dex on the hyposecretion of salivary glands could be related to the involvement of PDE4D and subsequently reduced cAMP level.
Key Words: NKCC, AE2, salivary gland, dexmedetomidine

P04-28(YP-03)

Mechanism of hERG current inhibition by 4-hydroxynonenal (4-HNE), a polyunsaturated fatty acid-derived electrophile

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Under oxidative stress, peroxidation of ω6-polyunsaturated fatty acids produces 4-Hydroxynonenal (4-HNE), a highly reactive electrophile forming 4-HNE-protein adducts. Here we investigate the effects of 4-HNE on cardiac hERG current that is a major target of arrhythmogenic conditions. Acute application of 4-HNE (30~100 μM) gradually decreased the tail current of hERG ($I_{hERG,tail}$), which was reversed by TCEP, a membrane impermeable reducing agent. The action potential duration (APD) of guinea-pig ventricular myocytes (GPVMs) was prolonged by 100 μM 4-HNE. Chronic incubation (1-3 h) with lower concentration of 4-HNE (10 μM) induced non-reversible, time-dependent suppression of $I_{hERG,tail}$ and prolongation of APD in GPCMs, which was not reversed by TCEP treatment. Western blot analysis revealed that mature glycosylated hERG (155 kDa) is reduced by chronic 4-HNE (10 μM, 1-12 h). In total cell preparations, the decrease of mature hERG was partly rescued by proteasome- and lysosome-dependent degradation inhibitors, bortezomib and bafilomycin, respectively. However, reduction of plasma membrane hERG proteins in the plasma membrane fraction and the chronic inhibition of IhERG,tail by 4-HNE were not restored by bortezomib and bafilomycin. Taken together, it is suggested that the electrophilic binding of 4-HNE with extracellular domain acutely inhibits hERG activity. Also, the sustained exposure to 4-HNE may accelerate the endocytosis and degradation of hERG proteins through proteasome- and lysosome-dependent process. The

suppression of hERG by 4-HNE may participate in proarrhythmic effects of endogenous lipid peroxidants under pathological conditions.
Key Words: hERG channel, electrophile, 4-hydroxynonenal, protein degradation

P04-29(YP-05)

Ethanol inhibition of KCNQ2/3 channel is regulated by plasma membrane PI(4,5)P_2

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KCNQ2/3 channel is known as M-type K^+ channel which is suppressed by muscarinic receptor stimulation. These channels broadly expressed in the central nerve systems including ventral tegmental area (VTA) dopamine neurons. Recently, it is reported that ethanol inhibits M-current in VTA neurons, and thus increases excitability of the cells and may reinforce the brain reward system. However, the molecular mechanism of the ethanol regulation of KCNQ2/3 channels and the effects on action potential firing in neurons have not been studied well. In this study, we investigated the mechanism by which alcohol modulate the KCNQ2/3 channel activity and neuronal excitability. We found that in superior cervical ganglia (SCG) neurons 200 mM ethanol inhibits M-current by ~20% and increases action potential firing about 1.5-fold. The alcohol inhibition was dependent upon carbon chain length and conformation of alcohols in tsA201 cell (200 mM methanol, ~18%; ethanol, ~22%; 2-propanol, 25%). The alcohol inhibition occurred rapidly (τ = < 4 s). We also found that the ethanol inhibition was decreased when the cells were co-transfected with PIPKIγ (~8%), suggesting that membrane phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) is an important regulator of the alcohol regulation of M-channels. In addition, KCNQ2 and KCNQ3 homomeric channel showed different alcohol sensitivity (~40% and 10%, respectively, at 200 mM ethanol). Therefore, these results indicate that alcohols could play an important regulator of neuronal excitability. We expect that understanding the mechanism will give some evidences to develop alcohol-selective therapy for overcoming or preventing alcohol addiction.
Key Words: KCNQ2/3 channel, Ethanol, Neuron, PI(4,5)P_2

P04-30(YP-09)

ATP release via gap junction hemichannels in rat atrial myocytes under shear stress

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We have previously shown that shear stress (~16 dyn/cm^2) induces longitudinal Ca^{2+} propagation wave with a 0.2- to 3-s delay in atrial myocytes through the activation of $P2Y_1$ purinergic signaling, and that the shear-mediated wave generation is inhibited by the blockade of gap junction hemichannels.[1] In the present study, we further examined whether atrial cells release ATP via the gap junction hemichannels. ATP releases of cells were directly measured with 2-s intervals as chemiluminescence emitted (at 562 nm) during ATP-driven luciferin-luciferase reaction. Shear stress was applied to fields of cells in a laminar flow chamber attached to the flow regulator. ATP was released from atrial myocytes by the application of shear stress in the range of 1-16 dyn/cm^2 in a strength-dependent manner. The shear-induced ATP release occurred transiently with a maximal release (~1.3 moles/μm^2) at about 2 s and 90% decay at 4-6 s after the shear application (~16 dyn/cm^2 for 10 s). Removal of external Ca^{2+} to enhance the activity of the gap junction hemichannels increased the shear-mediated ATP release to ≅ 320% of control. In cells pre-treated with carbenoxolone (50 μM for 10 min) or La^{3+} (2 mM for 5 min), the hemichannel blockers, ATP release by shear application was suppressed by ~50% and ~90%, respectively. The shear-induced ATP release was not affected by the pre-treatment of 50 μM Gd^{3+} (5 min) or 1 mM 9-anthracenecarboxylic acid (10 mM), suggesting no role of maxi anion channel and Cl^- channels in shear-induced ATP release in these myocytes. Our data suggest that shear stress induces ATP release through the gap junction hemichannels in atrial myocytes.

Reference

1) JC Kim and SH Woo. Shear stress induces a longitudinal Ca^{2+} wave via autocrine activation of P2Y1 purinergic signalling in rat atrial myocytes. *J Physiol* 593(23):5091-5109 (2015).

Key Words: Atrial myocyte, Shear stress, ATP, Gap junction hemichannel, Chemiluminescence

P05-01

The level of nitric oxide regulates lipocalin-2 expression under inflammatory condition in RINm5F beta-cells

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We previously reported that proinflammatory cytokines (interleukin-1β and interferon-γ) induced the expression of lipocalin-2 (LCN-2) together with inducible nitric oxide synthase (iNOS) in RINm5F beta-cells. Therefore, we examined the effect of nitric oxide (NO) on LCN-2 expression in cytokines-treated RINm5F beta-cells. Additionally, we observed the effect of LCN-2 on cell viability. First, we found the existence of LCN-2 receptor and the internalization of exogenous recombinant LCN-2 peptide in RINm5F and INS-1 beta-cells. Next, the effects of NO on LCN-2 expression were evaluated. Aminoguanidine, an iNOS inhibitor and iNOS gene silencing significantly inhibited cytokines-induced LCN-2 expression while sodium nitroprusside (SNP), an NO donor potentiated it. Luciferase reporter assay showed that transcription factor NF-κB was not involved in LCN-2 expression. Both LCN-2 mRNA and protein stability assays were conducted. SNP did not affect LCN-2 mRNA stability, however, it significantly reduced LCN-2 protein degradation. The LCN-2 protein degradation was significantly attenuated by MG132, a proteasome inhibitor. Finally, the effect of LCN-2 on cell viability was evaluated. LCN-2 peptide treatment and LCN-2 overexpression significantly reduced cell viability. FACS analysis showed that LCN-2 induced the apoptosis of the cells. Collectively, NO level affects LCN-2 expression via regulation of LCN-2 protein stability under inflammatory condition and LCN-2 may reduce beta-cell viability by promoting apoptosis.
Key Words: Lipocalin-2, Nitric oxide, Interleukin-1β, Interferon-γ, RINm5F cells

P05-02

Inhibition of neddylation facilitates cell migration through enhanced phosphorylation of caveolin-1 in PC3 and U373MG

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Protein neddylation is a post-translational modification by a covalent conjugation with the neural precursor cell expressed, developmentally downregulated 8 (NEDD8). Although this process has been reported to participate in diverse cellular signaling, little is known about its role in cancer cell migration. Given a recent proteomics report showing that NEDD8 is downregulated in prostate cancer tissues versus normal prostate tissues, we tested the possibility that neddylation plays a role in cancer evolution, and then tried to identify target proteins of the neddylation. The neddylation process was inhibited by transfecting cancer cells with NEDD8-targeting siRNAs or by treating the cells with a NAE1 inhibitor MLN4924. Cell migration was evaluated by an *in vitro* wound-healing assay and a Transwell migration assay. His/NEDD8-conjugated proteins were pulled down with nickel-affinity beads under a denaturing condition, and identified by Western blotting. Caveolin-1, which plays a critical role in cell migration, was identified to be conjugated with NEDD8. When the neddylation was inhibited, the phosphorylation of caveolin-1 at Tyr14 was augmented in PC3 and U373MG cells, thereby leading to increased cell migration. Such consequences by neddylation inhibition were abolished in the presence of a Src family kinase inhibitor PP2. NEDD8 seems to inhibit the Src-mediated phosphorylation of caveolin-1 by modifying the structure of caveolin-1 protein, which blocks the migration of cancer cells. Although the neddylation process is currently regarded as an emerging target for cancer therapy, our results suggest the possibility that the inhibition of neddylation could facilitate cancer invasion or metastasis at least in some types of cancers.

Key Words: Neddylation, NEDD8, Caveolin-1, Prostate cancer

P05-03

Necrox-5 attenuates ischemic heart injury via regulation of mitochondria biogenesis and inflammation response

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The aim of this study is to verify the effect of Necrox-5 on cardiac proteomic alteration and mitochondrial biogenesis, inflammation and fibrosis responses in a hypoxia-reoxygenation (HR) treated rat heart. Necrox-5 treatment (10 μM) and non-treatment were employed on isolated rat hearts during hypoxia/reoxygenation treatment using an *ex vivo* Langendorff system. Proteomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS) and non-labeling peptide count protein quantification. Proteomic results were confirmed by western blot and real-time PCR experiment. Mitochondrial functions and inflammation responses were analyzed by using complex activity assay, cytotoxicity assay, and cytokine ELISA assay were then assessed. HR treatment significantly decreased level of mitochondria biogenesis related proteins and increased level of pro-inflammatory proteins. NecroX-5 treatment significantly attenuated those HR-induced proteomic alterations, especially which are involved in oxidative phosphorylation and metabolic function. NecroX-5 post-hypoxic treatment also improved mitochondrial complex activities. Markedly higher PGC1α expression levels were found in NecroX-5 treatment group. In addition, HR- or LPS-induced TNF-α and TGF-β1 productions were significantly attenuated with NecroX-5 supplement. Furthermore, NecroX-5 suppressed phosphorylation of Smad2 production and increased expression level of decorin. The findings suggested the cardioprotective effect of NecroX-5 against cardiac HR injuries by modulating mitochondrial biogenesis and exerting anti-inflammation and anti-fibrosis effects. [This work was supported by the National Research Foundation of Korea (NRF), and the funding was granted by the Ministry of Science, ICT & Future Planning of Korea (2012R1A2A1A03007595) and by the Ministry of Education of Korea (2010-0020224)]

Key Words: NecroX5, Ischemic heart injury, Mitochondria, Inflammation

P05-04

Fetuin-B affects atherosclerotic plaque stability by regulating adipogenic differentiation of human mesenchymal stem cells

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Myocardial infarction is involved in the rupture of an atherosclerotic plaque that is one of the common causes of thrombotic occlusion in coronary artery. We reported that fetuin-B may participate in the development of myocardial infarction by decreasing atherosclerotic plaque stability in previous study using various cells. However, the possible role of fetuin-B in correlation between mesenchymal stem cell and atherosclerotic plaque stability remains to be elucidated. In this study, we investigated whether fetuin-B affects human mesenchymal stem cell (hMSCs) event associated with the rupture of an atherosclerotic plaque. Expression level in fetuin-B was enhanced in the atherosclerotic plaque. *In vivo* administration of recombinant human fetuin-B resulted in increased plaque rupture and the increased number of Sca1 (a stem cell maker)-positive cells in the atherosclerotic plaque. Treatment with recombinant human fetuin-B induced the increase in migration and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation and the decrease in cofilin phosphorylation in hMSCs. In addition, recombinant human fetuin-B also elevated the deposition of lipid, a vascular plaque-stabilizing risk factor, by upregulating the expression of adipogenic differentiation-linked factors of hMSCs. These results indicate that fetuin-B may participate in the change of atherosclerotic plaque stability by modulating adipogenic differentiation of hMSCs

Key Words: Atherosclerotic plaque stability, Fetuin-B, Human mesenchymal stem cell, Adipogenic differentiation

P05-05

Wnt, GSK-3β, JNK, and PKC pathway signaling regulates neural induction of human bone marrow-derived mesenchymal stem cells

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) are characterized by multipotency and self-renewal, and are responsible for tissue regeneration and repair. Following neural induction with supplementary factors, hBM-MSCs were differentiated into various types of neural cells *in vitro*. Understanding of multiple signal inputs to control of differentiation is important and the related mechanisms are not clear yet. We hypothesized that the Wnt and MAPK signaling pathway control stem cell maintenance and neural differentiation. We have observed the transcriptional expression of Wnt in neural induced-hBM-MSCs (NI-hBM-MSCs) compared to primary hBM-MSCs by RT-PCR, western blot, and immunocytochemistry assay. The expression of Wnt2 was decreased, but the expression of Wnt1, Wnt4, Wnt5a, and Wnt11 were increased after neural differentiation. In addition, the expression levels of Fzd4 and Fzd5 were increased, but not most of Fzds and LRP5/LRP6 ligand in neural differentiation. There were no changes in the expression of β-catenin. Interestingly, Wnt4, Wnt5a, and Wnt11 expressions were highly increased in NI-hBM-MSCs by real-time RT-PCR and western blot analysis. Finally, we found that the GSK-3β, JNK, and PKC expression levels were increased after neural induction and ERK and PI3K levels were decreased. Thus, this study shows how non-canonical Wnt ligands can regulate the neural differentiation through the activation of GSK-3β, JNK, and PKC pathway by binding Fzd4 and Fzd5 and directing Axin/GSK-3β in mesenchymal stem cells.

Key Words: Mesenchymal stem cell, Neural differentiation, Non-canonical Wnt pathway, GSK-3β, JNK, PKC

P05-06

Blockage of neddylation elevates c-Src stability and facilitates cancer cell migration

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c-Src, a well-known proto-oncogene, is activated or overexpressed in 50% of various tumors. As an upstream kinase of many signal pathways, c-Src mediates tumor progression properties including proliferation, angiogenesis, adhesion, and migration. It is well-characterized that the stability of active c-Src is regulated by ubiquitination. However, other post-translational modifications of c-Src are still unknown. Here, we figured out how the c-Src is regulated by another protein modification, neddylation. Conjugation of NEDD8 to c-Src is mediated by E3 ligase, c-Cbl, which is also act as an ubiquitin-E3 ligase of c-Src. Inhibition of c-Src neddylation markedly blocked its ubiquitination. Also blockages of neddylation by neddylation inhibitor MLN4924 or si-NEDD8 and si-APPBP1 elevated c-Src not only stability but also activity, promoting cancer cell migration and invasion. Inhibition of c-Src by siRNAs or PP2 significantly rescued the effect of neddylation defect, suggesting cancer cell migration through the change of neddylation is c-Src dependent. Taken together, c-Src was identified as a novel substrate for neddylation. c-Src neddylation is important for regulation of ubiquitination-dependent degradation pathway and cancer cell motility. Our study provided a new insight about the effect of c-Src modification in tumor progression

Key Words: Non-receptor tyrosine kinase, c-Src, Neddylation, Ubiquitination, Cell migration

P05-07

Ethanol extract of *Brassica rapa* subsp. *Pekinensis* suppress TNF-α-induced inflammatory response in human umbilical vein endothelial cells

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Brassica rapa L. ssp. *Pekinensis*, Chinese cabbage is one of the cruciferous vegetables that is traditionally ingested in the east Asia. Although its habitual consumption could be counted low incidence rate of chronic vascular inflammation, and effective compositions were already reported by species, protective and therapeutic and protective potentials of phytochemicals from Chinese cabbage has been poorly studied. In this present study, we identified polyphenols, gallic acid, kaempferol and quercetin from ethanol extraction of *B. rapa* L. (EtBR). We demonstrate for the first time that EtBR containing effective phytochemicals suppresses TNF-α-induced inflammatory response in human umbilical vein endothelial cells. The EtBR inhibited TNF-α-induced monocyte adhesion to endothelial cells in a dose-dependent manner. The anti-adhesive activity of EtBR directly correlated with suppressed expression and transcription of vascular cell adhesion molecule-1 (VCAM-1). When considered together, the results suggest that EtBR inhibit expression of TNF-α-induced adhesion molecules through the direct transcriptional modulation of *VCAM-1* in endothelial cells. In conclusion, we exhibit that ingestion of vegetables for a long time containing dietary phytochemicals might be a potential therapeutic strategy to protect against various stressors, preventing several pathological conditions and to treat chronic vascular inflammation, such as atherosclerosis.

