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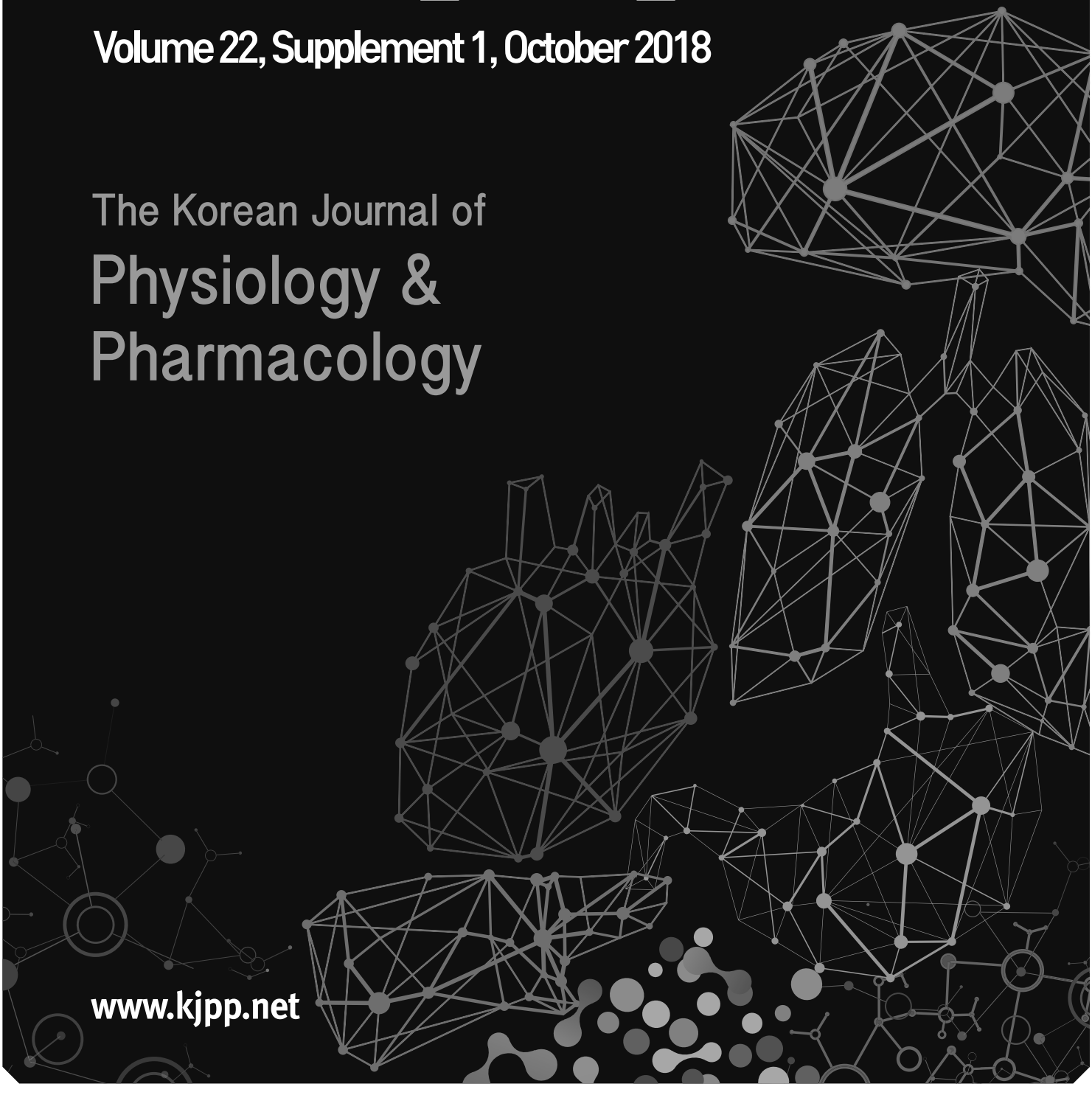

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Aims and Scope

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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김성주 김성준 김세훈 김양인 김영미 김용운 김원재 김의용 김재호
김정훈 김종연 김진혁 김창주 김형찬 나승열 나창수 나흥식 류판동
박경표 박규상 박명규 박병림 박사훈 박소라 박우현 박종성 박중진
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Contents

Welcome Message

대한생리학회 회원 여러분, 안녕하십니까?

우리 학회의 금년 학술 활동은 6월 29일에 서울대학교 의과대학에서 개최된 제26회 기초의학 학술대회로 시작되어 이제 10월 25~ 27일까지 연세대학교 원주의과대학 주관으로 원주 오크밸리에서 제 70회 대한생리학회 학술대회가 개최를 앞두고 있습니다.