Key Words: Chinese cabbage, Inflammatory response, *Brassica rapa*, Ethanol extract, HUVEC

P05-08(PO-03)

A Jumonji C (JmjC) domain-containing protein negatively regulates RANKL-mediated osteoclastogenesis

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The regulation of osteoclastogenesis is critical to maintain physiological bone homeostasis and prevent bone-destructive diseases. The nuclear factor of activated T-cells calcineurin-dependent 1 (NFATc1) plays an essential role in osteoclastogenesis, and its expression is induced during early osteoclastogenesis. On the other hand, the Jumonji C (JmjC) domain-containing protein (JHDM), a histone demethylase, catalyzes

histone 3 lysine 9 and is involved in osteoblastic bone formation. However, the mechanism for regulation of the enzymatic activity of JHDM in osteoclastogenesis is not yet well known. Here, we show that JHDM is a key negative regulator during receptor activator of nuclear factor-κB ligand (RANKL)-induced osteoclastogenesis. The expression level of JHDM gradually decreased during osteoclastogenesis in bone marrow macrophages (BMMs) treated with RANKL. Down-regulated expression of JHDM strongly facilitated osteoclast formation together with induction of several osteoclast-specific genes such as TRAP, Oscar and CathepsinK. NFATc1 proteins are ubiquitinated and rapidly degraded during late stage osteoclastogenesis. Interestingly, overexpression of JHDM induces NFATc1 degradation during late stage osteoclastogenesis. Taken together, the present study demonstrated that JHDM is a post-translational co-repressor for NFATc1 that attenuates osteoclastogenesis.
Key Words: Jumonji C (JmjC) domain-containing protein (JHDM), NFATc1, Stability, Osteoclastogenesis

P05-09

The role of 18-kDa translocator protein on protein kinase C-induced endothelial activation in HUVECs

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

P05-10

Pharmacologic activation of PGC-1α via AKT/mTOR signaling improves mitochondrial biogenesis in skeletal muscle cell mouse model

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Cancer wasting, also called cancer cachexia is marked by weakness and the progressive loss of body weight, fat, and muscle. The condition is responsible for 20-30% of cancer deaths and is currently untreatable. Knowing how tumors cause muscle loss could lead to life-saving treatments. Recently, studies have focused on addressing these by using drugs to target the mitochondria such as HS1793, a novel and potent analogue of resveratrol. This study aimed to determine how HS1793 takes effect in mouse myoblast C2C12 cells, and determine how the compound regulates mitochondrial biogenesis that could possibly lead to improved muscular conditions caused by oxidative stress and cancer drug treatment. Screening of various doses of HS1793 was performed in cultured C2C12 cells by evaluating for cytotoxicity and cell proliferation. Preliminary results show that dosages higher than 10 μM are detrimental to the cells, and were anti-proliferative. The succeeding experiments used dosages lower than 10 μM. Mitochondrial mass, mitochondrial membrane potential, reactive oxygen species (ROS) level, and mitochondria biogenesis-regulated genes were analyzed to determine the effects on mitochondrial biogenesis. HS1793 reduced ROS generation, but treatment did not interfere with cellular viability at low dosages. HS1793 also enhanced mitochondrial biogenesis function by increasing cellular and mitochondrial ATP synthesis function, but induced multinucleation in cells as an adaptive response. HS1793 also upregulated vital mitochondrial biogenesis-related genes such as PGC1-α, activated by AKT and mTOR, which are considered as important regulators of skeletal muscle function. When taken altogether, it shows the viability of HS1793 as a compound that can improve mitochondrial biogenesis that could potentially aid in muscle wasting.
Key Words: Mitochondria, Skeletal cell, HS1793, PGC-1α, AKT

P05-11

Enhanced store-operated calcium entry induced by over-expression of G-protein subunit beta 5

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Heterotrimeric G-protein consists of three subunits Gα, Gβ, and Gγ. Before discovery of direct activation and interaction of Gβγ and potassium channel in the heart, Gα subunit has been considered solely substantial effector, and Gβγ complex was considered as a signal turn-off molecule without specific function. In addition, Gβγ revealed tissue specific expression pattern, and specific coupling depend on type of G-protein coupled receptors suggesting that specific composition of Gβγ subunit has a unique role in certain tissues. In the present study, we present the enhanced store-operated calcium entry (SOCE) related over-expression of GNB5, a G-protein beta subunit 5. GNB5, STIM1, and Orai1 were transiently transfected in the HEK293T cell and calcium imaging was performed using Fura2 after 24 hrs transfection. SOCE stimulated GNB5 expression. Of note, Co-expression of Orai1 and STIM1, and GNB5 enhanced SOCE. Together, this suggests the novel role of GNB5 with the STIM1 and Orai1 and provides the insight to the novel regulation of SOCE.
Key Words: GNB5, Store-operated calcium entry, G-protein, Calcium, Cell signaling

P05-12

KSP inhibitor SB743921 induces death of multiple myeloma cells via inhibition of the NF-kB signaling pathway

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Multiple myeloma (MM) is a type of hematological malignancy characterized by abnormal plasma cells in the bone marrow. In order to treat MM, it is important to develop anticancer drugs targeting abnormal plasma cells. Since the introduction of combination therapy involving melphalan and prednisone, numerous multidrug chemotherapies employing agents such as dexamethasone, thalidomide, nitrosoureas, and bortezomib have been used to treat MM. However, many compounds have been reported to have resistance to anticancer activities when used as single treatment because of the multistep process of tumorigenesis. Kinesin spindle protein inhibitor (KSP) is a new generation of antimitotic agent with a novel mechanism of action for cancer therapy. SB743921 is being successfully investigated in ongoing clinical trials for the treatment of myeloma. However, little is known about the molecular events underlying the induction of cell death in MM by SB743921, alone or in combination treatment. In this study, we first investigated the antimyeloma activity and mechanism of action for SB743921 in human MM cells. We found SB743921 induces mitochondria-mediated cell death via inhibition of the NF-kB signaling pathway in KMS20 cells. SB743921-mediated inhibition of the NF-kB pathway results in reduced expression of SOD2 and Mcl-1, leading to mitochondrial dysfunction. We also found that combination treatment with SB743921 and bortezomib induces death in bortezomib-resistant KMS20 cells. However, more interestingly we investigated whether SB743921 did not induce cell death in human primary bone marrow mononuclear cells. Altogether, these data suggest that treatment with SB743921 alone or in combination with bortezomib offers excellent translational potential and promises to be a novel MM therapy. [This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare and Family affairs, Republic of Korea (0920040), a Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2010-0020224), and a Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2012R1A1A2041700)]

Key Words: SB743921, NF-kB, Multiple myeloma, Combination therapy, Superoxide dismutase 2, Mcl-1

P05-13

Novel function of histone demethylase of JHDM in spatial learning and memory

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Dynamic changes in histone modification play a role in regulating the gene expression program linked to memory formation. Among them, very little is known about the role of Jumonji-containing histone demethylases which erase methyl group of H3K4, H3K9, H3K27, H3K36, and H4K20- associated with memory formation. Here, we documented the physiological role of JHDM (H3K9 demethylase) in learning and memory. Surprisingly, we found that the JHDM overexpressed transgenic mice displayed enhancement of spatial-memory formation and contextual fear conditioning compared to wild-type mice. Conversely, JHDM-deficient mice derived by Lenti-viral vector in hippocampus showed the impairment in hippocampus-dependent memory function. We also observed that overexpression and knockdown of JHDM leads to changes in expression of neuronal target genes involved in memory formation. Taken together, our results suggest that JHDM has an important role of memory formation in the hippocampus. These findings suggest that JHDM might be a suitable therapeutic avenue for neurodegenerative diseases associated with learning and memory impairment.

Key Words: Jumonji-histone demethylase (JHDM), Learning and memory, Hippocampus

P05-14

Downregulation of HN1 is essential for the induction of autophagy in colorectal cancer cells

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Colorectal cancer is the third leading cause of cancer-related death in the USA and has a high mortality rate among cancers worldwide. Every year, the incidence of colorectal cancer is increasing dramatically in Asian countries including South Korea. Previous studies demonstrated that the hematopoietic- and neurologic-expressed sequence 1 (HN1) is strongly associated with survival of cancer patients and its depletion leads to cell cycle arrest in several cancer cells. Although it has been reported that HN1 is overexpressed in various cancers, the functional significance of HN1 in the adhesion and invasion of colorectal cancer cells remains largely unknown. In this study, we investigated the underlying molecular mechanisms by which HN1 regulates proliferation and metastasis in colorectal cancer cells. Knockdown of HN1 significantly decreased the viability of colorectal cancer cells. Knockdown of HN1 induced autophagy. Knockdown of HN1 decreased the expression of p62, whereas the LC3 II expression was increased. Moreover, knockdown of HN1 inhibited the invasion and metastasis of colorectal cancer cells. Therefore, our results suggest that HN1 regulates growth and metastasis of colorectal cancer cells and targeting HN1 may constitute a therapeutic strategy for colorectal cancer.

Key Words: HN1, Colorectal cancer, Proliferation, Metastasis, Autophagy

P05-15

Activation of hippo signaling by ursolic acid inhibits growth of human gastric cancer cells

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The hippo signaling pathway is frequently dysregulated in cancer cells leading to multiple aspects of tumor initiation and progression. Ursolic Acid (UA), a pentacyclic triterpene compound present in

many medicinal herbs and edible plants, is known to inhibit tumor growth and possesses anti-cancerous property in several human cancers. In this study, we investigated the molecular mechanisms underlying the function of UA in gastric cancer cells. To determine the gene expression patterns related to the effects of UA, a microarray analysis was performed. Gene ontology analysis revealed that several genes including the upstream regulator of the hippo pathway, ras association domain family (*RASSF1*), and its downstream target genes were significantly upregulated by UA. MTT assay showed decreased viability of gastric cancer cells by UA in a dose-dependent manner. Colony numbers and sizes of gastric cancer cells were diminished by UA treatment in time- and dose-dependent manner. Moreover, UA-induced apoptosis of gastric cancer cells was observed. The protein levels of caspase-3 were significantly decreased whereas those of cleaved-caspase-9 and cleaved-PARP were significantly increased in a dose-dependent manner. Cell invasiveness and migration were analyzed using wound healing assay and matrigel transwell assay, respectively. Invasion of gastric cancer cells was suppressed and their migration rates were decreased by UA in a dose-dependent manner. Taken together, our results suggest that UA inhibits the proliferation and metastasis of gastric cancer cells via activation of hippo pathway.
Key Words: Ursolic acid, Hippo signaling, Gastric cancer cells, Proliferation, Metastasis

P05-16

Anticancer effect of ursolic acid on esophageal cancer cells through ROS dependent autophagy

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Ursolic acid (UA) possesses various pharmacological activities, such as antitumor and anti-inflammation. In the present study, we investigated the underlying mechanisms of UA against esophageal squamous cell carcinoma (ESCC) using two ESCC cell lines (TE-8 cells and TE-12 cells). MTT assay and soft agar colony formation assay analyses showed that UA significantly suppressed proliferation of the cell lines and reduced colony numbers and size in a dose-dependent manner. After treatment of UA, large accumulated vacuoles and LC3 puncta, a marker of autophagosome, were detected and visualized by confocal microscopy. Induction of autophagy was confirmed by measuring expression levels of LC3 and p62 protein in ESCC cells. UA increased the protein levels of LC-3-II and decreased the p62 levels in ESCC cells. Upon the inhibition of autophagy by 3-methyladenine (3-MA), cell viability was reversed in the ESCC. In addition, UA dose-dependently inhibited Akt activation and increased p-Akt expression in ESCC cells. UA-induced accumulation of LC3 puncta was reversed by treatment with phosphoinositide 3-kinase inhibitor (PI3K), wortmannin. The protein levels of LC3-II were also decreased after treatment of Akt inhibitor and wortmannin. Moreover, the cellular reactive oxygen species (ROS) levels of ESCC were increased by UA in time- and dose-dependent manner. ROS inhibitor (diphenyleneiodonium, DPI) blocked the ROS and UA-induced accumulation of LC-3-II levels in ESCC cells, suggesting that UA-induced cell death and autophagy are mediated by ROS. Therefore, our data indicate that UA inhibits growth of ESCC cells by inducing ROS dependent autophagy.
Key Words: Ursolic acid, Esophageal squamous cell carcinoma, Autophagy, ROS, Cell death

P05-17

Altered secretory activity of APE1/Ref-1 D148E variants identified in human patients with bladder cancer

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Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) is a multifunctional protein involved in DNA repair and redox modulation. Recently, serum and urinary APE1/Ref-1 levels were reported to be increased in patients with bladder cancer. Genetic variations of APE/Ref-1 are associated with the risk of cancer. However, the effect of *APE1/Ref-1* variants on its secretory activity is yet unknown. *APE1/Ref-1* variants were evaluated by DNA sequencing analysis of reverse transcription polymerase chain reaction products in coding DNA sequences (CDS) of APE1/Ref-1 in bladder tissue samples from patients with bladder cancer (n=10). Secretory activity of *APE1/Ref-1* variants was evaluated with immunoblot and enzyme-linked immunosorbent assay of the culture medium supernatants. Four different substitution mutants (D148E, I64V/D148E, W67R/D148E, and E86G/D148E) of APE1/Ref-1 were identified in bladder cancer specimens. However, deletion mutants of APE1/Ref-1 CDS were not found. The secretory activity of the *APE1/Ref-1* variants (D148E, I64V/D148E, and E86G/D148E) was increased compared to that of wild type APE1/Ref-1. Furthermore, the secretory activity in basal or hyperacetylated conditions was much higher than that in *APE1/Ref-1* D148E-transfected HEK293 cells. Taken together, our data suggest that the increased secretory activity of D148E might contribute to increased serum levels of APE1/Ref-1 in patients with bladder cancer.
Key Words: Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1), Point mutation, Secretion, Enzyme-linked immunosorbent assay (ELISA), Bladder cancer

P05-18

Suberoylanlide hydroxamic acid inhibits the growth of lung cancer cells via caspase-dependent apoptosis

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Suberoylanlide hydroxamic acid (SAHA) is a pan-histone deacetylase (HDAC) inhibitor to have an anti-cancer effect. In the present study, we investigated the anti-growth effect of SAHA in lung cancer cell lines (A549, SK-LU-1, HCC-95, HCC-1588, NCI-H460, NCI-H1299, Calu-6, HCC-33 and NCI-H69) and normal lung cells (human pulmonary fibroblast and human small airway epithelial cells). SAHA inhibited the growth of lung cancer cells and induced apoptosis, as evidenced by sub-G1 cells and annexin V-FITC staining cells. In these cells, SAHA-induced apoptosis was accompanied by the loss of mitochondrial membrane potential (MMP; $\Delta\Psi_m$). However, this agent did not affect cell growth and apoptosis in the normal lung cells. Furthermore, murine experiments suggested that SAHA (25 mg/kg and 50 mg/kg) suppressed tumor growth in balb/c nude mice inoculated with NCI-H460 cells. In

conclusion, SAHA inhibited the growth of lung cancer cells via caspase-dependent apoptosis. In addition, SAHA maybe provide a potential therapeutic target in the lung cancer. [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 (2012-0009323) and the National Research Foundation of Korea Grant funded by the Korean Government (2016R1A2B4007773)]
Key Words: Lung cancer, Histone deacetylase, Suberoylanilide hydroxamic acid, Apoptosis

P05-19

Gallic acid inhibits the growth of calf pulmonary arterial endothelial cells through cell death and glutathione depletion

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Gallic acid (GA) has a variety of cellular effects including apoptosis, which is associated with oxidative stress. This study investigated the effects of GA on calf pulmonary arterial endothelial cells (CPAECs) with regard to cell growth, cell death, reactive oxygen species (ROS) and glutathione (GSH). GA inhibited the growth of CPAECs and the IC50 of it ranged between 25 and 50 μM at 24 hours. It induced cell death, which was accompanied by the loss of mitochondrial membrane potential (MMP; $\Delta\Psi_m$). GA generally decreased ROS levels including $O_2^{\cdot-}$ in CPAECs whereas it increased the number of GSH-depleted cells. A pan-caspase inhibitor (Z-VAD) and buthionine sulfoximine (BSO) did not affect cell growth inhibition, cell death, ROS and GSH levels in GA-treated CPAECs whereas N-acetyl-cysteine (NAC) enhanced cell growth inhibition, cell death and MMP ($\Delta\Psi_m$) loss in these cells. While NAC did not significantly influence ROS levels in GA-treated CPAECs, it strongly intensified GSH depletion in these cells. In conclusion, GA induced growth inhibition and death in CPAECs, which were related to GSH depletion rather than ROS level changes. [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 (2012-0009323) and the National Research Foundation of Korea Grant funded by the Korean Government (2016R1A2B4007773)]
Key Words: Gallic acid, Cell death, Calf pulmonary arterial endothelial cells, Reactive oxygen species, Glutathione

P05-20

Gallic acid induces HeLa cell death via increasing GSH depletion rather than ROS levels

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Gallic acid (GA; 3,4,5-triphydroxyl-benzoic acid) is widely dispersed in various plants, fruits and foods and it shows various biological properties including an anti-cancer effect. This study investigated the effects of GA on HeLa cervical cancer cells in relation to cell death, reactive oxygen species (ROS) and glutathione (GSH). GA dose-dependently inhibited the growth of HeLa cells and human umbilical vein endothelial cells (HUVEC) at 24 or 72 hours. The susceptibility of HeLa cells to GA was higher than that of HUVEC. GA induced apoptosis in HeLa cells, which was accompanied by the loss of mitochondrial membrane potential (MMP; $\Delta\Psi_m$). GA increased ROS levels including $O_2^{\cdot-}$ in HeLa cells at 24 hours and it also induced GSH depletion. N-acetyl-cysteine (NAC) increased the growth inhibition of GA-treated HeLa cells and enhanced the death of these cells. NAC differently influenced ROS levels in GA-treated HeLa cells and significantly increased GSH depletion in these cells. L-buthionine sulfoximine (BSO) increased MMP (ΔΨm) loss, ROS levels and GSH depletion in GA-treated HeLa cells. In conclusion, GA significantly inhibited the growth of HeLa cells. GA-induced HeLa cell death was tightly related to GSH depletion rather than ROS level changes. [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 (2012-0009323) and the National Research Foundation of Korea Grant funded by the Korean Government (2016R1A2B4007773)]
Key Words: Gallic acid, Cell death, HeLa cell, Reactive oxygen species, Glutathione

P05-21

Bitter taste receptors expression levels analysis of exocrine glands in mice

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Mammalian type 2 taste receptors (T2Rs) are mainly found in the oral cavity, where they serve as bitter taste receptors. Recent findings indicate that T2Rs are expressed outside the gustatory system, including in the gastrointestinal tracts, respiratory organs and exocrine glands such as submandibular (SM), parotid (P), lacrimal (L) glands and pancreas (PC). T2Rs are found in some gastrointestinal endocrine cells and these cells secreted the peptide hormones in responses to stimulation by bitter-tasting compounds. Also, stimulation of T2Rs expressed in respiratory epithelia and smooth muscle has been implicated in protective airway reflexes. It shows that T2Rs may have significant physiological roles besides bitter taste reception. However, functions of T2Rs in exocrine glands remain poorly understood. Expression levels analysis of T2Rs are help to determine those functions in exocrine glands. Expression levels of T2Rs in exocrine glands were found out by qPCR. C57BL/6J mice of 42~60-day-old were used. Messenger RNAs were extracted from S, P, L and PC. Cloned DNAs were synthesized by reverse transcription. Quantitative PCRs were performed using SYBR Green method. Expression levels of T2Rs were calculated as relative expression levels to that of GAPDH. Statistical significance among observed exocrine glands was tested using the variance analysis (ANOVA test). T2r108, out of murine 35 T2Rs, was the most highly expressed in every observed exocrine gland. This finding was similar to previous result in tongue papillae, but expression levels were lower than that of tongue papillae. In addition, T2r137 of SM, P, L and PC was expressed to far lower than that of tongue papillae. Taken together, T2Rs in exocrine glands may play slightly different roles from those in tongue. We suggest that physiological studies such as patch clamp and functional Ca^{2+} imaging of acinar cells are necessary for understand T2r108 functions.
Key Words: Exocrine glands, qPCR, Bitter taste receptors, Mammalian type 2 taste receptors

P05-22

Activation of TGFß/ERK signaling by CTHRC1 promotes growth and metastasis in esophageal adenocarcinoma

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The incidence of esophageal adenocarcinoma (EAC) has risen dramatically worldwide in the last three decades without clear etiology. Collagen triple-helix repeat-containing 1 (CTHRC1) has been identified via genome-wide multiplatform approaches as risk alleles or mutated genes associated with EAC. In the present study, we aimed to investigate the underlying molecular mechanisms by which CTHRC1 regulates growth and metastasis using two EAC cell lines (BE3 and OE-33). MTT assay and colony formation assay analyses demonstrated that knockdown of CTHRC1 significantly decreased the cell viability and the colony numbers and size in EAC cells. To identify important gene expression patterns related to CTHRC1 in EAC, we utilized genome-wide transcriptome analyses. Statistical analyses of gene expression data revealed a total of 739 genes significantly associated with CTHRC1 knockdown EAC cell lines (567 genes from BE-3 and 194 genes from OE-33). Both cell lines shared 22 common genes and among them, several oncogenes (TGFß1, JUN and FOS) were identified to be significantly suppressed. The protein levels of apoptotic proteins including cleaved-caspase9 and cleaved-PARP were significantly increased whereas those of caspase-3 were decreased after silencing of CTHRC1. Knockdown of CTHRC1 suppressed the invasion of EAC cells and decreased their migration rates. The mRNA and protein levels of vimentin, twist, MMP9, and uPA were also decreased in CTHRC1 knockdown EAC cells. Taken together, our results suggest that targeting TGFß/ERK pathway via CTHRC1 may constitute a potential strategy for the prevention and treatment of EAC.
Key Words: Esophageal adenocarcinoma cells, Microarray, Collagen triple-helix repeat-containing 1, TGFß/ERK, Metastasis

P05-23(PO-06)

WNK1 promotes renal tumor progression by TRPC6-NFAT pathway via activating phosphatidylinositol 4-kinase IIIα

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Deregulation of Ca^{2+} signaling and altered phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) level have been postulated as a feature of cancer progression. It is well characterized that WNK1 (with-no-lysine (K)) is a major regulator of renal ion transport whose perturbation by *WNK1* mutation causes hypertension and hyperkalemia. However, if and/or how WNK1 signaling contributes renal cancer development beyond regulation of ion homeostasis remains poorly understood. Here, we show that WNK1 activates TRPC6 channel/NFATc1 pathway by stimulating phosphatidylinositol 4-kinase IIIα (PI4KIIIα) promoting $PI(4,5)P_2$ synthesis. Compared with that of adjacent non-neoplastic tissue, the expression levels of WNK1, PI4KIIIα and NFATc1 in tumor tissues were elevated. Notably, blockade of WNK1-mediated TRPC6 activation and its downstream substrate calcineurin attenuated NFATc1 activation, proliferation and migration of renal cell carcinoma cell confirming the pivotal role of WNK1 signaling cascades in aggravating tumor development. These findings reveal novel perspectives on WNK1-dependent signaling targeting TRPC6 channel/NFATc1 pathway involving tumor progression and offers an attractive targets for therapeutic intervention. [This study was supported by NRF-2015R1D1A1A01060454]
Key Words: TRPC6, WNK1, NFATc1, Renal cell carcinoma, Phosphatidylinositol 4-kinase IIIα

P05-24

Secreted APE1/Ref-1 inhibits TNF-α-stimulated endothelial inflammation via thiol-disulfide exchange in TNF receptor

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Apurinic apyrimidinic endonuclease redox factor-1 (APE1/Ref-1) is known as multifunctional protein with redox activity. Now APE1/Ref-1 was proved to be secreted from stimulated cells in previous our study. The aim of this study was to evaluate the functions of extracellular APE1/Ref-1 with respect to leading anti-inflammatory signaling in TNF-α-stimulated endothelial cells in response to acetylation. During an inhibitor of histone deacetylase, trichostatin A (TSA)-mediated intracellular acetylation, a time-dependent increase in secreted APE1/Ref-1 was confirmed by an enzyme-linked immunosorbent assay and immunoblotting. TSA-induced intracellular acetylation in culture significantly suppressed vascular cell adhesion molecule-1 (VCAM-1). Interestingly, secreted APE1/Ref-1 was acetylated and rapidly converted to the nonacetylated, native form base on the removal kinetics of the acetyl moiety. Additionally, recombinant human (rh) APE1/Ref-1 with reducing activity induced a conformational change in TNF-α receptor 1 (TNFR1) by thiol-disulfide exchange, according to biochemical reducing activity and a biotin-switch assay. Following treatment with the neutralizing anti-APE1/Ref-1 antibody, inflammatory signals via the binding of TNF-α to TNFR1 were remarkably recovered, leading to up-regulation of reactive oxygen species generation and VCAM-1, in accordance with the activation of p66shc and p38 MAPK. APE1/Ref-1 secreted from TNF-α-stimulated endothelial cells in response to intracellular acetylation functions as an endogenous inhibitor of vascular inflammation, leading to the inhibition of TNF-α binding to TNFR1 by reductive conformational change.
Key Words: Secreted APE1/Ref-1, Acetylation, Reducing activity, TNF-α, Anti-inflammation.

P05-25

Role of oxidative stress via TGF-β-ERK-mTOR-NOX4 pathway in transdifferentiation of mouse hepatic stellate cells

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Liver cirrhosis results from chronic hepatotoxic injuries, characterized by fibrotic changes with accumulation of extracellular matrix. The principal mediator of fibrosis is known as transdifferentiation of hepatic stellate cells (HSCs) into myofibroblasts. Oxidative stress is involved in the initiation of this process; however, the molecular mechanism of reactive oxygen species (ROS) generation from HSCs has not been clearly identified. Liver fibrosis in mice was developed by thioacetamide (TAA) administration. Mechanistic studies were conducted using primary HSCs isolated and purified from Balb/C mice of 20 weeks of age. RNA and protein levels were quantified by real-time PCR and western blotting, respectively. ROS generation was measured by a confocal imaging system with DCF fluorescence dye. We observed consistent and marked upregulation of NADPH oxidase 4 (NOX4) along with α-smooth muscle actin (α-SMA) and plasminogen activator inhibitor 1 (PAI-1) in the process of hepatic fibrosis development. Increased expression of TGF-β and activation of its downstream signaling cascades including extracellular signal-regulated kinases (ERK) and mammalian target of rapamycin (mTOR) were prominent at the early period of TAA treatment. In primary mouse HSCs, upregulation of α-SMA, PAI-1 and vimentin were evident during culture. We also observed time-dependent increase in TGF-β and NOX4 protein levels as well as activation of ERK1/2 and mTOR pathways. Consistent with NOX4 upregulation, cytosolic ROS was elevated during myofibrotic changes in primary HSCs, which was attenuated by SB431542 (TGF-β receptor blocker), PD184352 (ERK inhibitor) or rapamycin (mTORC1 inhibitor). We suggest that oxidative stress during transdifferentiation of HSCs may be originated from increased NOX4 protein triggered by TGF-β-ERK-mTOR axis, inhibition of which could be an effective therapeutic target to prevent the progression of liver cirrhosis.

Key Words: Hepatic stellate cell, Transforming growth factor-β, mTOR, ERK, NADPH Oxidase 4

P05-26

VEGFR2 aptamer as tool of commitment for hPSC-derived endothelial progenitor cells

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Aptamers are 40-120mer nucleotides that bind to a specific target molecule, as antibodies do. Endothelial progenitor cells (EPCs) contribute to neovascularization and vascular endothelial growth factor receptor2 (VEGFR2) known as KDR or CD309 VEGF receptor. VEGFR is receptor tyrosine kinase that promotes cell proliferation, migration, survival, and permeability. VEGF signaling contributes to angiogenesis and endothelial cell integrity. VEGFR2 expression in adult endothelial cells appears to account for most of the mitogenic and chemotactic effects of VEGF. Parenthetically, the widely used term 'EPC' is a misnomer. The surface markers that are commonly used for identification of human EPCs include markers that are not specific for endothelial lineage, such as CD133 and VEGFR2. In addition, methods for harvesting, purifying, and culturing EPCs have not been standardized. Thus, semantic confusion is compounded by methodological variation. Casual readers of the literature may not recognize that EPCs are a mixed population of progenitor cells of different lineages. Functional human endothelial cells were differentiated from hPSCs could be beneficial for many potential clinical applications including engineering new blood vessels, endothelial cell transplantation into the heart for myocardial regeneration, and induction of angiogenesis for treatment of regional ischemia. hPSC-derived endothelial progenitors and endothelial cells may provide building blocks for the establishment of in vitro disease models for screening and development of drugs to treat these diseases. Functionality of hPSC-derived endothelial cells has been shown using in vitro cell culture platforms and in vivo animal models. And Human pluripotent stem cell (hPSC)-derived endothelial cells and their progenitors may provide the means for vascularization of tissue-engineered constructs and can serve as models to study vascular development and disease. We successfully generated aptamers that recognize human VEGFR2 with cord blood derived EPC and hPSC derived EPC. However, VEGFR2 aptamers showed nonspecific interaction with VEGFR2-negative 293T cells. Addition of polyanionic competitor dextran sulfate resolved the problem of nonspecific interaction. And also VEGFR2 aptamers have function as a receptor agonist.

Key Words: VEGFR2, Aptamer, Human pluripotent stem cells, Endothelial progenitor cells

P05-27

Echinochrome A prevents vascular smooth muscle cell proliferation via mTOR/Akt-OPN signaling pathway in atherosclerosis

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Proliferation of vascular smooth muscle cells (VSMC) is a key process in the pathogenesis of various vascular diseases including atherosclerosis. Osteopontin (OPN), implicated in vascular disease, has a capacity to regulate the proliferation of VSMC. It is well known that Echinochrom A (Ech A) plays an important role in the inhibiting cardiovascular disease factors, but the potential protective role of Ech A on VSMC proliferation. Therefore, we investigated the effect of Ech A on VSMC proliferation and the molecular mechanisms involved. Primary VSMC was isolated from aorta of Wistar rats to investigate whether Ech A modulates proliferation of VSMC and expression of OPN (a marker of VSMC synthetic state). MTT, trypan blue and migration assay were employed to determine proliferation of VSMC. OPN expression was assessed using western blotting and OPN promoter activity was measured by luciferase assay. Ech A attenuated PDGF-BB-induced VSMC proliferation in a dose-dependent manner. Moreover, PDGF-BB-induced expression of OPN was inhibited by Ech A. Interestingly, Ech A directly reduced mTOR kinase activity. Under Ech A treatment, VSMC exhibited decreased phosphorylation of mTOR and protein kinase B (Akt), a downstream effector of mTOR. In line with these results, both pharmacological inhibitor of mTOR (rapamycin) and Akt (AI) blocked PDGF-BB-induced OPN expression. Further increased OPN transcriptional activation by PDGF-BB was significantly inhibited by Ech A. Ech A treatment suppresses PDGF-BB-induced proliferation of VSMC via the mTOR/Akt/OPN signaling cascade. Thus, our results suggest that Ech A might be useful treatment of vascular disease such as atherosclerosis. [This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2015M3A9B6029133 and 2010-0020224)]

Key Words: Echinochrome A, Vascular smooth muscle cell, Osteopontin, Proliferation, mTOR

P05-28

Endothelin-1 and phenylephrine activate cAMP response element binding protein via IP3 receptor/ Ca^{2+}-dependent PKC-CaMKII signaling in ventricular myocytes

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Endothelin-1 (ET-1) and α_1-adrenoceptor agonist phenylephrine (PE) activate cAMP response element binding protein (CREB) a transcription factor in cardiac myocytes, which is implicated in hypertrophy. Signaling pathways for the CREB activation in cardiac myocytes by these hypertrophic stimuli remain to be understood. Here, we examined signal transduction pathways for the ET-1 or PE-induced CREB activation by assessing phosphorylation of key signaling molecules using western blotting in rat ventricular myocytes. Both ET-1 (10-100 nM) and PE (100 μM) increased the phosphorylation of CREB, which was inhibited by the antagonists for the ETA receptor and α_1-adrenoceptor, respectively. The activation of CREB by ET-1 or PE was suppressed by pharmacological inhibition of phospholipase C, extracellular-signal-regulated kinase 1/2 (ERK1/2) pathway, protein kinase C (PKC) or Ca^{2+}-calmodulin-dependent protein kinase II (CaMKII). Intracellular Ca^{2+} buffering decreased ET-1- and PE-induced CREB phosphorylation by 80-90%. In the cell pretreated with sarcoplasmic reticulum (SR) Ca^{2+} pump inhibitor or inositol 1,4,5-trisphosphate receptor (IP_3R) blocker, the increases of phosphorylated CREB by ET-1 and PE were not observed. Consistently, in the type 2 IP3R (IP_3R2)-deficient myocytes, ET-1 and PE did not enhance CREB phosphorylation. Both ET-1 and PE increased phosphorylation of CaMKII and ERK1/2, which was also removed by IP_3R blockade/knock-out or by PKC inhibition. Interestingly, the activation of only CaMKII by these agonists, but not ERK1/2, was sensitive to Ca^{2+} buffering or blockade of Ca^{2+}-dependent PKC. These results suggest that CREB phosphorylation by both ET-1 and PE is mainly mediated by CaMKII activated by IP_3R2/Ca^{2+}-dependent PKC signaling in ventricular myocytes, and that ERK1/2, linked to IP_3R2 and Ca^{2+}-independent PKC in a microdomain, also contributes to CREB phosphorylation during these hormonal stimulation.

Key Words: CREB, IP_3R2, PKC, CaMKII, Ventricular myocytes

P05-29

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

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TRPML3 is an intracellular Ca^{2+} channel that is expressed in endocytic and autophagic pathways. Although TRPML3 is suggested to regulate autophagy as an autophagosomal Ca^{2+} channel, it is still not clear whether TRPML3 directly provides Ca^{2+} for the process. To image a TRPML3-specific Ca^{2+} efflux from the organelles, we used a GCaMP6-fusion protein, a genetically encoded calcium indicator attached to C-terminus of TRPML3. We found that TRPML3-GCaMP6 hardly overlapped with LC3 or LAMP1, indicating that subcellular localization of a functional TRPML3 is totally different from previously reported TRPML3 distribution. Strikingly, TRPML3-GCaMP6 was mainly localized in ATG5-positive phagophores, suggesting that TRPML3 supplies Ca^{2+} for the early autophagosome biogenesis. Lipid binding assay revealed that both N-terminus and 1st extracytosolic loop of TRPML3 interact with phosphatidylinositol-3-phosphate (PI3P) which is essential for the initiation of autophagy and enriched in early autophagosomes. Confocal imaging and electrophysiological experiments showed that TRPML3 is directly activated by both cytosolic and extracytosolic PI3P, resulting in increased autophagy. Moreover, activation of TRPML3 by a synthetic agonist, ML-SA1 rescued inhibition of autophagy by PI3K antagonist, whereas dominant-negative TRPML3 (D458K) inhibited autophagy even in the presence of excess PI3P. Taken together, these results suggest that TRPML3 is a key regulator for autophagy process as a downstream effector of PI3P, providing Ca^{2+} during early steps of autophagosome formation.

Key Words: TRPML3, Autophagy, Autophagosome, GCaMP6, Phosphoinositide

P06-01

The effect of the fully lengthened immobilization on the change of the apoptotic and myosin heavy chain expression in soleus muscle in rats

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Clinically, muscle immobilization can be used to prevent secondary injury following skeletal injuries and is often implemented in neutral position. The lengthened immobilization can be applied in spastic muscle and contracture for sustaining normal muscle length, however the effect remains controversial. This study aims to examine the effects of lengthened immobilization on dynamic weight bearing during freely walking and on the changes of apoptosis-related proteins, muscle cell nuclei and muscle-forming protein. To lengthen the right soleus of male SD rat (weighing 200-250g) right ankle was casted and immobilized in the condition of neutral position (= 0° ankle angle, NEUT) and full lengthened position (= 55° ankle angle, LENG), whereas the control (CONT) group was not casted. Using dynamic weight bearing device in walking rats, we repeatedly measured the weight load of ipsilateral foot at 7, 14 and 21 days after immobilization. The expression of Bax (pro-apoptotic Bcl-2 family member), MyoD (myogenic differentiation factor D) and MYH (myosin heavy chain) protein in soleus were measured using western blot, and apoptotic nuclei change was observed using TUNEL assay at 7, 14 and 21 day post-immobilized periods. In dynamic weight load, both NEUT and LENG groups showed a significant reduced weight load in the ipsilateral side compared to the control group. In addition, the soleus of LENG group showed a significant increase of both Bax and MyoD and a significant reduction of MYH compared to those of NEUT and CONT group. There was an increased expression in apoptotic nuclei of LENG group compared to NEUT group. Therefore, our findings show that fully lengthened immobilization can not only increase the apoptotic expression in muscle with weight-loss behavior but also decrease muscle forming protein. These results suggest that fully lengthened immobilization induces severe muscle atrophy due to the increase of apoptosis in muscle.