이번 정기학술대회는 “자연에서 미래로(from nature to future)” 라는 큰 주제하에 마코토 구로오 교수의 Plenary lecture를 비롯해 2개의 워크샵, Satellite meeting, 젊은 과학자 세션, 그리고 연구주제별 9개의 심포지움으로 구성되어 있습니다. 특히 이번 학회는 모든 회원이 관심을 갖고 참여하는 학술대회를 만드는데 주안점을 두고 프로그램을 구성하였습니다. 본 학술대회를 통해 새로운 과학 지식의 습득 및 회원간의 활발한 소통을 바탕으로 친목을 도모하는 좋은 기회가 될 것 입니다. 끝으로, 회원 여러분들의 적극적인 참여를 다시 한번 부탁드리며, 건강과 가정의 행복이 함께하시기를 기원합니다.

대한생리학회 회 장 **박 경 표**
대한생리학회 이사장 **서 인 석**

주관교 환영사

제70회 대한생리학회 정기학술대회 개최를 학회 회원 여러분과 함께 진심으로 축하하며, 특히 좋은 계절에 이곳 강원도 원주시 오크 밸리 리조트에서 학술대회의 주관교가 되었음을 무한히 기쁘게 생각합니다. 1978년 강원도 최초의 의사 양성기관으로 출발한 연세원주의대는 올해로 꼭 개교 40년이 되었습니다. 그간 많은 분들의 수고와 헌신으로 오늘에 이르렀다고 믿으며, 앞으로도 잘 이어 갈 수 있으리라 소망합니다. 되돌아 보니 25년전인 1993년 처음 우리 대학이 생리학회를 유치하여 용평리조트에서 함께 했던 일들이 지금도 생생합니다. 시간이 흘렀지만 같은 마음으로 이번 제70회 대한생리학회 학술대회를 맞고자 합니다.

올해 학술대회 또한 학문적 호기심을 키우고, 흥미롭고, 즐겁게 교류하는 것으로 진행되리라 봅니다. “탐험하라, 꿈꾸라, 발견하라” 라는 마크 트웨인의 말처럼 우주의 신비와도 같은 우리 몸의 생리학적 영역에의 도전이 이번 학회에서도 계속해서 이어질 것으로 기대합니다. 연세원주의대 생리교실의 교수진과 교실원은 모두 이번 학회를 준비하고 돕는 마음에 소홀함이 없도록 최선을 다하도록 하겠습니다. 앞으로도 우리 생리학교실에 많은 관심과 성원 부탁드립니다. 끝으로 제70차 대한생리학회 정기학술대회를 위해 수고해주신 대한생리학회 회장, 이사장, 그리고 학술 이사를 포함한 임원진과 학회 관계자 여러 분들께 감사의 말씀 드립니다.

연세대학교 원주의과대학 생리학교실 주임교수 **공 인 덕**

Schedule (일정표)

▶ 10월 25일 목요일

Time	Contents
10:30–11:00	Registration
11:00–12:30	**Workshop 1: Optical and Electrophysiological Tools**
12:30–13:10	Luncheon
13:10–14:40	**Workshop 2: Drug Discovery: A How-To Guide**
14:40–15:00	Coffee Break
15:00–17:00	**Satellite Meeting: Physiome research and Physiome-based educational program**
17:00–18:30	Welcome Reception (오크뷰 식당)
18:30–18:45	Opening Ceremony
18:45–20:00	**Young Scientist Session**
20:00–22:00	**Poster Session and Beer Time**