Key Words: lengthened immobilization; apoptosis; lengthening; soleus; casting

P06-02

Expression profile of mitochondrial voltage-dependent anion channel-1 influenced genes is associated with pulmonary hypertension

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Mitochondrial voltage-dependent anion channel-1 (VDAC1) is one of the major components of the outer mitochondrial membrane and plasma membrane. Several human diseases have been associated with VDAC1 due to its role in calcium ion transportation and apoptosis. Recent studies suggest that VDAC1 interacts with endothelium-dependent nitric oxide synthase (eNOS). Decreased VDAC1 expression may limit the physical interaction between VDAC1 and eNOS and thus impair nitric oxide production, leading to cardiovascular diseases, including pulmonary arterial hypertension (PAH). We conducted transcriptomic meta-analysis to identify VDAC1 influenced genes implicated in PAH pathobiology. First, we identified the genes differentially expressed between wild-type and Vdac1 knockout mouse embryonic fibroblasts in hypoxic conditions. These genes were deemed to be influenced by VDAC1 deficiency. Gene ontology analysis indicates that the VDAC1 influenced genes are significantly associated with PAH pathobiology. Second, a molecular signature derived from the VDAC1 influenced genes was developed. We suggest that this gene expression signature can be used to i) predict severity of pulmonary hypertension secondary to pulmonary diseases, ii) differentiate idiopathic pulmonary artery hypertension (IPAH) patients from controls, and iii) differentiate IPAH from connective tissue disease associated PAH. Our study suggests the protective role of VDAC1 in PAH pathobiology despite unclear molecular mechanism.
Key Words: VDAC1, nitric oxide synthase, transcriptomic meta-analysis, pulmonary hypertension

P06-03

Mitsugumin 53 regulates extracellular Ca^{2+} entry and intracellular Ca^{2+} release via Orai1 and RyR1 in skeletal muscle

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Mitsugumin 53 (MG53) participates in the membrane repair of various cells, and skeletal muscle is the major tissue that expresses MG53. Except for the binding and regulatory effects of MG53 on SERCA1a, the role(s) of MG53 in the unique functions of skeletal muscle such as muscle contraction have not been well examined. Here, a new MG53-interacting protein, Orai1, is identified in skeletal muscle. To examine the functional relevance of the MG53-Orai1 interaction, MG53 was over-expressed in mouse primary or C2C12 skeletal myotubes and the functional properties of the myotubes were examined using cell physiological and biochemical approaches. The PRY-SPRY region of MG53 binds to Orai1, and MG53 and Orai1 are co-localized in the plasma membrane of skeletal myotubes. MG53-Orai1 interaction increases extracellular Ca^{2+} entry via Orai1 by store-operated Ca^{2+} entry (SOCE) mechanism in skeletal myotubes. Interestingly, skeletal myotubes over-expressing MG53 or PRY-SPRY displays reduced intracellular Ca^{2+} release in response to K+-membrane depolarization or caffeine stimulation, suggesting reduced RyR1 channel activity. Expressions of TRPC3, TRPC4, and calmodulin 1 are increased in the myotubes, and MG53 directly binds to TRPC3, which suggests a possibility that TRPC3 also participates in the enhanced extracellular Ca^{2+} entry. Thus, MG53 could participate in regulating extracellular Ca^{2+} entry via Orai1 during SOCE and also intracellular Ca^{2+} release via RyR1 during skeletal muscle contraction.
Key Words: Mitsugumin 53, Orai1, ryanodine receptor, store-operated Ca^{2+} entry, TRPC3, TRPC4, calmodulin, skeletal muscle

P06-04(PO-10)

The maintenance ability and Ca^{2+} availability of skeletal muscle are enhanced by sildenafil

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Sildenafil relaxes vascular smooth muscle cells, and is used to treat pulmonary artery hypertension as well as erectile dysfunction. However, the effectiveness of sildenafil on skeletal muscle and the benefit of its clinical use have been controversial, and most studies have focused primarily on tissues and organs from disease models, without cellular examinations. Here, the effects of sildenafil on skeletal muscle at the cellular level were examined using mouse primary skeletal myoblasts (the proliferative forms of skeletal muscle stem cells) and myotubes along with single- cell Ca^{2+} imaging experiments and cellular and biochemical examinations. The proliferation of the skeletal myoblasts was enhanced by sildenafil, without dose-dependency. In the skeletal myotubes, sildenafil enhances the activity of ryanodine receptor 1, an internal Ca^{2+} channel, and Ca^{2+} movements that promote skeletal muscle contraction, possibly due to an increase in the resting cytosolic Ca^{2+} level and a unique microscopic shape in the myotube membranes. Therefore, these results suggest that the maintenance ability of skeletal muscle mass and the contractility of skeletal muscle could be improved by sildenafil via enhancing the proliferation of skeletal myoblasts and increasing the Ca^{2+} availability of skeletal myotubes, respectively.
Key Words: skeletal muscle, myoblast, skeletal muscle stem cell, myotube, proliferation

P06-05

$K_{Ca}3.1$ upregulation preserves endothelium-dependent vasorelaxation during aging and oxidative stress

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Endothelial oxidative stress develops with aging and reactive oxygen species (ROS) impair endothelium-dependent relaxation (EDR) by decreasing nitric oxide (NO) availability. Endothelial $K_{Ca}3.1$, which contributes to EDR, is upregulated by H_2O_2. We investigated whether $K_{Ca}3.1$ upregulation compensates for diminished EDR to NO during aging-related oxidative stress. Previous studies identified that the levels of ceramide synthase 5 (CerS5), sphingosine, and sphingosine 1-phosphate were increased in aged wild-type and CerS2 mice. In primary mouse aortic endothelial cells (MAECs) from aged wild-type and CerS2 null mice, superoxide dismutase (SOD) was upregulated, and catalase and glutathione peroxidase 1 (GPX1) were downregulated, when compared to MAECs from young and age-matched wild-type mice. Increased H_2O_2 levels induced Fyn and extracellular signal-regulated kinases (ERK) phosphorylation and $K_{Ca}3.1$ upregulation. Catalase/GPX1 double knockout (catalase$^{-/-}$/GPX1$^{-/-}$) upregulated $K_{Ca}3.1$ in MAECs. NO production was decreased in aged wild-type, CerS2 null, and catalase$^{-/-}$/ GPX1$^{-/-}$ MAECs. However, $K_{Ca}3.1$ activation-induced, N^G-nitro-L-arginine and indomethacin-resistant EDR was increased without a change in acetylcholine-induced EDR in aortic rings from aged wild-type, CerS2 null, and catalase$^{-/-}$/ GPX1$^{-/-}$ mice. CerS5 transfection or exogenous application of sphingosine or sphingosine 1-phosphate induced similar changes in levels of the antioxidant enzymes and upregulated $K_{Ca}3.1$. Our findings suggest that, during aging-related oxidative stress, SOD upregulation and downregulation of catalase and GPX1, which occurs upon altering the sphingolipid composition or acyl chain length, generates H_2O_2 and thereby upregulates $K_{Ca}3.1$ expression and function via a H_2O_2/Fyn-mediated pathway. Altogether, enhanced $K_{Ca}3.1$ activity may compensate for decreased NO signaling during vascular aging.

Key Words: endothelium, nitric oxide, oxidative stress, K+ channel

P06-06(YP-04)

Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells

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Mitochondria are crucial for maintaining the properties of embryonic stem cells (ESCs) and for regulating their subsequent differentiation into diverse cell lineages, including cardiomyocytes. However, mitochondrial regulators that manage the rate of differentiation or cell fate have been rarely identified. This study aimed to determine the potential mitochondrial factor that controls the differentiation of ESCs into cardiac myocytes. We induced cardiomyocyte differentiation from mouse ESCs (mESCs) and performed microarray assays to assess messenger RNA (mRNA) expression changes at differentiation day 8 (D8) compared with undifferentiated mESCs (D0). Among the differentially expressed genes, Pdp1 expression was significantly decreased (27-fold) on D8 compared to D0, which was accompanied by suppressed mitochondrial indices, including ATP levels, membrane potential, ROS and mitochondrial Ca^{2+}. Notably, Pdp1 overexpression significantly enhanced the mitochondrial indices and pyruvate dehydrogenase activity and reduced the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate compared to a mock control. In confirmation of this, a knockdown of the Pdp1 gene promoted the expression of cardiac differentiation marker mRNA and the cardiac differentiation rate. In conclusion, our results suggest that mitochondrial PDP1 is a potential regulator that controls cardiac differentiation at an early differentiation stage in ESCs.

Key Words: mitochondria, stem cells, ROS, differentiation, cardiomyocyte, pyruvate dehydrogenase phosphatase

P06-07

Mechanistic differences in calcium handling and contractility of the left and right ventricles of rat heart

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Differences of the left (LV) and right ventricles (RV) have been described across different species; and with the complexity of recent methods used in cardiac research, from the organ down to the molecular level, differences beyond structural and functional properties have been identified. However, differences in their protein profiles that focus on excitation-contraction coupling remain unclear. Therefore, the present study aims to provide a molecular basis on the differences in Ca^{2+} handling and contractile properties between LV and RV. This study characterized the rat LV and RV proteome by 1D-LC-MS/MS, and expression level of the protein of interest was verified by western blotting. Functional analyses on Ca^{2+} handling and contractility were also performed on isolated cardiomyocytes. The proteomic analysis revealed 728 unique proteins; 47 of these were differentially expressed between the LV and RV. Interestingly, the protein abundance of ryanodine receptor 2 (RyR2) was higher in the RV than that in the LV, and this result was verified using western blot. Majority of the Ca^{2+} used for contraction comes from the sarcoplasmic reticulum (SR), so other SR Ca^{2+} handling proteins were also examined. RyR2 regulator FKBP12.6, SR protein SERCA2a, and phospholamban (PLB) all showed higher protein expressions in the RV than in the LV. Since SERCA2a activity is dependent on PLB phosphorylation, phosphosites on PLB were also checked, which showed a higher level of phosphorylation on the Thr17 residue of PLB in the LV than in the RV. Using line scanning, Ca^{2+} transient analysis showed shorter time to peak and faster Ca^{2+} decay in LV myocytes than RV myocytes. RyR2 was inhibited by keeping it in an open subconductance state using ryanodine, and this significantly reduced time to peak in LV, but not in RV. Measurement of sarcomere shortening using an edge-detector system also showed that LV myocytes show greater contractility, but RV myocytes displayed shorter shortening and relaxation times. Taken together, variations in protein levels of SR calcium handling proteins could explain the differences in Ca^{2+} handling and contractile properties between LV and RV myocytes. Differences in protein expression could be useful in improving therapeutic strategies for region-specific cardiomyopathies. (This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2015M3A9B6029133 and 2010-0020224))

Key Words: calcium handling, contractility, left and right ventricles, proteomics, ryanodine receptor 2

P06-08

Functional analysis of colonic motility in response to Ang III, Ang IV, and Ang-(1-7) in rats

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Renin-angiotensin system is involved in the pathophysiology of colonic inflammation. There are many active angiotensin (Ang) fragments but their effects on colonic motility are not clear. This study investigated the pathophysiological role of Ang fragments in the regulation of colonic motility in control and experimental colitis. Experimental colitis was induced by an intake of 5% dextran sulfate sodium (DSS) dissolved in tap water for 7 days. After sacrifice, plasma hormone concentrations, mRNAs for RAS were measured. Functional analysis of colonic motility in response to Ang III, Ang IV, and Ang-(1-7) was performed using Taenia coli. Ang III and Ang IV (1, 3, and 10 uM) stimulated the frequency of colonic motility in a dose-dependent manner and the order of potency was Ang II >>> Ang IV > Ang III=Ang-(1-9). Ang-(1-7) did not affect colonic motility. The effect of Ang III and Ang IV on motility was inhibited by the pretreatment of AT2R or AT4R, respectively. DSS-treated colon showed an increased necrosis with massive infiltration of inflammatory cells. The colonic mRNA level for Ang converting enzyme (ACE)-2 was markedly decreased but that for ACE was not in experimental colitis. The colonic mRNA levels for AT1R, AT2R, and Mas R were markedly increased. However, the stimulated responses of frequency of basal motility to Ang IV and Ang-(1-7) did not affect in DSS-treated rat colon. These data suggest that Ang fragments are partly involved in the regulation and colonic motility and may play a role in pathophysiology of experimental colitis. (supported by NRF of Korea grant funded by the Korea government, No 2008-0062279)
Key Words: colitis, truncated angiotensin, renin, motility, mRNA

P06-09

Insulin signaling targeting WNK1 kinase promotes GLUT4 trafficking in skeletal muscle

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WNK kinase 1 is a substrate of insulin receptor-phosphatidylinositol 3-kinase (PI3K) signaling pathway and stimulates endocytosis of ion channels and transporters to regulate ion homeostasis. Impaired insulin signaling in skeletal muscle disturbs trafficking of glucose transporter 4 (GLUT4) associated with the onset of type 2 diabetes (T2D). WNK1 is highly expressed in skeletal muscle and is known to regulate trafficking of transporters including GLUT1. It is currently unknown how insulin signaling targeting WNK1 regulates GLUT4 abundance in skeletal muscle and whether this regulation is perturbed in T2D. Here, we found that compared with control mice, T2D *db/db* mice exhibited significant insulin resistance and decreased level of WNK1 phosphorylation, TBC1D4 and GLUT4. Insulin increased phosphorylation of the downstream kinase Akt as well as WNK1 in a PI3K-dependent mechanism. A biotinylation assay demonstrated that insulin stimulates GLUT4 surface expression by promoting its exocytosis via stimulating PI3K-Akt pathway. Notably, increased Akt phosphorylation and cell surface abundance of GLUT4 by insulin was blunted by knockdown of WNK1 suggesting that WNK1 is a novel regulator of insulin-stimulated GLUT4 trafficking in skeletal muscle. These results provide a new perspective on WNK1 function beyond regulation of endocytosis of ion channels and transporters and offer new insights for pathogenesis of hyperglycemia in T2D. [This research was supported by NRF-2013R1A1A2060764 and NRF-2015R1D1A1A01060454]
Key Words: WNK1, GLUT4, Type 2 diabetes, TBC1D4, Skeletal muscle

P06-10

Sphingosylphosphorylcholine induces differentiation of human mesenchymal stem cells into smooth muscle cells by regulating ROS-mediated DJ-1 oxidation

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Although many proteins/factors were known to contribute to differentiation of human mesenchymal stem cells (hMSCs) into various types of cells, the proteins/factors remain to be identified. It is reported at reactive oxygen species (ROS) may participate in differentiation of hMSCs and that sphingosylphosphorylcholine (SPC) stimulates differentiation of hMSCs into smooth muscle cells (SMCs). This study aimed to investigate correlation between altered proteins, especially those related to ROS, and SMC differentiation in SPC-stimulated hMSCs. Proteomics analysis showed that an antioxidant DJ-1 protein has significant expression change in oxidized form and reduced from in SPC-exposed hMSCs. Increment of oxidized DJ-1 expression in SPC-stimulated hMSCs was validated by immunoblot analysis. Treatment with SPC resulted in increased expressions of SMC markers (α-smooth muscle actin [SMA] and calponin) and ROS production in hMSCs. SPC-increased α-SMA expression was stronger in DJ-1-knocked down hMSCs than in nonsilencing control cells. Moreover, the expressions of α-SMA and calponin and ROS generation in hMSCs in response to SPC were weaker in normal control cells compared with DJ-1-overexpressing hMSCs. These results indicate that hMSCs may be differentiated into SMCs through ROS-regulated DJ-1 pathway in response to SPC. Therefore, DJ-1 may act as a molecule contributing to SPC-stimulated differentiation of hMSCs into SMCs.
Key Words: Human mesenchymal stem cell, Sphingosylphosphorylcholine, Reactive oxygen species, DJ-1, Differentiation

P06-11

Nafamostate Mesilate improves endothelium-dependent vascular relaxation via the Akt-eNOS dependent pathway

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Nafamostat mesilate (NM), a synthetic serine protease inhibitor, has anticoagulation and anti-inflammatory properties. However, the function of NM in vascular endothelial cells has not been well determined. In this study, we examined whether NM had effect on vascular relaxation in human umbilical vein endothelial cells (HUVECs) and *in vivo*. NM increased Akt and endothelial nitric oxide synthase (eNOS) phosphorylation in a dose and time dependent manner in HUVECs. NM phosphorylated Akt and eNOS expression and decreased arginase activity, leading to NO production in HUVECs. Furthermore, the effects of NM on Akt-eNOS phosphorylation and NO production were investigated *in vivo*. NM was intravenously administered with different concentrations, and the aortic tissue was extracted for molecular, histological and biological detection. Western blot and histology data showed that NM significantly increased Akt-eNOS phosphorylation and NO production in a concentration dependent manner in the aortic tissue, showing the same tendency as in HUVECs. The concentration of bioavailable NO and endothelium-dependent vasorelaxation in the aortic ring were also increased in the NM administration mice. These results suggest that NM activates Akt-eNOS phosphorylation and increases NO production in HUVECs and *in vivo*, that promotes endothelium-dependent vascular relaxation.
Key Words: Akt, endothelial nitric oxide synthase, nafamostat mesilate, nitric oxide, vascular relaxation

P06-12

Repaglinide, an anti-diabetic drug, induces vasorelaxation by activation of PKA and PKG in aortic smooth muscle

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We investigated the vasorelaxant effect of repaglinide and its related signaling pathways using phenylephrine (Phe)-induced pre-contracted aortic rings. Repaglinide induced vasorelaxation in a concentration-dependent manner. The repaglinide-induced vasorelaxation was not affected by removal of endothelium. Pre-treatment with adenylyl cyclase inhibitor or the PKA inhibitor effectively reduced repaglinide-induced vasorelaxation. Also, pretreatment with guanylyl cyclase inhibitor or the PKG inhibitor effectively inhibited repaglinide-induced vasorelaxation. However, pretreatment with voltage-dependent K^+ channel inhibitor (4-AP), ATP-sensitive K^+ channel inhibitor (glibenclamide), big-conductance Ca^{2+}-activated K^+) channel inhibitor (paxilline), and the inwardly rectifying K^+ channel inhibitor (Ba^{2+}) did not affect the vasorelaxant effect of repaglinide. Furthermore, pretreatment with Ca^{2+} inhibitor (nifedipine) and SERCA inhibitor (thapsigargin) also did not affect the vasorelaxant effect of repaglinide. From these results, we concluded that repaglinide induced vasorelaxation by activation of adenylyl cyclase/PKA and guanylyl cyclase/PKG signaling pathway independently of endothelium, K^+ channels, Ca^{2+} channel and intracellular Ca^{2+} ($[Ca^{2+}]_i$).
Key Words: Repaglinide, diabetes, protein kinase A, protein kinase G

P06-13

Reversal of levosimendan-induced suppression of ANP secretion in the presence of PDE 4 inhibitor via PKA pathway

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Levosimendan is known as a calcium sensitizer as well as a phosphodiesterase (PDE) 3 inhibitor. We aimed to define the effects of levosimendan on atrial contractility and atrial natriuretic peptide (ANP) secretion and its mechanism using isolated perfused rat beating atria. Levosimendan (0.01 to 10 μM) and its metabolite (OR-1896, 0.01 to 10 μM) caused a positive inotropic effect (PIE) and a suppression of high stretch-induced ANP secretion in a dose-dependent manner. The selective inhibitor of PDE3 (cilostamide, 0.1 μM) or PDE4 (rolipram, 1 μM) suppressed ANP secretion similarly to levosimendan without changes in atrial contractility and cAMP efflux. In the presence of PDE3 inhibitor, PIE and the inhibitory effect of ANP secretion induced by levosimendan (1 μM) were abolished. The cAMP efflux was not changed by levosimendan with and without PDE3 inhibitor. In the presence of the selective PDE4 inhibitor, levosimendan or OR-1896 stimulated high stretch-induced ANP secretion and increased cAMP efflux over 400% compared with control period. The potentiation of ANP secretion induced by levosimendan plus PDE4 inhibitor was attenuated by the protein kinase A (PKA) inhibitor (H89) but not by exchange protein directly activated by cAMP (Epac)-1 inhibitor and PKG inhibitor. Cacium-induced PIE and suppression of ANP secretion did not alter in the presence of levosimendan plus PDE4 inhibitor. Levosimendan-induced suppression of ANP secretion and PIE in isoproterenol-treated atria was not different from sham atria. This study suggest that reversal effect of LEVO on ANP secretion in the presence of PDE4 inhibition may be due to activation of PKA in isolated rat atria. (supported by NRF grant funded by the MSIP, No 2008-0062279)
Key Words: calcium sensitizer, PDE3, PDE4, atrium, rat, ANP, PKA

P06-14

Scoparone, the bioactive component of chestnut inner shell ethanol extract, suppresses neointima formation by inhibiting migration via mitogen-activated protein kinase signaling pathway in vascular smooth muscle cells

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The chestnut (*Castanea crenata*) inner shell extract has anticancer, anti-hepatic steatosis, and antioxidative activities. However, it has not been investigated whether the extract from chestnut inner shell affects the function of vascular smooth muscle cells (VSMCs). In this study, we tested the effect of chestnut inner shell ethanol extract (CISEE) on VSMC migration and proliferation and determine its bioactive component. CISEE inhibited platelet-derived growth factor (PDGF)-BB-induced migration in VSMCs. GC/MS analysis showed that the CISEE contains 10 compounds. Among these 10 compounds, we examined the bioactivity in VSMCs of the scoparone (6,7-dimethoxycoumarin) that is known to affect vascular contractility and VSMC proliferation at high dose. Scoparone dose-dependently suppressed migration

and phosphorylation of mitogen-activated protein kinases (MAPKs), p38MAPK and extracellular signal-regulated kinase (ERK)1/2, in VSMCs in response PDGF-BB. Treatment with scoparone resulted in the decreased neointima formation in balloon-injured rat carotid artery. These findings demonstrate that scoparone inhibits migration by suppressing the phosphorylations of p38MAPK and ERK1/2 in VSMCs, thereby leading to reduction of neointima formation and that scoparone may be a main bioactive component of CISEE, exerting anti-migration action in VSMCs. Therefore, scoparone as well as CISEE may be potential candidates for prevention or treatment of vascular disorders such as vascular restenosis or atherosclerosis.

Key Words: Chestnut inner shell ethanol extract, Scoparone, Vascular smooth muscle cells, Migration, Restenosis, Atherosclerosis

P06-15

Serum response factor regulates smooth muscle contractility via myotonic dystrophy protein kinases and L-type calcium channels

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Serum response factor (SRF) transcriptionally regulates expression of contractile genes exclusively in smooth muscle cells (SMCs). Lack or decrease of SRF is directly linked to a phenotypic change of SMCs, leading to hypomotility of smooth muscle in gastrointestinal (GI) tract. However, a molecular mechanism of SRF-induced hypomotility in GI smooth muscle is largely unknown. We describe here how SRF plays a functional role in the regulation of the SMC contractility via myotonic dystrophy protein kinase (DMPK) and L-type calcium channel CACNA1C. Deficiency of SRF in SMCs of *Srf* knockout (KO) mice leads to reduction of SRF-dependent DMPK, which downregulates the expression of CACNA1C. Reduction of CACNA1C in KO SMCs not only decreased intracellular Ca2+ spikes but also disrupted their coupling between cells resulting in decreased contractility. The role of SRF in the regulation of SMC phenotype and function provides new insight into how SMCs lose their contractility leading to hypomotility in pathophysiological conditions within the gastrointestinal tract.

Key Words: serum response factor, calcium channel, smooth muscle, motility, calcium channel

P06-16

Effects of high glucose and metabolic substrates on vascular reactivity in rat mesenteric and deep femoral arteries

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Hyperglycemia causes vascular damage with several underlying mechanisms and increased risk of cardiovascular diseases. Chronic exposure to high glucose impaired endothelial function has been demonstrated in several studies, however effects of short-term exposure to high glucose on vascular reactivity are controversial. Moreover, the combined effects of other metabolic substrates such as free fatty acids (FFA) on vascular reactivity remain poorly understood. Here we investigate the effects of short-term incubation to high glucose and metabolic substrates termed "nutrition full" (NF) solution in mesenteric (MA) and deep femoral arteries (DFA) of rats. In the myograph study of endothelium-intact rat MA and DFA, dose-response to phenylephrine (PhE) was observed in control (5 mM) and high glucose (23 mM, HG) environments over a 30 min period. PhE-induced contraction in both arteries was enhanced by pre-incubation with HG. A combined incubation with HG and palmitic acid (100 μM) induced similar sensitization of PhE-induced contraction in both arteries. In contrast, high K^+-induced contractions were not affected by HG. Interestingly, pre-incubation with NF solution decreased PhE-induced contraction in DFA but increased in MA. In NF solution, the HG-induced facilitation of PhE-contraction was not observed in MA. Furthermore, the PhE-induced contraction of DFA was attenuated by HG in NF solution. Our results demonstrate that the sensitization of PhE-induced arterial contraction by HG is differentially affected by other metabolic substrates. The conversation of skeletal arterial contractility by HG in NF solution requires careful interpretation. Further studies are required to elucidate the mechanism underlying the inconsistent effect of NF solution on MA and DFA.

Key Words: vasoconstriction; fatty acids; isometric contraction; hyperglycemia; phenylephrine

P06-17

The characteristics of morphology and respiration of the cryopreserved cardiac myocytes isolated from adult rat heart

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Cryopreservation of cardiac myocytes will be useful when the preservation of viable cardiac myocytes is required. Recently we developed a cryopreservation method of cardiac myocytes showing similar morphology and L-type Ca2+ currents. In this study, we would like to check the microstructure using electron microscopy (EM) and the mitochondrial function. In EM photos, there were clear differences in mitochondrial density and the mitochondria of the cryopreserved cardiac myocytes were swollen. However, the cryopreserved cells showed clear mitochondrial cristae without signs of disruption. The oxygen consumption of the cryopreserved cardiac myocytes was significantly decreased in the presence of 1 mM pyruvate/malate/glutamate. We tried the high osmotic solutions to reduce mitochondrial swelling to test the reversibility of mitochondrial functions, however, there were no differences in difference osmotic conditions. When the uncoupler, 5 μM FCCP, was applied, the oxygen consumption of the control cardiac myocytes increased two times, however, there were no changes in cryopreserved cardiac myocytes. In conclusion, the present method of the cryopreservation could maintain the electrophysiological characteristics, however, the mitochondrial functions are somehow

attenuated. Further study to find the way to normalize mitochondrial functions of the cryopreserved cardiac myocytes is definitely required. (supported by the grant (R0005739)from KIAT).
Key Words: cardiomyocyte, cryopreservation, mitochondria respiration, uncoupled respiration

P06-18

Vascular sheet of hPSC-derived endothelial progenitor cells and smooth muscle cells using 3D culture

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The blood vessels are the part of the circulatory system that transports blood throughout the human body. The circulatory system is an organ system that permits blood to circulate and supply nutrients, oxygen to tissue and organ. For that reason, neovasculogenesis and angiogenesis are an important process that occurs both during health and disease such as formation of new tissues involves formation of new blood vessels, tissue regeneration and repair. Cell sheet engineering allows for tissue regeneration by either direct transplantation of cell sheets to host tissues. Reconstructing a vascular network is a common task for three-dimensional (3D) tissue engineering. hPSC-derived endothelial cells and their progenitors may provide the means for vascularization of tissue-engineered constructs and can serve as models to study vascular development and disease. In this study, to serve as models to study vascular development and treat to disease, we differentiated to endothelial progenitor cells (EPC) and contractile state smooth muscle cells (SMC) from hPSC. To make 3D vascular sheet, we cultured mixed cell population of EPC and SMC in the ratio of certain concentration in 20% matrigel for 3days, which confirmed that cells formed tubular structure and vascular network dispersedly in matrigel by way of 3D. Finally, we engineered reconstructing a vascular network using 3D culture of hPSC-derived EPC and SMC.
Key Words: 3D culture, vascular sheet, blood vessel, vascular tissue engineering, endothelial progenitor cells, human pluripotent stem cell

P06-19(PO-07)

Gross morphological properties of the primo-vascular system and its relations with the acupuncture meridian

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The primo-vascular system (PVS) is a novel vascular network that was first reported in the 1960s by Bong-Han Kim, who claimed that the tissue corresponded with the acupuncture meridians. The PVS has been identified in various sites in several animal species. However, the PVS in subcutaneous tissues, which was related the acupuncture meridians, was not well identified. Here, we examined the subcutaneous PVS (scPVS) on the surface of abdominal subcutaneous tissue in rats using Hemacolor staining. The scPVS consisted of vessels that connect the node parts and that the vessels were frequently sub-branched. In the Hemacolor-stained whole scPVS tissue in isolation, the cells of the scPVS were found to be composed of leucocytes and mast cell. The inner space-channel (20-60 μm) structure containing PVS cells along the inside of a vessel of the scPVS was identified. Electron microcopy revealed that the scPVS has a bundle structure of subducts and round cells on the surface and that these were not found in the lymphatic vessel. In addition, the distribution pattern of scPVS tissues in the ventral midline was similar to the route of the conception vessel meridian. In conclusion, our results newly revealed that the PVS is present in the abdominal subcutaneous tissue layer, and indicate that the scPVS tissues are closely correlated with acupuncture meridians. The scPVS has major features in common with the well-established organ-surface PVS tissue in terms of gross morphology and cellular/structural characteristics. These findings may help to identify other PVS tissues in the body and further elucidate their pathophysiological roles in healthy and disease states.
Key Words: subcutaneous tissue, hemacolor staining, mast cell, electron microscopy, conception vessel meridian

P06-20(PO-04)

IDH2 Inhibition impairs endothelium-dependent vasomotor function via mitochondrial function in endothelial cells

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Mitochondrial NADP(+)-dependent isocitrate dehydrogenase (IDH2) plays an essential role protecting cells against oxidative stress-induced damage. A deficiency in IDH2 leads to mitochondrial dysfunction and the production of reactive oxygen species (ROS) in cardiomyocytes and cancer cells. In this study the effects of IDH2 deficiency on mitochondrial and vascular function were investigated in endothelial cells. IDH2 knockdown decreased the expression of mitochondrial oxidative phosphorylation (OXPHOS) complexes I, II and III, which lead to increased mitochondrial ROS (mtROS). In addition, the levels of fission and fusion proteins (Mfn-1, OPA-1, and Drp-1) were significantly altered and MnSOD expression also was decreased by IDH2 knockdown. Knockdown of IDH2 decreased eNOS phosphorylation and nitric oxide (NO) concentration in endothelial cells. Interestingly, treatment with Mito-TEMPO, a mitochondrial-specific superoxidescavenger, recovered mitochondrial fission- fusion imbalace and blunted mt superoxide production, and reduced the IDH2 knockdown-induced decrease in MnSOD expression, eNOS phosphorylation and NO production in endothelial cells. Endothelium-dependent vasorelaxation was impaired, and the concentration of bioavailable NO decreased in the aortic ring in IDH2 knockout mice. These findings suggest that IDH2 deficiency induces endothelial dysfunction through the induction of dynamic mitochondrial changes and impairment in vascular function.
Key Words: IDH2, mitochondrial dysfunction, ROS, eNOS, vascular function

P06-21(YP-08)

Impaired myogenic responses in the skeletal arteries from denervated hind limbs and their recovery by exercise training; underlying changes of ion channel currents in the arterial myocytes

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K^+ channel currents such as voltage-gated K^+ current (I_{Kv}) and inwardly rectifying K^+ current (I_{Kir}) set the negative membrane potential of arterial myocytes, inhibiting voltage-operated L-type Ca^{2+} currents ($I_{Ca,L}$). Previous studies demonstrated that both I_{Kv} and I_{Kir} in deep femoral arterial smooth muscle cells (DFASMCs) are significantly increased in the rats underwent endurance exercise training (ET). In this study, a unilateral sciatic nerve was severed to induce lower hindlimb paralysis and atrophy. In deep femoral arteries (DFAs) and the myocytes isolated from DFAs (SkASMCs), pressurized artery video-analysis and whole-cell patch clamp were applied, respectively. The myogenic response (MR) of DFA from paralyzed legs disappeared in 5 wk of denervation. In DFASMCs from the paralyzed legs, both $I_{Ca,L}$ and TRPC-like current induced by intracellular GTPγS ($I_{GTPγS}$) were decreased. In addition, I_{Kir} and I_{Kv} became decreased in the DFASMCs from atrophic legs. When combined with ET (4 weeks) after the sciatic nerve injury, the MR of DFAs showed almost complete recovery with associated increase of $I_{GTPγS}$ while not $I_{Ca,L}$ in DFASMCs. I_{Kir} and I_{Kv} also showed recovery in the ET-combined rats. The atrophy side-specific decreases of MR and ion channel currents might reflect the importance of blood flow-induced mechanical stimuli to the arterial smooth muscle function. The recovery of MR and ion channel currents by ET in skeletal arteries suggest beneficial effects on the blood pressure regulation as well as the regional blood flow control in motor nerve injury patients.

Key Words: denervation, atrophy, skeletal artery, myogenic response, smooth muscle, ion channel

P06-22(YP-10)

Rapamycin ameliorates the portal hypertension, cardiovascular autonomic dysfunction, and alterations in the excitability of sympathetic and parasympathetic cardiac neurons in cirrhotic rats

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Cardiovascular autonomic dysfunction (CAD), which is manifested by the decreased baroreflex sensitivity (BRS) and heart rate variability (HRV), is prevalent irrespective of etiology and contributes to the increased morbidity and mortality in patients with liver cirrhosis. Recently, we have observed that liver cirrhosis and portal hypertension-induced CAD is attributable to an imbalance of excitability between sympathetic and parasympathetic cardiac neurons. A previous study has shown that rapamycin improves liver function by limiting inflammation, fibrosis, and portal pressure in the early phase of cirrhotic portal hypertension. Thus, we examined whether rapamycin ameliorates development of the portal hypertension, the CAD, and the functional plasticity of the cardiac autonomic neurons in cirrhotic rats. Biliary cirrhotic rats were generated via common bile duct ligation (CBDL). From the first day of CBDL, rats orally received 0.5 mg/kg/day for 2 weeks. Picrosirius red and Masson staining showed that rapamycin inhibited heavy collagen deposition, as verified by broad fibrotic septa that surround abnormal nodules. In accordance with the histological examination, rapamycin significantly inhibited CBDL-induced increase in the transcripts that encode alpha-smooth muscle actin, TGF-beta, and type I collagen. Development of the portal hypertension was prevented in the rapamycin-treated CBDL rats. More importantly, rapamycin inhibited the CBDL-induced decreases in the BRS and HRV. Under the current clamp mode of the gramicidin-perforated patch-clamp technique, cell excitability was recorded in sympathetic stellate ganglion (STG) and parasympathetic intracardiac ganglion (ICG) neurons. Rapamycin inhibited the CBDL-induced increase and decrease in the frequency of action potential discharge in the STG and ICG neurons, respectively. Taken together, these results suggest that cirrhotic portal hypertension is associated with CAD and functional plasticity of the cardiac autonomic neurons. Importantly, anti-fibrotic agents might be considered as potential therapeutics for the CAD in cirrhotic patients. (This research was supported by Basic Science Research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2013424)).

Key Words: arterial baroreflex, autonomic, cardiovascular autonomic dysfunction, excitability, heart rate variability, liver cirrhosis, portal hypertension, rapamycin

P06-23

Necrox-5 attenuates ischemic heart injury via regulation of mitochondria biogenesis and inflammation response

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The aim of this study is to verify the effect of Necrox-5 on cardiac proteomic alteration and mitochondrial biogenesis, inflammation and fibrosis responses in a hypoxia-reoxygenation (HR) treated rat heart. Necrox-5 treatment (10 μM) and non-treatment were employed on isolated rat hearts during hypoxia/reoxygenation treatment using an ex vivo Langendorff system. Proteomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS) and non-labeling peptide count protein quantification. Proteomic results were confirmed by western blot and real-time PCR experiment. Mitochondrial functions and inflammation responses were analyzed by using complex activity assay, cytotoxicity assay, and cytokine ELISA assay were then assessed. HR treatment significantly decreased level of mitochondria biogenesis related proteins and increased level of pro-inflammatory proteins. NecroX-5 treatment significantly attenuated those HR-induced proteomic alterations, especially which are involved in oxidative phosphorylation and metabolic function. NecroX-5 post-hypoxic treatment also improved mitochondrial complex activities. Markedly higher PGC1α expression levels were found in NecroX-5 treatment group. In addition, HR- or LPS-induced TNF-α and TGF-β1 productions were significantly attenuated with NecroX-5 supplement. Furthermore, NecroX-5 suppressed phosphorylation of Smad2 production and increased expression level of decorin. The findings suggested the cardioprotective effect of NecroX-5 against cardiac HR injuries by modulating mitochondrial biogenesis and exerting anti-inflammation and anti-fibrosis effects.