▶ 10월 26일 금요일

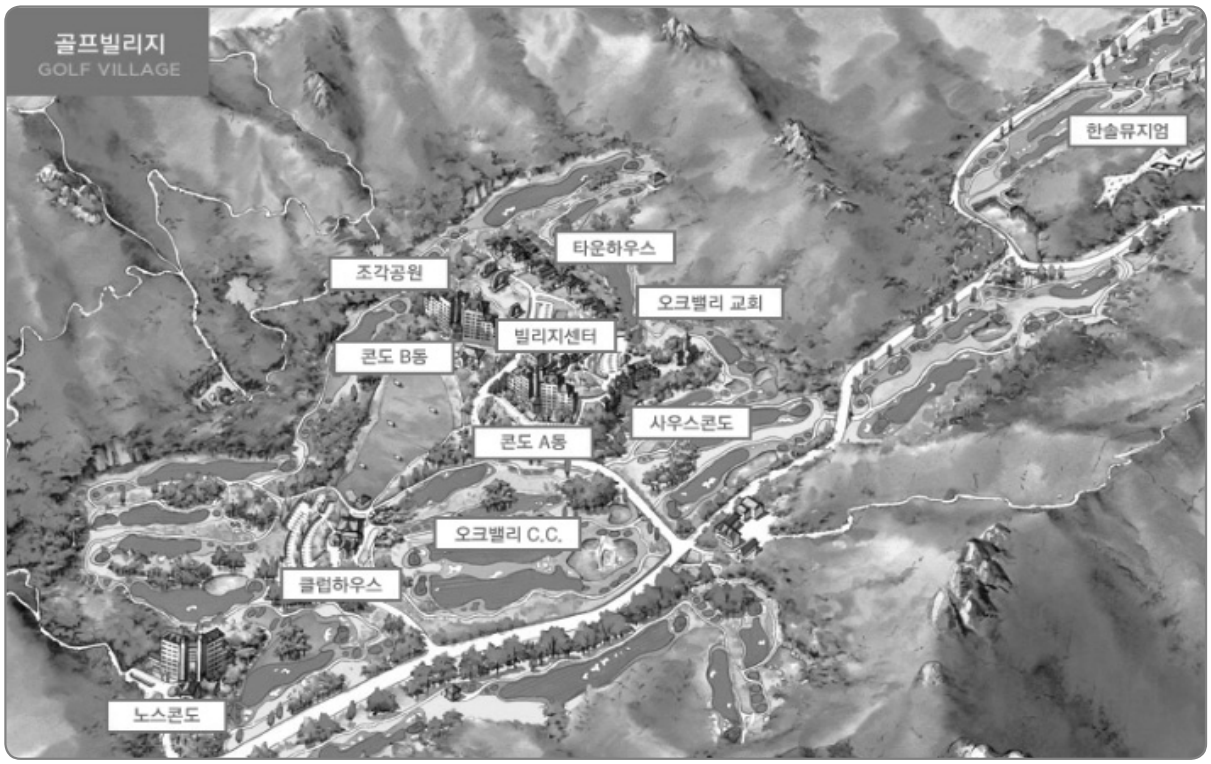
Time	Contents		
	Room A	Room B	Room C
08:30–11:00	**Symposium 1:** Pathophysiology of Cognitive Disorder	**Symposium 2:** Pathophysiology of Potassium Channels	**Symposium 3:** Exercise Physiology / IMPACT
11:00–11:50	**Plenary Lecture:** Makoto Kuro-O 'Aging and chronic kidney disease: phosphate connection'		
11:50–12:00	단체사진 촬영		
12:00–13:00	Lunch (오크뷰 식당) 및 이사회 회의 (퍼시몬 C)		
13:00–15:00	'70세 생리학회 70분 걷기' 행사 또는 '뮤지엄 산' 관람		
15:00–17:30	**Symposium 4:** Pathophysiology of Trigeminal Somatosensation	**Symposium 5:** Heart and Circulation	**Symposium 6:** Organellar Physiology and Metabolism
17:30–18:30		Poster-Oral	Poster-Oral
19:00–21:00	Official Buffet (그랜드볼룸)		

▶ 10월 27일 토요일

Time	Contents		
	Room A	Room B	Room C
09:00–11:30	**Symposium 7:** Neuronal Regulation	**Symposium 8:** Stem Cells and Differentiation	**Symposium 9:** Skin and Epidermis Research
11:30–12:00	Yudang Lecture		
12:00–12:30	General Assembly		

Venue Guide (학술대회장 안내)

리조트(골프빌리지 맵)