Key Words: NecroX5, ischemic heart injury, mitochondria, inflammation

P07-01

Regulation of voltage-gated Ca^{2+} channels by DREADD system

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Voltage-gated calcium channels (Ca_V channels) play essential roles in adjusting calcium ion influx upon membrane depolarization. In synapse, an electrical signal of presynaptic neuron is converted to and released as neurotransmitter. Specifically, the presynaptic Ca_V channels gated by depolarized membrane potential take Ca^{2+} intracellularly, and then, the depolarization of membrane potential is accelerated. These series of process cause further neurotransmitter release. The Ca_V channels which control neuronal signal transduction can be modulated by transmembrane proteins called G protein-coupled receptors (GPCRs). Previous experiments have shown that gating of Ca_V channels is inhibited by activating muscarinic acetylcholine receptor (mAchR). According to the subtypes of mAchR, the signaling pathways to inhibit Ca_V channels are different; one is G_qPCR-mediated voltage-independent inhibition and the other $G_{i/o}$PCR-mediated voltage-dependent inhibition. However, in neuron Oxo-M, a ligand of mAchR, cannot selectively activate these separate GPCRs. To solve this problem, we used a chemogenetic system called DREADD (designer receptors exclusively activated by designer drugs), which is able to activate the signaling pathways selectively and artificially. At the present stage, we compare the kinetic differences of PIP_2 depletion and Ca_V channel current inhibition depending on the G_qPCRs (mAchR or DREADD). By using confocal microscopy, we confirmed that DREADD needs longer time to deplete PIP_2 and DREADD depletes less PIP_2. With FRET (fluorescence resonance energy transfer) and Ca^{2+} imaging experiments, it was also confirmed that a series of intracellular signal transduction pathways starting from PIP_2 depletion takes a longer time in DREADD. Lastly, we confirmed DREADD has less and slower effects on voltage-independent inhibition of Ca_V channels.

Key Words: PIP_2, DREADD, Voltage-gated calcium channel

P07-02

Endogenous progenitors on regeneration and repair after spinal cord injury: JAK3-dependent microgliosis

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Endogenous progenitor cells that express nestin, in spinal cord injury (SCI), migrate into the lesion to aid in repair and regeneration. These cells can transform into neurons, astrocytes and other cells. Microglial activation helps control injury progression and motor function, which are affected by axonal survival and outgrowth. Inhibition of the tyrosine kinase JAK3 induces neurogenesis with neurite elongation in cortical neuroprogenitor cells. We study tested the hypothesis that JAK3 plays a vital role in SCI progression by altering neurite extension or by controlling the microglial response. Inhibitor of JAK3, WHI-P131, improved motor function in our right-sided spinal cord hemisection rat model. This improvement was related to reduce microglia activation in the lesion epicenter. Interestingly, $nestin^+$ cells in the lesion center could differentiate into $Iba1^+$ microglia which is JAK3-dependent. JAK3 inhibition markedly increased neurogenesis and promoted longer neurite outgrowth across the lesion while reducing microgliogenesis and the levels of M1 and M2 macrophages. Moreover, WHI-P131 reduced microglial migration and phagocytic activity. Increased neurite outgrowth was inversely related to microglial activation. We conclude that JAK3 signaling helps to recruit endogenous $nestin^+$ progenitors and that microgliogenesis via activated JAK3 might be harmful to neuronal regeneration. [This study was supported by a Korean Research Foundation grant from the Korean government (2012R1A2A01011417) and the Chronic Inflammatory Disease Research Center (NRF-2012R1A5A204183).]

Key Words: Spinal cord injury, JAK3, Microglia, Neuroprogenitor cell, Regeneration

P07-03

Adrenergic modulation of glial activity during noxious information processing in the cerebellum

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Cerebellar activation and increase in metabolic changes during pain processing have been reported in lots of brain imaging study. However, the activation of Bergmann glia (BG), the cerebellar astrocytes which may support cerebellar metabolic needs during noxious information processing and its physiological outcome were not investigated. To monitor the calcium activity of BG in intact cerebellar cortex lobule IV/V, we performed two-photon calcium imaging using a calcium dye OGB-1 and astrocyte marker SR101 in anesthetized mice. Various noxious electrical stimuli were delivered to anesthetized mice during calcium imaging and pharmacological modulation. To monitor BG calcium activity in response to physiological pain stimuli, formalin was injected in hind-paw during imaging sessions. We show that 1) Noxious electrical stimulation (ES) in anesthetized mice results in norepinephrine release and subsequent activation of BG network in the cerebellum. 2) The ES-induced BG calcium response was completely blocked by tetrodotoxin and alpha1-adrenergic receptor blocker while attenuated by glutamate receptor blockers. 3) Formalin injection induces BG calcium responses, during the early phase other than the late phase. Taken together, we suggest that noradrenergic signaling mediates activation of the glial network during noxious information processing in the cerebellum.

Key Words: Cerebellum, Pain, Noxious information processing, Norepinephrine, Bergmann Glia

P07-04

Dapoxetine induces neuroprotective effects against glutamate-induced neuronal cell death through inhibiting calcium signaling and mitochondrial depolarization in cultured rat hippocampal neurons

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Selective serotonin reuptake inhibitors (SSRIs) have inhibitory effects on various ion channels including Ca^{2+} channels. We examined whether dapoxetine, a novel rapid acting SSRI, affects glutamate-induced calcium signaling, mitochondrial depolarization and neuronal cell death in cultured rat hippocampal neurons using fluorescent dye-based digital imaging methods, a whole cell patch clamping and cytotoxicity assays. Pretreatment with dapoxetine (0.1 μM to 300 μM) for 10 min inhibited glutamate-induced $[Ca^{2+}]_i$ increases in a concentration-dependent manner (IC_{50}= 4.79 μM). Dapoxetine (5 μM) markedly inhibited glutamate-induced $[Ca^{2+}]_i$ increases, whereas other SSRIs fluoxetine and citalopram slightly inhibited the $[Ca^{2+}]_i$ responses. Dapoxetine significantly inhibited the glutamate-induced $[Ca^{2+}]_i$ responses following depletion of intracellular Ca^{2+} stores by treatment with thapsigargin. Dapoxetine also markedly inhibited the metabotropic glutamate receptor agonist, DHPG-induced $[Ca^{2+}]_i$ increases. Dapoxetine significantly inhibited the glutamate and AMPA-induced $[Ca^{2+}]_i$ responses in the presence or absence of nimodipine. Dapoxetine also significantly inhibited AMPA-evoked currents. However, dapoxetine slightly inhibited NMDA-induced $[Ca^{2+}]_i$ increases. Dapoxetine markedly inhibited 50 mM K^+-induced $[Ca^{2+}]_i$ increases. Dapoxetine significantly inhibited glutamate-induced mitochondrial depolarization. Moreover, dapoxetine significantly inhibited glutamate-induced neuronal cell death and increased the cell survival. The neuroprotective effect of dapoxetine was significantly greater than fluoxetine. All these data suggest that dapoxetine reduces glutamate-induced $[Ca^{2+}]_i$ increases by inhibiting multiple pathways mainly through AMPA receptors, voltage-gated L-type Ca^{2+} channels (VOCCs) and metabotropic glutamate receptors, which is involved in neuroprotection against glutamate-induced cell death through Ca^{2+}-mitochondrial depolarization. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022015)" Rural Development Administration, Republic of Korea and the Development Fund for Department of Physiology, College of Medicine, The Catholic University of Korea.

Key Words: $[Ca^{2+}]_i$, Dapoxetine, Glutamate, Hippocampal neurons, Neuroprotection, Selective serotonin reuptake inhibitor

P07-05

Neonatal maternal separation enhances $GABA_A$ receptor-mediated inhibitory currents of ventral hippocampal dentate granule cells in adolescent

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Neonatal maternal separation (MS) has been shown to be associated with an increased vulnerability to psychiatric illnesses such as anxiety, depression later in life. Previously, we have shown that adolescent mice show anxiety- and aggressive-like behavior and reduction of long-term potentiation (LTP) in mossy fiber-CA3 synapses after neonatal MS. The present study investigated the mechanism of the reduction of LTP in mossy fiber-CA3 synapses after neonatal MS. After MS procedure for 19 days, we measured excitatory postsynaptic current (EPSC) and inhibitory postsynaptic current (IPSC) (spontaneous-, miniature- postsynaptic current, respectively) in hippocampal granule cell by voltage clamp technic. We also evaluated intrinsic membrane property of granule cell using current clamp technic. As results, EPSC and intrinsic membrane property such as resting membrane potential, input resistance, membrane time constant, action potential amplitude and mean discharge frequency did not significantly different between handling group and MS group. However, neonatal MS showed significantly elevated $GABA_A$ receptor-mediated currents. This observation suggests that reduction of LTP in mossy fiber-CA3 synapses after neonatal MS may come from the increased GABAergic neurotransmission in the hippocampal granule cell. **References or Acknowledgement:** The research was supported by Basic Science Research foundation of Korea (NRF) funded by the Ministry of Education (2015R1D1A1A01061326)

Key Words: maternal separation, GABA, patch clamp, hippocampal granule cell

P07-06

Encoding strategy for sensory information processing with different stimuli features in Primary somatosensory cortex

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The primary somatosensory (S1) cortex has a key role in the perception of both painful and non-painful somatic sensations, including discrimination of stimulation modality, quality and intensity. However, unlike other sensations (vision, hearing, taste and smell), how touch and pain somatic sensations are coded in the cortex at the cellular level is largely unknown. To identify encoding strategies adopted by S1 cortex for processing the sensory information, we investigated the tuning profiles of layer 2-3 neurons in the mouse S1 cortex in response to several stimuli features. We found that responding neurons of layer 2/3 in S1 were separated by brushing and forceps touch/pinch stimuli, but a majority of neurons were highly overlapped to forceps touch and pinch (intensity feature). And these neurons respond selectively to either of texture and dynamics (quality feature), but more tuned to texture. Taken together, we conclude that quality feature of the sensory information is coded selectively by highly tuned neurons while intensity feature is coded by broadly tuned neurons, suggesting that S1 uses different encoding strategies for processing the sensory information with different stimuli features.

Key Words: pain, sensory systems, in vivo imaging, neural coding

P07-07

Progesterone metabolite suppresses visceral afferent signal transduction on medial nucleus tractus solitarius (mNTS) neurons

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Pregnancy accompanies changes in hemodynamics by blunting baroreflex response in many species including human. Suppressed baroreflex response impairs the ability to maintain blood pressure

during obstetrical hemorrhage. Recent reports suggested that elevated level of 3α-hydroxy-dihydroprogesterone (3α-OH-DHP), a major metabolite of progesterone, in maternal plasma and brain may contribute to pregnancy associated changes in baroreflex response. 3α-OH-DHP is one of the most potent endogenous positive allosteric modulator of GABAA receptor and exerts diverse physiological functions by acting GABA receptors in various brain regions. However, 3α-OH-DHP action on central baroreceptor processing is not known yet. In this experiment, 3α-OH-DHP effect on visceral afferent signal transduction in mNTS is tested using female adult rat hindbrain slice preparations. In those neurons, 3α-OH-DHP increased gabaergic inhibition by increasing decay-time of gabaergic inhibitory postsynaptic currents (IPSCs). 3α-OH-DHP also suppressed mNTS neurons activity by inducing gabaergic tonic inhibitory current or suppressing evoked EPSCs. These results suggest that elevated level of 3α-OH-DHP during pregnancy may suppress baroreflex response by inhibit mNTS neurons activity.

Key Words: mNTS, Progesterone metabolite, 3α-OH-DHP, evoked EPSCs

P07-08

TRPC3 channels drive pacemaking and regulate tonic firing rate in nigral dopamine neurons

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Dopamine neurons in the substantia nigra pars compacta (SNc) are slow pacemakers that generate spontaneous action potentials (APs) regularly. Although spontaneous action potentials are essential for maintenance of background dopamine levels and proper functioning of basal ganglia, it is not clear what channels are responsible for pacemaking and determine basal firing rate in the SNc dopamine neurons. In this study, we report that TRPC3 channels drive pacemaking and regulate basal firing rate via type 1 metabotropic glutamate receptors (mGluR1) in the SNc dopamine neurons. Specific TRPC3 channel blockers, pyr10 and pyr3, stopped spontaneous firing and Ca^{2+} oscillations in dopamine neurons. However, spontaneous firing was conserved in dopamine neurons of TRPC3 knockout (KO) mice and there was no significant difference in spontaneous firing rates between the TRPC3 KO and wild type mice. However, application of pyr10 did not affect spontaneous firing and Ca^{2+} oscillations in TRPC3 KO mice, suggesting that pyr10 blocked spontaneous firing by specifically blocking TRPC3 channels in normal dopamine neurons. TRPC3 channel blockade with pyr10 hyperpolarized membrane potentials, but somatic current injection regenerated pacemaker activity again, suggesting that TRPC3 channels are constitutively active and helps to maintain membrane potential depolarized within pacemaking ranges. In addition, stimulation of mGluR1 induced sustained Ca^{2+} influxes together with enhancement of spontaneous firing. When TRPC3 channels were blocked by pyr10, mGluR1-induced slow Ca^{2+} influxes were dramatically reduced. Furthermore, in TRPC3 KO mice, firing rates did not significantly enhanced by activation of mGluR1 in SNc dopamine neurons. From these experimental results, we conclude that TRPC3 channels are not only essential for pacemaking, but also determine basal firing rate in mGluR1-dependent ways in SNc dopamine neurons.

Key Words: dopamine neuron, mGluR1, TRPC3, spontaneous firing

P07-09

Differential density of IP_3 receptors confers dendritic domain-dependency on muscarinic modulation in layer 2/3 pyramidal neurons of the primary visual cortex

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Previously, we demonstrated that muscarine-induced Ca^{2+} release from IP_3-sensitive stores and induction of LTP was restricted in basal dendrites but not in distal apical dendrites. The mechanism of this dendritic domain dependency is still not known. Therefore, here we investigated which components of the processes contribute for the nature of dendritic domain-dependent muscarinic LTP. Subthreshold EPSPs were evoked by localized single-shock electric stimulation (SES) with a glass pipette located ~10 μm from dendrite. SES on basal dendrites released Ca^{2+} from IP_3-stores and evoked LTP with the bath application of muscarine. When the cells were loaded with IP_3-3F (100 μM), a Ca^{2+}-releasing analogue of IP_3 but not metabolized by IP_3-3-kinase, SES released Ca^{2+} and evoked LTP in the absence of muscarine. The magnitude of LTP by IP_3-3F was comparable to those of muscarine treatment. However, there was no Ca^{2+} release and induction of LTP at distal apical dendrite by SES either inclusion of IP_3-3F in the pipette solution or bath application of muscarine. These results imply that density of IP_3 receptors may different among the dendrites. Immunoreactivities of IP_3 receptor type 1 were strongest at the soma and the apical trunk. In fine basal dendrites, immunoreactivities were successfully obtained and showed even signal along dendrites. However, immunoreactivities at distal apical dendrite were scarce. Interestingly, this dendritic area was consistent with layer 1. Meanwhile, immunoreactivities for mAChR were strongly detected at this layer 1. It was not clear whether ER structure extended to whole dendrite of layer 2/3 pyramidal neurons since the immunoreactivities of calnexin, ER marker protein, were very weak at both layer 1 and basal dendrite. Taken together, these results indicate that dendritic domain-dependent muscarinic effects on Ca^{2+} release and LTP might be caused by differential density of IP_3 receptor in dendrites rather than by that of mAChR. Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).

Key Words: IP_3 receptor, Visual cortex, LTP, Muscarinic, Pyramidal neuron, Dendrite.

P07-10

Estrogen blocks the inhibitory-to-excitatory switch in GABAergic action in the vasopressin neurons of salt-dependent hypertension model rats

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Background: Abundant evidence indicates that estrogen plays a protective role against hypertension. To date, however, central mechanism underlying the antihypertensive action of estrogen has not been identified. Estrogen receptors are expressed in the hypothalamic magnocellular neurons secreting vasopressin, a molecule involved in

the pathogenesis of salt-dependent hypertension. And, in the male rat models of salt-dependent hypertension ("salt loading" and "DOCA-salt" models), GABA functions as an excitatory, rather than inhibitory, neurotransmitter in vasopressin neurons, and therefore, helps hypertension to develop and/or be maintained. In the current study we raised the hypothesis that, in the female rat, estrogen suppresses salt-dependent hypertension by preventing GABA from functioning as an excitatory neurotransmitter in vasopressin neurons. **Methods and Results:** To test this hypothesis, we examined GABAergic transmission in vasopressin neurons recorded in the supraoptic nucleus (SON) slices prepared from two different female rat models of salt-dependent hypertension (salt loading and DOCA-salt), which were subjected to: 1*) sham ovariectomy (sham group), 2) bilateral ovariectomy (OVX group)* or *3) estrogen capsule implantation after bilateral ovariectomy (OVX+E_2 group),* a week prior to the commencement of treatment for the generation of the models. Gramicidin-perforated recordings in the SON slices revealed that, in both of the models, GABA was mainly inhibitory in the vasopressin neurons of sham group of rats, whereas it usually acted as an excitatory transmitter in the cells of the OVX group. Also, the recordings showed that, in the DOCA-salt model, GABA was mostly inhibitory in the neurons of OVX+E_2 group as in the cells of sham group. Collectively, these findings indicated that estrogen suppresses the transition of GABAergic inhibition to excitation in the vasopressin neurons of the female rat models of salt-dependent hypertension and, therefore, restrains the output of these cells. To provide further evidence for this idea, we next examined the effects on the blood pressure (BP) of intravenously injected V1a vasopressin receptor antagonist in the sham and OVX groups of DOCA-salt model rats and found that this drug lowered the BP to a lesser extent in the sham, than OVX, group of rats. **Conclusions:** The prevention by estrogen of the emergence of GABAergic excitation in hypothalamic magnocellular vasopressin neurons may be an important central mechanism underlying the action of estrogen against salt-dependent hypertension.
Key Words: DOCA-salt, Estrogen, Electophysiology, GABA, Hypertension, Salt loading, Vasopressin

P07-11

Suppression of the Sweat Gland Sensitivity to QSART in Tropical Africans Compared to Temperate Koreans

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During heat stress, tropical natives are reported to economize body fluid by exhibiting suppressed sweating compared to temperate natives. However the mechanisms involved in this suppressed sweating has not been not fully understood. In this study, we elucidate the peripheral mechanisms of the suppressed thermal sweating in tropical natives, sweating responses to acetylcholine (ACh), a primary transmitter of the sudomotor innervation, were compared between temperate Koreans (41 healthy males) and tropical Africans (36 healthy males). ACh was iontophoretically administered on the forearm. Directly activated and axon reflex-mediated sweat responses were evaluated by quantitative sudomotor axon reflex test (QSART). Directly activated (DIR) and axon reflex-mediated (AXR) sweat responses during ACh were evaluated by QSART were measured by capacitance hygrometer. The sweat onset-time during QSART was shorter (P< 0.01) and the sweat volume (AXR and DIR) higher (P< 0.001) in the temperate natives compared to the tropical Africans. The number of sweat glands actively secreting sweat was higher (P < 0.01) and active sweat output per gland was larger (P< 0.01) in the temperate natives compared to the tropical Africans. These results indicate that suppressed thermal sweating in Africans is, at least in part, attributed to the suppressed glandular sensitivity to ACh through both recruitment of sweat glands and sweat output per each gland.
Key Words: QSART, Sweating, Active sweat glands, Acclimatization, Tropical, Temperate.