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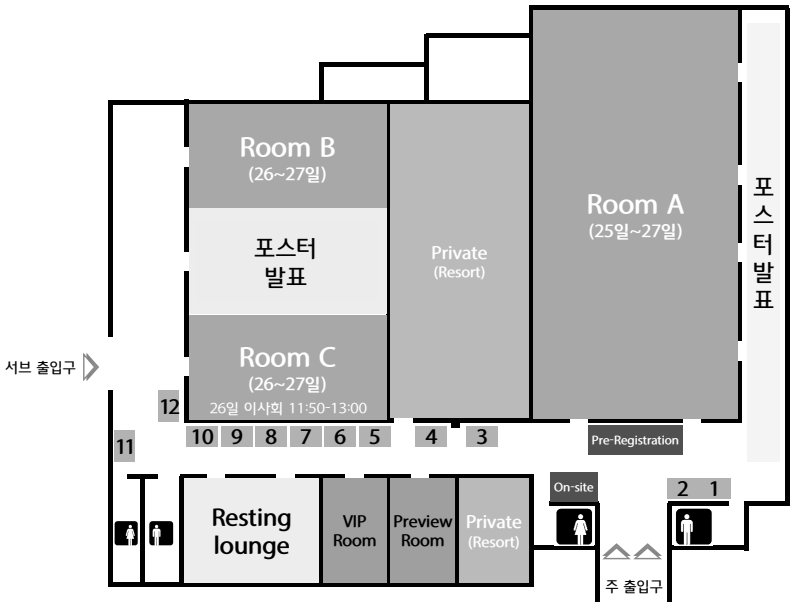

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12	삼우과학

Scientific Program (학술프로그램)

▶ Workshop (10월 25일 목요일)

Contents		
Workshop 1: Optical and Electrophysiological Tools (11:00–12:30)		**Chair: *Seung-Kuy Cha, Hyoung Kyu Kim***
11:00–11:30	Novel methods for clearing and labeling the tissues for 3D high-resolution microscopy	*장성호 (서울대 의대)*
11:30–12:00	Electrophysiological approach and methods for research in cardiac arrhythmias	*최종일 (고려대 의대)*
12:00–12:30	Nikon microscopes for life science	*Fukui Tatsuo (Nikon, Japan)*

Workshop 2: Drug Discovery: A How-To Guide (13:10–14:40)		**Chair: *Sun-Hee Woo, Wan Namkung***
13:10–13:32	High throughput screening for drug discovery of protease-activated receptor 2 modulators	*남궁완 (연세대 약대)*
13:32–13:55	Considerations in the preclinical studies for drug development	*서정욱 (안전성평가연구소)*
13:55–14:17	Chondroitin sulfate-based nanoparticles for tumor penetration	*이재영 (충남대 약대)*
14:17–14:40	New drug development of SP-8203(otaprimastat) in ischemic stroke	*유제만 (신풍제약)*

▶ Satellite Meeting (10월 25일 목요일)

Contents		
Physiome Research and Physiome-based Educational Program		**Chair: *Jae Boum Youm***
15:00–15:30	EKG training using three-dimensional heart model	*심은보 (강원대 공대)*
15:30–16:00	Simulation-based general electrophysiology	*임채헌 (울산대 의대)*
16:00–16:30	Integrative understanding of human circulatory functions in students practicum of physiology using quantitative circulation physiology program (QCP-2005)	*김성준 (서울대 의대)*
16:30–17:00	Simulation-based lecture on muscle physiology using EDISON software	*염재범 (인제대 의대)*

▶ Young Scientist Session (10월 25일 목요일)

Contents		
		Chair: *Kyu-Sang Park*
18:45–19:10	Brain mechanisms of neuropathic pain and interaction between chronic pain, depression and empathic distress	*정지훈 (경희대 한의대)*
19:10–19:35	Tissue-to-tissue communication of mitochondrial function in the model organism C. elegans	*윤경혜 (연세대 원주의대)*
19:35–20:00	SHANKs deficiency impairs hyperalgesia in neuropathic and inflammatory pain models	*김용호 (가천대 의대)*

▶ Symposium (10월 26일 금요일)

Contents	
Symposium 1: Pathophysiology of Cognitive Disorder	**Chair: *Seung-Hee Lee, Yong-Seok Lee***
Cortical circuits for the multi-sensory integration: role of inhibition	*이승희 (KAIST 생명과학과)*
Cellular and molecular mechanisms of epilepsy in focal brain malformations	*백승태 (포항공대)*
Lithium-responsive and layer-specific prefrontal dysfunction in a mouse model of mania	*한기훈 (고려대 의대)*
Synaptic dysfunction of mild intellectual disability	*최세영 (서울대 치대)*
Cell type-specific signaling networks in learning disabilities	*이용석 (서울대 의대)*
Symposium 2: Pathophysiology of Potassium Channels	**Chair: *Tong Mook Kang***
Blockade of Kv1.5 by PCP derivatives and its clinical implications	*배영민 (건국대 의대)*
Identification of C-terminal domains regulating TREK K^+ channels	*김성준 (서울대 의대)*
Regulation of endothelial Ca^{2+}-activated K^+ channels in health and vascular diseases	*서석효 (이화여대 의대)*
KCNQ channel methylation in control of neuronal excitability	*조하나 (성균관대 의대)*
KCNQ4 potassium channelopathy and hearing loss	*강동묵 (성균관대 의대)*
BK_{Ca} channel drug discovery targeting overactive bladder	*박철승 (GIST)*
Symposium 3: Exercise Physiology / IMPACT	**Chair: *In Deok Kong, Hyo-Bum Kwak***
Single nucleotide polymorphisms and world-class Korean athletes	*박동호 (인하대 스포츠과학과)*
Inter-individual variation in the changes in insulin sensitivity in response to regular exercise	*이소정 (경희대 스포츠의과학)*
Beneficial role of HIT exercise on hippocampal plasticity	*이민철 (차의대 스포츠의학과)*
Investigation of vitamin D level and its role in inactive submariner	*박은미 (한남대 식품영양학과)*
Regulation of hepatic stellate cells by the factors derived from contracting skeletal muscle cells	*장재승 (연세대 원주의대)*
The role of echinochrome A for exercise capacity	*서대윤 (인제대 의대)*
Symposium 4: Pain Physiology	**Chair: *Seog Bae Oh, Sun Wook Hwang***
Ultrastructural basis for craniofacial sensory processing in the brainstem (Expression of glycine receptor alpha 3 in the primary sensory neurons	*배용철 (경북대 치대)*
Role of mechanosensitive ion channels in tooth pain	*오석배 (서울대 치대)*
Maresin 1 inhibits TRPV1 in temporomandibular joint (TMJ)-related trigeminal nociceptive neurons and TMJ inflammation-induced synaptic plasticity in the trigeminal nucleus	*박철규 (가천대 의대)*
Experimental animal models for trigeminal neuralgia	*안동국 (경북대 치대)*
Possible involvement of oral dysfunction in inducing stress disorder	*Youngnam Kang (Osaka Univ., Japan)*

Symposium 5: Heart and Circulation	Chair: *Suhn Hee Kim, Sung Joon Kim*
Structural and functional significances of the atrial T-tubules	*강동묵 (성균관대 의대)*
Ca^{2+} signaling triggered by shear-autocrine P2X receptor pathway in rat atrial myocytes	*우선희 (충남대 약대)*
Atrial fibrillation and atrial electrophysiology	*정보영 (연세대 의대)*
Atrial natriuretic peptide in cardiovascular biology and diseases	*김선희 (전북대 의대)*

Symposium 6: Organellar Physiology and Metabolism	Chair: *Kyu-Sang Park*
Role of lysosomal Ca^{2+} in mitophagy	*이명식 (연세대 의대)*
Regulation of PDK activity on mitochondrial quality control & metabolic flexibility	*이인규 (경북대 의대)*
Transfer of isolated mitochondria: uptake mechanism and therapeutic application	*김영미 (경희대 의대)*
Cellular mechanism of over-exercise	*한 진 (인제대 의대)*
PGC-1α functions as a co-suppressor of XBP1s to regulate glucose metabolism	*이재민 (대구경북 과학기술원)*

▶ Poster-Oral (10월 26일 금요일)

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		Room B
17:30-17:40	P1-06 (PO-A-1): *In vivo* voltage-sensitive dye imaging of the insular cortex after mTOR inhibition in nerve-injured rats	*Kyeongmin Kim (Yonsei University)*
17:40-17:50	P1-07 (PO-A-2): Pain alleviation via inhibition of mTOR pathway in the insular cortex	*Songyeon Choi (Yonsei University)*
17:50-18:00	P1-10 (PO-A-3): Acute fasting induced-analgesia	*Jeong-Yun Lee (Seoul National University)*
18:00-18:10	P1-18 (PO-A-4): The role of Intrinsic plasticity of cerebellar Purkinje cell in cerebellum-dependent motor learning	*Dong Cheol Jang (Seoul National Universiy)*
18:10-18:20	P3-18 (PO-A-5): Translocatable voltage-gated Ca^{2+} channel β subunits in $\alpha1$-β complexes reveal competitive replacement yet no spontaneous dissociation	*Jun-Hee Yeon (DGIST)*
18:20-18:30	P3-29 (PO-A-6): Contribution of transient receptor potential channels to store-operated calcium entry in autonomic neuron-satellite glia unit	*Sohyun Kim (Yonsei University Wonju College of Medicine)*