P07-12

Effects of acute ingestion of caffeine on the blood levels of dopamine and serotonin (5-HT) in exercising humans

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The aim of this study was to investigate the blood levels of dopamine (DA) and serotonin (5-HT) in humans with and without caffeine ingestion during treadmill running (30 min, 75% $\dot{V}O_{2max}$). Thirty male volunteers (22.2 ± 3.12 yr; BMI, 22.1 ± 1.11 kg/m^2) participated in the randomized experiment (CON; n=15, tap water vs. CAFF; n=15, 3 mg·kg^{-1} caffeine and tap water). After treadmill running (Post-EX), DA was significantly increased in CAFF (P < 0.01) and significantly higher compared to the CON group (P < 0.01). 5-HT was significantly increased in both groups at Post-EX (P < 0.05). However, there was no significant statistical difference between groups. Prolactin and cortisol were significantly increased in both groups at Post-EX (P < 0.01), but there were no significant statistical differences between groups. β-endorphin significantly increased in CAFF at Post-EX (P < 0.01) and significantly higher than in CON at Post-EX (P < 0.01). In conclusion, these data suggest that 3mg·kg^{-1} caffeine ingestion prior to treadmill running can increase DA, but has no effect of 5-HT inhibition.
Key Words: caffeine, running, blood, dopamine, serotonin

P07-13

Bidirectional synaptic plasticity in late-spiking neurons of layer 2/3 in rat visual cortex

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Characterization of cell types in the primary visual cortex is prerequisite to understand the processing of sensory information. Various types of inhibitory neurons have been known to exert critical function in visual coding in cortical circuits. Moreover, inhibitory circuits play an important role in synaptic plasticity of excitatory pyramidal neurons during postnatal development and adulthood. However, whether these inhibitory neurons themselves exhibit long-term synaptic plasticity in the visual cortex remains unknown. Therefore, using intracellular recordings and confocal reconstruction, we investigated the mechanism of long-term synaptic plasticity in late-spiking (LS) neurons of layer 2/3, induced by stimulation of underlying layer 4 in the rat visual cortex. Inhibitory interneurons in layer 2/3 of primary visual cortex were classified into four types according to the electrophysiological and

morphological characteristics: fast-spiking (FS), LS, burst-spiking (BS) and regular-spiking non-pyramidal (RSNP) neurons. We used different conditioning stimulation to induce long-term depression (LTD) and potentiation (LTP). All the inhibitory neurons showed LTD with low-frequency stimulation (1 Hz, 15 min) except FS neurons. LTD induced in LS neurons was blocked by intercellular application of BAPTA (10 mM) and bath application of nimodipine (10 μM), but not by bath application of D-AP5 (50 M). Theta-burst stimulation (5 shocks at 100 Hz, 5 times at 5 Hz) did not change the synaptic efficacy in LS neurons. Tetanic stimulation (5 x 20 Hz trains for 1s at 0.5 Hz, 5 times at 2 min interval) reliably induced LTP in LS neurons as well as pyramidal neurons. LTP in LS neurons was blocked by intracellular application BAPTA. Therefore, we suggest that LS neurons in layer 2/3 of the visual cortex exhibit bidirectional long-term changes in synaptic strength, which are dependent on the increase in intracellular calcium via L-type calcium channels. This bidirectional modification of synaptic transmission may be important in visual information processing and postnatal development. Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).
Key Words: Late-spiking neurons, Visual cortex, LTP, LTD, L-type calcium channel.

P07-14

Thalamic miR-338-3p mediates auditory thalamocortical disruption and its late onset in 22q11.2 microdeletion models

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The 22q11.2 deletion syndrome (22q11DS) is associated with high risk of developing schizophrenia symptoms, including psychosis, later in life. Auditory thalamocortical projections recently emerged as a circuit specifically disrupted in 22q11DS mouse models. Haploinsufficiency of the microRNA-processing gene Dgcr8 results in the elevation of the dopamine receptor Drd2 in the auditory thalamus, an abnormal sensitivity of thalamocortical projections to antipsychotics, and an abnormal acoustic-startle response. Here we show that these auditory abnormalities have a delayed onset in 22q11DS mouse models and are associated with age-dependent reduction of the microRNA miR-338-3p, which targets Drd2 and is enriched in the thalamus of humans and mice. Replenishing depleted miR-338-3p in the mature 22q11DS mice rescued the thalamocortical abnormalities, and miR-338-3p deletion/knockdown mimicked thalamocortical and behavioral deficits and eliminated their age dependence. Thus, miR-338-3p depletion is necessary and sufficient to disrupt auditory thalamocortical signaling in 22q11DS mouse models and may therefore mediate the pathogenic mechanism of 22q11DS-related psychosis and control its late onset.
Key Words: 22q11 deletion syndrome, Dgcr8, Drd2, microRNA, Schizophrenia

P07-15

Critical role of muscarinic acetylcholine receptors in synaptic depotentiation at the hippocampal SC-CA1 synapses

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Modification of synaptic strengths through activity-dependent synaptic plasticity such as long-term potentiation (LTP), long-term depression (LTD), and depotentiation, is believed to be the cellular basis of learning and memory. In contrast to LTP and LTD, little is known about the mechanisms of depotentiation (reversal of LTP). Here we show that co-activation of NMDARs (N-methyl-D-aspartate receptors), mGluRs (metabotropic glutamate receptors), and mAChRs (muscarinic acetylcholine receptors) is necessary and sufficient to reverse stably expressed LTP. At hippocampal Schaffer collateral (SC)-CA1 synapses, paired-pulse low frequency stimulation (PP-LFS) reversed LTP that was induced by high-frequency stimulation. PP-LFS-induced depotentiation was inhibited by the NMDAR antagonist AP-5, the mGluR5 antagonist MPEP, and the mAChR antagonist atropine. Conversely, co-activation of NMDARs, mGluRs, and mAChRs with low doses of agonists reversed stably expressed LTP. Activation of mAChRs caused depolarization of membrane potential and allowed sufficient activation of NMDARs. In addition, mAChR stimulation enhanced Gq-mediated ERK activation, a common downstream signaling pathway with mGluRs. These results suggest that activation of mAChRs amplifies glutamate-mediated signaling and mediates synaptic depotentiation.
Key Words: hippocampus, depotentiation, mAChR, NMDAR, mGluR

P07-16

Effects of acute hypoxic stimulus with inflammation and reperfusion on excitability and synaptic transmission of the hippocampal CA1 neurons

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Hypoxia, often observed together with inflammatory neuropathology such as trauma, stroke, and neurodegenerative diseases, is thought to exacerbate inflammatory responses and synergistically enhance brain damage. Recent studies showed microglia - driven AMPA receptor internalization upon inflammatory stimulus and hypoxia. However, direct physiological impact on neuron and circuit are not studied thoroughly. In this study, we investigated whether hypoxia and an inflammatory stimulus with lipopolysaccharide (LPS) act synergistically to regulate properties of neuronal excitability and presynaptic neurotransmitter release properties. To elaborate the impact of the combined insults, we recorded in the hippocampal CA1 region of acute brain slices from 12-21 days old SD rat. We found various physiological changes that can cause significant neuronal excitability and synaptic connections upon incubation with hypoxia (8% O_2, 5% CO_2, 87% N_2) and LPS (10 μg/ml) for 30 min; Hyperpolarization of resting membrane potentials (RMPs), decreased input resistant (R_{in}) and AP frequency and I_h current and increase of AP onset time were observed. Also, we

found significant changes in presynaptic release efficacy measured by short-term plasticity and mEPSC frequency. On the other hand, during reperfusion we found dramatic rebound activity including significant depolarization and increased AP frequency, which may be a major excitotoxicity during re-oxygenation after a period of hypoxia.
Key Words: Hypoxia, inflammation, Action potentials, Synaptic transmission, I_h channels

P07-17

Suppression of spinal IL-1β facilitates gap junction-mediated production of contralateral astrocyte D-serine leading to the early development of contralateral mechanical allodynia in carrageenan rats

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Previously we have reported that spinal IL-1β mediated modulation of spinal astrocyte gap junctions play a critical role in development of mirror-image pain (MIP). However, it is still remain to be discovered the underlying mechanisms how contralateral astrocytes stimulate the contralateral nociceptive neurons to produce MIP. In this study, we demonstrated the role of spinal astrocyte derived D-serine on development of MIP and the effect of spinal IL-1β on astrocyte gap junction in production of contralateral D-serine. Peripheral carrageenan (CA) injection induced upregulation of ipsilateral serine racemase (SeRa) and D-serine levels in earlier phase of post-carrageenan injection, while, contralateral SeRa and D-serine levels were upregulated in later phase showing similar pattern with the timepoint of MIP development. Intrathecal (i.t.) administration of LSOS, a SeRa inhibitor, or DAAO, a D-serine degrading enzyme, significantly blocked the development of MIP. Meanwhile, the i.t. injection of IL-1ra, an IL-1 receptor antagonist, notably advanced the development of MIP and increased the expression levels of spinal SeRa and D-serine. These effects of i.t. IL-1ra on behavioral or molecular changes were significantly inhibited by administration of carbenoxolone, a gap junction decoupler, or Gap43, a connexin 43 mimetic blocking peptide. These results demonstrated that contralateral spinal astrocyte D-serine plays a key role in the development of MIP. Furthermore, it is suggested that blocking of spinal IL-1β facilitates the astrocyte gap junction mediated signal spreading which advances of contralateral D-serine synthesis and ultimately evokes the early development of MIP.
Key Words: Mirror-image pain, D-serine, astrocyte, Interleukin-1β

P07-18

The nociceptive effect of dephosphorylated astrocytic aromatase in mice formalin model is mediated by spinal sigma-1 receptor through calcineurin pathway

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Aromatase is a key enzyme that carries out the biosynthesis of estrogen from testosterone, which then participates in various biological processes including pain sensitization. It has been discovered that the short term changes of aromatase activity are regulated with rapid and transient cellular processes, such as phosphorylation at different activation sites. We have previously established that Sigma-1 receptor, a unique ligand-regulated molecular chaperone, can inflict changes in the phosphorylation states of various receptors and enzymes in pain sensation through activating calcineurin pathway. On this note, we examined whether spinal Sigma-1 receptor can modulate the activity of aromatase through modifying phosphorylation states of the enzyme, leading to nociceptive effect in mice formalin model. Intrathecal (i.t.) injection of letrozole, an aromatase inhibitor, has dose-dependently reduced the nociceptive responses in the second phase of formalin test and the expression of spinal Fos, as compared with those of control group. After showing that aromatase was co-localized with Sigma-1 receptor in spinal astrocytes, we confirmed through co-immunoprecipitation that the level of phosphorylated serine in spinal aromatase was down-regulated in control mice. This dephosphorylation of aromatase was, however, significantly restored by i.t. administration of BD-1047, a sigma-1 receptor antagonist. Moreover, co-administration of sub-effective doses of letrozole and BD-1047 showed anti-nociceptive effect in formalin injected mice. Finally, we have found that co-administration of sub-effective doses of BD1047 and CsA, a calcineurin inhibitor, reduced the formalin-induced nociception and restored the down-regulated phosphor-serine level of spinal aromatase. These results demonstrate that spinal Sigma-1 is an important factor that mediates dephosphorylation of astrocytic aromatase which leads to the induction nociceptive effect in formalin mice.
Key Words: aromatase, nociception, sigma-1 receptor, astrocyte.

P07-19(YP-11)

A Study for Electrophysiological Characteristics of Neural Circuit between Ventral Tegmental Area and Medial Prefrontal Cortex in Rats

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The ventral tegmental area (VTA) is a vital part of brain that involve in avoidance, fear conditioning, addiction and other neuropsychiatric illnesses. The medial prefrontal cortex (mPFC) is a key structure for executive functions of the brain. Some papers has been shown that mPFC takes part in the modulation of the firing pattern of VTA dopaminergic neurons. However, the functional connectivity between mPFC and VTA GABAergic neuronal activities is still unclear. The purpose of this study was to observe electrophysiological characteristics of neural circuit between the GABAergic neurons in the VTA and mPFC. Spontaneous activities of VTA GABAergic neurons induced by Electrical stimulation (200Hz, 10 pulses) of mPFC were recorded using extracellular recording technique in Sprague - Dawley rats. The electrical stimulation to mPFC resulted in 3 different patterns of spontaneous firings in the VTA GABAergic neurons, in which main effect of the electrical stimulation was increase in firing rate. In order to see effects of neurochemical modulations of mPFC on VTA GABAergic neuronal activities. PEPA, glutamate receptor agonist and a mixture of muscimol, $GABA_A$ receptor agonist and baclofen, $GABA_B$ receptor

agonist were injected through a cannula inserted into the mPFC, respectively. After injecting the PEPA into the mPFC, the spontaneous activities of 80% VTA GABAergic neurons was gradually increased for more than one hour. The increasing rate of the spontaneous activity was 30.9% following PEPA injection. Oppositely, the injection of a GABA mixture resulted in the reduction of the spontaneous firings of VTA GABAergic neurons for more than one hour. According to these results, we suggest that excitatory synapse is major component in neural circuit between the mPFC and VTA GABAergic neurons.
Key Words: ventral tegmental area, medial prefrontal cortex, GABAergic neurons.

P07-20(YP-12)

Intrinsic plasticity amplifies long-term depression in parallel fibre input gain in cerebellar Purkinje cells

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Cerebellar Purkinje cells (PCs) are the sole output neuron in the cerebellar cortical area and endowed with the neural plasticity, most investigation accounting for cerebellum-dependent motor learning, however, have been intensively focused on the synaptic plasticity between parallel fibre and the PCs. Here, we describe how PCs recognise learned the pattern and generate their output signal during long-term depression at parallel fibre to PC synapses occur using patch-clamp whole cell recordings from mouse Purkinje cells. Both parallel fibre tetanisation and burst stimulation with climb fibre reliably induce synaptic depression accompanied by long-term depression of intrinsic excitability (LTD-IE). Interestingly, the dendritic temporal summation of EPSP shows no significant alteration although the synaptic gain is attenuated after LTD induction. In addition, the ratio of spike frequency adaptation is also not changed after the manifestation of LTD-IE. Collectively, our findings suggest that LTD-IE of PCs enable to amplify the modification of synaptic weight, rather the strategy for information processing when the novel pattern of the parallel fibre input is delivered after neuronal plasticity induction.
Key Words: Long-term depression, intrinsic plasticity, cerebellar Purkinje cells

P07-21(YP-07)

Analgesic effect of endogenous oxytocin was attributed to AVP1a receptor-mediated hyperpolarization of dorsal root ganglion cells

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Recent studies have provided several lines of evidence that peripheral administrations of oxytocin (OT) induced analgesia in human and rodents. However, the exact underlying mechanism of the analgesia still remains elusive. In the present study, we investigated to identify which receptor mediated analgesic effect of intraperitoneal (IP) injection of OT in thermal pain behavior, and to verify the expression patterns of mRNA of OT-related receptors in dorsal root ganglion (DRG) neurons. We interrogated whether OT affected capsaicin-induced intracellular calcium transients and the membrane excitability in peripheral sensory neurons. If so, we examined which receptor mediated these changes. For these aims, we examined whether the IP injection of specific antagonist for oxytocin and vasopressin (AVP) receptors reversed the OT-induced analgesia during the pain behavioral tests such as Hargreaves' in rats. We determined mRNA expression patterns of transient receptor potential vanilloid 1 (TRPV1) and the oxytocin-related receptors, such as OT, AVP1a, AVP1b, and AVP2 receptors, in the individual cells by single cell RT-PCR. We explored the effect of oxytocin and its antagonists on capsaicin-induced intracellular calcium transient using fluorescent microscopy in the dissociated DRG cells. In addition, we also investigated the effect of oxytocin and its antagonists on the membrane excitability using patch clamp. The behavioral results showed that the antagonist for AVP1a receptor reversed the oxytocin-induced analgesia. Single cell RT-PCR discovered predominnt co-expression of mRNA of TRPV1 and AVP1a receptor in the DRG cells. Also, the number of cell expressing mRNA of AVP1 receptor was more predominant than that of OT, AVP1b, and AVP2 receptors. The fluorescent imaging experiments demonstrated that oxytocin partially inhibited capsaicin-induced intracellular calcium transient through AVP1a receptor in the DRG cells. The electrophysiological study also demonstrated that OT hyperpolarized the membrane potential by increasing the permeability of potassium channel via AVP1a receptor in the DRG cells. Taken together, our findings possibly suggested that OT-induced membrane hyperpolarization of the DRG cells contributed to the analgesic effects of peripheral administration of OT through AVP1a receptor.
Key Words: analgesia, oxytocin, vasopressin, single cell rt pcr, electrophysiology, calcium imaging

P07-22(PO-08)

Salt loading recruits Mg^{2+}-resistant extrasynaptic NMDA receptors in supraoptic nucleus neurons

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Supraoptic nucleus (SON) of the hypothalamus containing magnocellular neurosecretory neurons (MNC) consists of vasopressin (VP) and oxytocin (OT) neurons and plays fundamental roles in reproduction and fluid balance homeostasis. Accumulating evidences suggest that ambient glutamate of synaptic and non-synaptic origin activate extrasynaptic NMDA receptors (NMDAR) mediating a persistent tonic excitatory current (tonic I_{NMDA}) in the brain. However, the physiological role(s) of tonic I_{NMDA} have not been well established. In this study, we show that 7 day of salt loading (SL) recruited a Mg^{2+}-resistant tonic I_{NMDA} in SON NMCs. While AP5, a NMDARs antagonist, failed to affect holding current ($I_{holding}$) in normal aCSF at holding potential of -70 mV in SON NMCs from euhydarted (EU) animals, it uncovered tonic I_{NMDA} shown by an outward shift in $I_{holding}$ in ~45% of the SL SON MNCs. SL recruited the Mg^{2+}-resistant tonic I_{NMDA} in ~90% of VP neurons genetically labelled with eGFP, but none of OT neurons labeled with mRFP. Extrasynaptic NMDAR inhibitor, mematine and PPDA mimicked the AP5-induced outward shift in $I_{holding}$ of SL VP neurons (AP5, 14.5 ± 3.0 pA, n = 9; memantine, 13.7 ± 3.3, n = 4; 12.7± 1.6, n = 5). In low Mg^{2+} aCSF, AP5-sensitive I_{NMDA} were significantly larger in SL VP neurons (24.3 ± 3.1, n = 6) than in EU VP neurons (14.2 ± 1.6, n =7, $p < 0.05$). Sequential application of PPDA and PPDA+AP5 further suggested that SL recruited

additional PPDA-sensitive NMDARs in SL VP neurons. While PPDA induced outward shift in $I_{holding}$ in SL (15.0 ± 2.4, n =7) but not in EU VP neurons, additional application of PPDA (PPDA+AP5) induced $I_{holding}$ shift in both neurons (EU, 13.2 ± 3.8, n = 8; SL, 8.9 ± 1.3, n=7). Finally, PPDA significantly inhibited the neuronal firing rates in SL VP neurons, but not in EU VP neurons. Taken together, our results suggest that altered expression and composition of extrasynaptic NMDARs generating Mg^{2+}-resistant tonic I_{NMDA} regulate VP neuronal activity and, in turn, the hormone secretion and fluid balance during osmotic challenges.
Key Words: extrasynaptic NMDA receptors, supraoptic nucleus, osmotic challenge, salt loading

P07-23(PO-09)

Low Intensity Ultrasound Decreases α-Synuclein Aggregation in PC12 Cells: Potential action on mitochondrial reactive oxygen species

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by the loss of nigrostriatal dopaminergic neurons. Several mechanisms have been proposed to explain the underlying pathogenic mechanism of PD that include increase of reactive oxygen species (ROS), mitochondrial dysfunction, α-synuclein deposition, and apoptosis. α-Synuclein aggregates are the major protein component of the Lewy bodies, the neuro-pathological hallmark of PD. α-Synuclein aggregation has been shown to occur after the increase in the oxidative stress. Here, we studied the effects of low intensity ultrasound (LIUS) stimulation on the mitochondrial ROS-dependent α-synuclein aggregation in neurotoxin-treated PC12 cells. To induce oxidative stress and subsequent α-synuclein aggregation, we treated PC12 cells with a neurotoxin 1-methyl-4-phenylpyridinium ion (MPP^+); and then we exposed the cells to LIUS stimulation. We found that LIUS stimulation decreased α-synuclein aggregates in intensity-dependent manner. We hypothesized that LIUS stimulation initially suppressed the mitochondrial ROS generation and inhibited the neurotoxin-induced exacerbation of mitochondrial complex I activity, thereby causing the attenuation of α-synuclein aggregates in PC12 cells. Consequently, LIUS stimulation resulted in a better cell survival than that of the neurotoxin-treated and non-LIUS-stimulated PC12 cells.
Key Words: Low intensity ultrasound (LIUS); 1-methyl-4-phenylpyridinium ion (MPP^+); α-Synuclein; Parkinson's disease (PD); mitochondrial dysfunction; and reactive oxygen species (ROS).

P08-01(YP-13)

Mitochondrial calcium uniporter inhibition attenuates mouse bone marrow-derived mast cell degranulation induced by beta-1,3-glucan

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Mast cells are primary mediators of allergic inflammation. Beta-1,3-glucan (BG) protects against infection and shock by activating immune cells. Activation of the BG receptor induces an increase in intracellular Ca^{2+}, which may induce exocytosis. However, little is known about the precise mechanisms underlying BG activation of immune cells and the possible role of mitochondria in this process. The present study examined whether BG induced mast cell degranulation, and evaluated the role of calcium transients during mast cell activation. Our investigation focused on the role of the mitochondrial calcium uniporter (MCU) in BG-induced degranulation. Black mouse (C57) bone marrow-derived mast cells were stimulated with 0.5 μg/ml BG, 100 μg/ml peptidoglycan (PGN), or 10 μM A23187 (calcium ionophore), and dynamic changes in cytosolic and mitochondrial calcium and membrane potential were monitored. BG-induced mast cell degranulation occurred in a time-dependent manner, and was significantly reduced under calcium-free conditions. Ruthenium red, a mitochondrial Ca^{2+} uniporter blocker, significantly reduced mast cell degranulation induced by BG, PGN, and A23187. These results suggest that the mitochondrial Ca^{2+} uniporter has an important regulatory role in BG-induced mast cell degranulation.
Key Words: Beta-1,3-glucan, Mast cell degranulation, Mitochondrial calcium uniporter

P08-02(PO-05)

Computational analysis of cerebrovascular flow reserve using patient-specific medical images

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Here, we provide a novel method to evaluate the cerebrovascular flow reserve (CFR) by using a computer simulation model. Then, the hyperemic blood flows of the 25 patients with neurovascular stenotic diseases were simulated to test the efficacy of the present method as a non-invasive screening technique of stroke. We have implemented a novel computational method to compute the CFR by coupling computational fluid dynamics (CFD) with the lumped parameter model (LPM) of the neurovascular system. In the CFD model, we used a finite element method to solve blood flow in three dimensional (3D) geometry of patient-specific cerebral arteries that was obtained from patients' magnetic resonance angiography (MRA). In the LPM, autoregulation mechanism to maintain cerebral flow homeostasis was included. Computed blood pressure distribution showed an abrupt decrease through stenosed cerebral arteries. This trend was more remarkable in hyperemic cases than non-hyperemic ones. Increased blood flow in internal carotid arteries was induced by the hyperemic condition, but it was comparably reduced in case of stenosed cerebral arteries. The CFRs of the patients were obtained and the physiological significances of their cerebrovascular stenotic lesions were analyzed. The present simulation method provided non-invasively the CFR values of the patients and showed the functional significances of their stenotic lesions, indicating its clinical efficacy.
Key Words: Computational analysis, cerebrovascular flow reserve, patient-specific method

P08-03

CT image-based computational fluid dynamic three-dimensional modeling in coronary artery atherosclerosis

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Hemodynamics is an important factor in coronary artery atherosclerosis (CAA) to assess myocardial infarction risk. Computational fluid dynamic (CFD) analysis based on human three-dimensional (3D) CAA reconstructions provides hemodynamic data that can be used in the prognosis of vascular disease. The objective of this study is to develop an anatomically accurate 3D CAA model and to describe image-based CFD for patients with complex CAA. Based on the computed tomography (CT) images, regions of interest were segmented using the Mimics 19.0 software (Materialise, Leuven, Belgium). The CAA models were solved using the Ansys 17.0 software (ANSYS Inc., Canonsburg, PA, USA). Time-varying inlet/outlet boundary flow rates were used as input for CFD simulation of the flow patterns. Stress variation on critical sites such as plaque in coronary artery was higher than in normal sites. Hemodynamic parameters in the plaque were quantified by the evaluation of flow rates, pressure, and stress. Hemodynamic analysis using CFD improved the accuracy of plaque vulnerability assessment. Thus, the 3D CAA model might facilitate the hemodynamic investigation. This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2015M3A9B6029133 and 2011-0028925).
Key Words: Image-based model, Hemodynamics, Coronary artery, Atherosclerosis, Computational fluid dynamics

P08-04

A computational fluid dynamics study on the image-based three-dimensional aorta

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Developments in image-based three-dimensional (3D) modeling have been producing increasingly realistic representations of hemodynamics. However, recent studies have not performed hemodynamic analysis on specific lesions. It is difficult to measure blood flow on a lesion *in vivo* when assessing its influence in the aortic atherosclerosis. The aim of this study is to evaluate how lesion specific hemodynamics affects aortic hemodynamics using computational fluid dynamics (CFD). Reconstructed aortic computed tomography (CT) images were converted into DICOM format, and then imported into 3D segmentation using both Mimics v.19.0 and 3-matics v.11.0 software. Using the 3D reconstructed aorta, a pulsatile blood pressure waveform was applied to the ascending aorta to provide a biomimetic environment by ANSYS v.17.0 software. The CFD simulation allowed the detailed characterization of complex flow velocity, pressure, and wall shear stress. There were significant differences in shear stress between the control and the atherosclerotic aorta. These results indicated that plaques were associated with low shear stress. This simulation provided an assessment of the lesion specific rheological properties such as stress. This approach exhibited the complex geometry of the aorta wherein areas with stenosis and rupture are exposed to variations in stress. Conclusively, an optimized analysis method has been suggested that it integrates advanced image processing strategies and computational techniques based on finite element method to perform hemodynamics analysis based on CT images. Image-based CFD analysis might provide an enhanced understanding of the relationship between aortic hemodynamics and aortic diseases. This research was supported by the Bio & Medical Technology Development Program of the NRF funded by the Korean government, MSIP (2015M3A9B6029133 and 2011-0028925).
Key Words: Image based model, Computational Fluid Dynamics, Hemodynamics, Vascular diseases

P08-05

A comparison of extracellular matrix components produced by 2D and 3D cultured human conjunctival fibroblasts

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Conventional two-dimensional (2D) cell culture is commonly used to study cell behavior and function providing a basic method to explore biological mechanisms. However, cells cultured in three dimensional (3D) models show different cell behaviors such as differentiation, proliferation, and gene expression when compared to cells cultured in 2D. Here, we focus on microenvironments formed by human conjunctival fibroblasts by comparing a difference in extracellular matrix (ECM) components secreted by cells cultured either in 2D or 3D conditions. A primary culture of human conjunctival fibroblasts was prepared form surplus tissue obtained from surgical resection in three cataract patients using the explant technique. Cells were cultured either on the traditional polystyrene plastic surface (2D) or electrospun poly-ε-caprolactone (PCL) nanofiber scaffold (3D) for 6 days and the culture supernatants were harvested for the determination of ECM components. Hydroxyproline assay revealed that collagen level in the culture supernatants increased depending on the duration of culture and was higher in 3D culture than that of 2D culture throughout all the passages we tested. Collagen level in the culture supernatants from 6-day culture of fibroblasts at 9th passage had 4 times higher in 3D conditions than that in 2D conditions. RT-PCR analysis of human conjunctival fibroblasts revealed that expressions of collagen I, Fibronectin and CTGF were dramatically increased in nanofiber-based 3D culture condition compared to those in 2D culture condition. Interestingly, TGF-β expression started to increase after 3 days of 2D culture condition whereas it remained low even after 6 days of 3D culture conditions. These data suggest that stroma formed by human conjunctival fibroblasts cultured in 3D conditions using PCL nanofiber scaffold provides more suitable biomimetic microenvironments for the conjunctival epithelial cells to reconstruct 3D human conjunctiva. This work was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009583)
Key Words: Human conjunctival fibroblasts, 3D culture, PCL nanofiber, Collagen, Fibronectin

P08-06

A comparison of cell morphology and behaviors between 2D and 3D cultured human conjunctival fibroblasts

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Our understanding of many biological processes is largely based on studies with a homogenous population of cells cultured on traditional two-dimensional (2D) plastic surface. However, recently cells cultured in three dimensional (3D) microenvironments have shown a striking dissimilarity in physiological behavior of cells when compared with those cultured in 2D condition. Here, we investigated the differences in morphology and proliferation between human conjunctival fibroblasts grown in 2D and 3D cultures. A primary culture of human conjunctival fibroblasts was prepared form surplus tissue obtained from surgical resection in three cataract patients using the explant technique. Cells were cultured either on the traditional polystyrene plastic surface (2D) or electrospun poly-ε-caprolactone (PCL) nanofiber scaffold (3D) up to 14 days and cell proliferation was assessed by CCK-8. Continuous cell proliferation was observed on both 2D and 3D cultures, but cells on PCL nanofiber proliferated faster than those on plastic surface making a bigger difference over time. Human conjunctival fibroblasts grown in 3D condition showed more elongated cell morphology attached to the nanofiber with less stress fibers and focal adhesion complexes than those in 2D condition. Intracellular levels of procollagen and collagen were increased in 3D condition compared to those of 2D condition. These data suggest that human conjunctival fibroblasts cultured in nanofiber scaffold might capture more faithfully the physiological behavior of cells in vivo and provide more suitable biomimetic microenvironments for studying cellular responses such as migration and proliferation by epithelial cell-fibroblast and immune cell-fibroblast crosstalk in human conjunctiva stroma. This work was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009583)
Key Words: Human conjunctival fibroblasts, 3D culture, PCL nanofiber, Proliferation, Morphology

P08-07

Computational Simulations of noninvasive Instant Flow Reserve by using Coronary Computed Tomographic Angiograms in non-hyperemia

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Fractional flow reserve (FFR) is a hyperemic pressure ratio of distal coronary pressure to aortic pressure during cardiac cycle, while instantaneous wave-free ratio (iFR) is a recently-introduced vasodilator-free index to assess a coronary stenosis severity, the ratio of mean distal coronary pressure (Pd) to an aortic pressure during a pre-specified period in mid to late diastole of cardiac cycle with the lowest resting resistance in a non-hyperemic condition. The purpose of this study is to compare a computed CT-iFR with a clinically measured iFR. And it is to evaluate the computed CT-iFR for a clinical 0.8 M-FFR cutoff and to present the usefulness of CT-iFR as the prognostic index of outcome of post-stenting. In this work, patient-specific 3D coronary arterial model were reconstructed from CT. Simulation of flows in coronary artery were performed by using Navier-Stoke solver based on finite element method. 3-D coronary arterial models are coupled with a 0-D lumped parameter coronary vascular bed models in a basal state. The computed CT-iFR shows the good agreements with the clinically measured iFR by using Bland-Altman plot. The results from this study show that CT-iFR is not inferior to currently well-known CT-FFR. Knowledge from computational simulations might assist or obviate the need for a clinical iFR and FFR in selected patients for better revascularization strategy.
Key Words: coronary artery, instant flow reserve, non-hyperemia, hemodynamics, wall shear stress

P08-08

Deep learning and neuroscience: Bridging the gap between artificial neural networks and natural neural networks

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Artificial neural networks (ANN) were originally inspired by neuroscience, but for decades, the major development of the ANN was guided by mathematical optimization methods and algorithms, rather than biological knowledge about the brain network. Neuroscience has accumulated a wealth of findings about the molecular characteristics of the neurons, synapses, and neuronal circuits, but these findings are rarely considered in terms of the principal knowledge for developing machine learning algorithms for many years. Recent advances of deep learning, however, are giving a perspective of re-convergence of these two fields, artificial neural networks and natural neural networks (NNN). In this study we compared the major classes of deep learning architectures with the plausible biological neural architectures in the brain. The results offer neuroscientists the opportunity to understand their findings in terms of artificial intelligence research, bridging the gap between ANN and NNN.

P08-09

Inflammatory regulation of spinal PPAR-gamma in contusive spinal thoracic injury

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Peroxisome proliferator-activated receptors (PPARs) control processes such as inflammation, oxidative stress and apoptosis in several injured tissues. PPARs have been studied which focused on the potential to be neuroprotective in spinal cord injury (SCI) because the complex of those pathological events are presented in injured spinal cord tissue. In particular, Pathways in biosynthesis of eicosanoids which result from membrane breakdown extremely increase immediately after SCI. Products of those are the ligands of peroxisome proliferator activated receptors (PPARs), which are a nuclear receptor to regulate gene transcription related to glucose and lipid metabolism. Above all, PPAR-γ is well studied as regulation of inflammation and neuroprotection in various neurological degenerative diseases. Thus, we investigated a

neuroprotective role of PPAR-γ after SCI in rats. We used male Sprague-Dawley rats weighing 220-250 grams in this study. The dorsal surface of the spinal cord was exposed by T10 vertebral laminectomy, and a 10-g rod was dropped on the spinal cord from a height of 12.5 mm by a New York University (NYU) impactor device under anesthesia with ketamine/rompun mixture (1:4) in rats. We examined protein expression of PPAR-γ in the injured epicenter, rostral, caudal and L4-5 region of the spinal cord at various times after SCI (6h, 12h, 24h, 3d, 1w, 3w and 5w). Pioglitazone (PPAR-γ agonist), G3335 (PPAR-γ antagonist) or vehicle (100% DMSO or saline) were administered by intrathecal injection in the early phase or the late phase after SCI. We assessed locomotor behavior using a BBB rating scale, and analyzed mRNA expression of inflammatory mediators using real-time PCR after the drugs administration. The expression of PPAR-gamma increased rapidly from 6 hours, was maintained for 3 days of SCI and then returned to basal levels in all regions, including the remote site. Intrathecal administration of pioglitazone in the early phase improved the joint movement, however, no difference after that. Interestingly, G3335 reduced spontaneous locomotion recovery after SCI. mRNA expression of inflammatory mediators and chemoattractant were further increased after G3335 administration in the late phase than in the early phase. These results demonstrated that additionally elevated PPAR-γ may not have benefits to recover motor function anymore, however, deficit of PPAR-γ activity affects to exacerbate it through enhancement of inflammatory reaction.

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