		Room C
17:30-17:40	P9-04 (PO-B-1): Determining the deubiquitinating enzymes regulating the adipose derived mesenchymal stem cells senescence	*Jongbeom Oh (CHA University)*
17:40-17:50	P9-07 (PO-B-2): EPHB6 mutation induces cell adhesion-mediated paclitaxel resistance via EPHA2 and CDH11 expression	*Sarah Yoon (Ajou University)*
17:50-18:00	P9-11 (PO-B-3): Cardiovascular drug, echinochrome A enhances cardiac differentiation from embryonic stem cell via PKCiota inhibition	*Hyoung Kyu Kim (Inje University)*
18:00-18:10	P9-13 (PO-B-4): Influence of pharmacological inhibition of AKT by novel inhibitor HS1793 in relapsed multiple myeloma	*Amy Kim (Inje University)*
18:10-18:20	P9-32 (PO-B-5): Impairment of NHE6 recruitment to synaptic vesicle by SCAMP5 deficiency decreases quantal size at glutamatergic synapses	*Unghwi Lee (Seoul National University)*

▶ Symposium (10월 27일 토요일)

Contents	
Symposium 7: Neuronal Regulation	**Chair: *Youngho Jin, Seungsoo Chung***
The neural mechanisms underlying cardiovascular autonomic dysfunction in rodent models of cirrhosis and portal hypertension	*정성우 (연세대 원주의대)*
Neuronal regulation of the gastrointestinal defense mechanisms	*진영호 (경희대 의대)*
Reactivation of critical period-like plasticity at adult TC input in neocortex	*정승수 (연세대 의대)*
Neurourologic research resources	*김계환 (가천대 의대)*
Symposium 8: Stem Cells and Differentiation	**Chair: *Sang-Mo Kwon***
Direct reprogramming for in vivo therapy and disease modeling	*김종필 (동국대 바이오시스템대)*
Discovery of new regulators in HSCs and hematological malignancies	*이동준 (부산대 의대)*
Direct conversion of fibroblast into endothelial cells	*한정규 (서울대 의대)*
Highly accurate prediction of CRISPR-Cpf1 activity	*김형범 (연세대 의대)*
Organoid technologies: current limitations and challenges	*유종만 (차의과대 의대)*
Symposium 9: Skin and Epidermis Research	**Chair: *Joohyun Nam***
Calcium – the central regulator of the extrinsic skin aging	*남주현 (동국대 의대)*
TRPV channels and post-burn pruritus	*김혜원 (한림대 의대)*
Keratinocytes in house dust mite-induced atopic skin inflammation	*장용현 (경북대 의대)*
A novel synthetic Piper amide derivative NED-180 inhibits hyperpigmentation by activating the PI3K and ERK pathways and by regulating Ca^{2+} influx via TRPM1 channels	*김선여 (가천대 약대)*
Calcium ion on skin barrier	*최응호 (연세대 원주의대)*
Clinical application of ion channels in skin	*김우경 (동국대 의대)*

Workshop

Workshop 1: Optical and Electrophysiological Tools

S 26 W-1-1 Novel methods for clearing and labeling the tissues for 3D high-resolution microscopy
Sunghoe Chang
Department of Physiology and Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea

S 26 W-1-2 Electrophysiological approach and methods for research in cardiac arrhythmias
Jong-Il Choi
Division of Cardiology, Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea

S 26 W-1-3 Nikon microscopes for life science
Fukui Tatsuo
Nikon Corporation, Japan

Workshop 2: Drug Discovery: A How-To Guide

S 26 W-2-1 High throughput screening for drug discovery of protease-activated receptor 2 modulators
Yohan Seo[1,2], Jiwon Chang[1], Wan Namkung[1,2]
[1]College of Pharmacy and Yonsei Institute of Pharmaceutical Sciences, [2]Interdisciplinary Program of Integrated OMICS for Biomedical Science Graduate School, Yonsei University, Incheon, Korea

S 27 W-2-2 Considerations in the preclinical studies for drug development
Joungwook Seo
Center of Safety Pharmacology, Division of Integrated Toxicity, Korea Institute of Toxicology, Daejeon, Korea

S 27 W-2-3 Chondroitin sulfate-based nanoparticles for tumor penetration
Jae-Young Lee
College of Pharmacy, Chungnam National University, Daejeon, Korea

S 27 W-2-4 New drug development of SP-8203(otaprimastat) in ischemic stroke
Jeiman Ryu
CEO/Shinpoong Pharmaceutical Company, Ansan, Korea

Satellite Meeting

Physiome research and Physiome-based educational program

S 28 SM-1 EKG training using three-dimensional heart model
Eun Bo Shim
Department of Mechanical & Biomedical Engineering, Kangwon National University, Chuncheon, Korea

S 28 SM-2 Simulation-based general electrophysiology
Chae Hun Leem
Department of Physiology University of Ulsan College of Medicine, Seoul, Korea

S 28 SM-3 Integrative understanding of human circulatory functions in students practicum of physiology using quantitative circulation physiology program (QCP-2005)
Young-Keul Jeon[1,2], Sung Joon Kim[1,2,3]
[1]Department of Physiology, [2]Department of Biomedical Sciences, [3]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, Korea

S 28 SM-4 Simulation-based lecture on muscle physiology using EDISON software
Jae Boum Youm
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

Young Scientist Session

S 29 YS-1 Brain mechanisms of neuropathic pain and interaction between chronic pain, depression and empathic distress
Geehoon Chung
Department of Physiology, Kyung Hee University, College of Korean Medicine, Seoul, Korea

S 29 YS-2 Tissue-to-tissue communication of mitochondrial function in the model organism C. elegans
Kyoung-hye Yoon
Mitohormesis Research Center, Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 29 YS-3 SHANKs deficiency impairs hyperalgesia in neuropathic and inflammatory pain models
Yong Ho Kim
Department of Physiology, College of Medicine, Gachon University, Incheon, Korea

Plenary Lecture

S 30 Plenary Lecture Aging and chronic kidney disease: phosphate connection
Makoto Kuro-O
Division of Anti-aging Medicine, Center for Molecular Medicine, Jichi Medical University, Tochigi, Japan, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, USA

Symposium

Symposium 1: Pathophysiology of Cognitive Disorder

S 33 S-1-1 Cortical circuits for the multi-sensory integration: role of inhibition
Seung-Hee Lee
Department of Biological Sciences, KAIST, Daejeon, Korea

S 33 S-1-2 Cellular and molecular mechanisms of epilepsy in focal brain malformations
Ye Eun Kim, Chang Hyun Shin, Seung Tae Baek
Department of Life Sciences, Pohang University of Science and Technology (POSTECH), Pohang, Korea

S 34 S-1-3 Lithium-responsive and layer-specific prefrontal dysfunction in a mouse model of mania
Kihoon Han
Department of Neuroscience, College of Medicine, Korea University, Seoul, Korea

S 34 S-1-4 Synaptic dysfunction of mild intellectual disability
Se-Young Choi
Department of Physiology, Seoul National University School of Dentistry, Seoul, Korea

S 34 S-1-5 Cell type-specific signaling networks in learning disabilities
Hyun-Hee Ryu[1,2], TaeHyun Kim[3], Jung-Woong Kim[2], Bong-Kiun Kaang[3], Yong-Seok Lee[1]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Life Science, Chung-Ang University, [3]School of Biological Sciences, College of Natural Sciences, Seoul National University, Seoul, Korea

Symposium 2: Pathophysiology of Potassium Channels

S 34 S-2-1 Blockade of Kv1.5 by PCP derivatives and its clinical implications
Jae Gon Kim[1], Sang Woong Park[2], Hyunju Noh[1], Bok Hee Choi[3], Haiyue Lin[1], Young Min Bae[1]
[1]Department of Physiology, Konkuk University School of Medicine, Chungju, [2]Department of Emergency Medical Services, Eulji University, Seongnam, [3]Department of Pharmacology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 35 S-2-2 Identification of C-terminal domains regulating TREK K^+ channels
Joohan Woo[1], Young Keul Jeon[1], Yin-Hua Zhang[1], Joo Hyun Nam[2], Dong Hoon Shin[3] , Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, [2]Department of Physiology & Ion Channel Disease Research Center, Dongguk University College of Medicine, [3]Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

S 35 S-2-3 Regulation of endothelial Ca^{2+}-activated K^+ channels in health and vascular diseases
Shinku Choi, Ji Aee Kim, Suk Hyo Suh
Department of Physiology, College of Medicine, Ewha Womans University, Seoul, Korea

S 35 S-2-4 KCNQ channel methylation in control of neuronal excitability
Hana Cho
Department of Physiology, Single Cell Network Research Center, Sungkyunkwan University School of Medicine, Suwon, Korea

S 36 S-2-5 KCNQ4 potassium channelopathy and hearing loss
Hyun Been Choi[1], Byung Yoon Choi[2], Jinsei Jung[3], Jae Young Choi[3], Heon Yung Gee[4], Tong Mook Kang[1]
[1]Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, [2]Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Bundang, [3]Department of Otorhinolaryngology, Yonsei University College of Medicine, [4]Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

S 36 S-2-6 BK_{Ca} channel drug discovery targeting overactive bladder
Chul-Seung Park
School of Life Sciences and National Leading Research Laboratory, Gwangju Institute of Science and Technology (GIST), Gwangju, Korea

Symposium 3: Exercise Physiology / IMPACT

S 36 S-3-1 Single nucleotide polymorphisms and world-class Korean athletes
Kwang-Jun Kim[1], Chang-Sun Kim[2], Jung-Jun Park[3], Ju-Hee Kang[4], Dong-Ho Park[5]
[1]Korea Institute of Sports Science, [2]Department of Physical Education, Dongduk Women's University, [3]Division of Sport Science, Pusan National University, Busan, [4]Department of Pharmacology and Medicinal Toxicology Research Center, College of Medicine, Inha University, [5]Department of Kinesiology, Inha University, Incheon, Korea

S 37 S-3-2 Inter-individual variation in the changes in insulin sensitivity in response to regular exercise
SoJung Lee
Division of Sports Medicine, Graduate School of Physical Education, Kyung Hee University, Seoul, Korea

S 37 S-3-3 Beneficial role of HIT exercise on hippocampal plasticity
Min Chul Lee
Department of Sports Medicine, College of Health Science, CHA University, Pocheon, Korea

S 37 S-3-4 Investigation of vitamin D level and its role in inactive submariner
Eunmi Park
Department of Food and Nutrition, Hannam University, Daejeon, Korea

S 37 S-3-5 Regulation of hepatic stellate cells by the factors derived from contracting skeletal muscle cells
Soo-Jin Kim[1,2], Jihye Kim[1,3], Jun Namkung[1,3], In Deok Kong[2], Jae Seung Chang[1]
Mitohormesis Research Center[1], Department of Physiology[2], Department of Biochemistry[3], Yonsei University Wonju College of Medicine, Wonju, Korea

S 38 S-3-6 The role of echinochrome a for exercise capacity
Dae Yun Seo[1], Hyo-Bum Kwak[3], Hyun Seok Bang[4], Jin Han[1,2]
[1]National Research Laboratory for Mitochondrial Signaling, Department of Physiology, BK21 Plus Team, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, [2]Department of Convergence of Biomedical Science, Inje University, Busan, [3]Department of Kinesiology, Inha University, Incheon, [4]Department of Physical Education, Tong Myong University, Busan, Korea

Symposium 4: Pain Physiology

S 38 S-4-1 Ultrastructural basis for craniofacial sensory processing in the brainstem (Expression of glycine receptor alpha 3 in the primary sensory neurons
Yong Chul Bae
Department of Anatomy and Neurobiology, School of Dentistry, Kyungpook National University, Daegu, Korea

S 38 S-4-2 Role of mechanosensitive ion channels in tooth pain
Seog Bae OH[1,2]
[1]Department of Neurobiology and Physiology School of Dentistry, [2]Department of Brain and Cognitive Sciences College of Natural Sciences, Seoul National University, Seoul, Korea

S 38 S-4-3 Maresin 1 inhibits TRPV1 in temporomandibular joint (TMJ)-related trigeminal nociceptive neurons and TMJ inflammation-induced synaptic plasticity in the trigeminal nucleus
Chul-Kyu Park
[1]Department of Physiology, College of Medicine, Gachon University, Incheon, Korea

S 39 S-4-4 Experimental animal models for trigeminal neuralgia
Dong-Kuk Ahn
Department of Oral Physiology, School of Dentistry, Kyungpook National University, Daegu, Korea

S 39 S-4-5 Possible involvement of oral dysfunction in inducing stress disorder
Jonhwa Won[1], Seog Bae Oh[1], Youngnam Kang[1,2]
[1]Department of Neurobiology and Physiology, School of Dentistry, Seoul National University, Seoul, Korea, [2]Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka, Japan

Symposium 5: Heart and Circulation

S 39 S-5-1 Structural and functional significances of the atrial T-tubules
Jieun An, Ami Kim, Sun Hwa Park, Hyun Bin Choi, Tong Mook Kang
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

S 39 S-5-2 Ca^{2+} signaling triggered by shear-autocrine P2X receptor pathway in rat atrial myocytes
Joon-Chul Kim, Min-Jeong Son, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, Korea

S 40 S-5-3 Atrial fibrillation and atrial electrophysiology
Boyoung Joung
Cardiology Division, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea

S 40 S-5-4 Atrial natriuretic peptide in cardiovascular biology and diseases
Suhn Hee Kim
Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea

Symposium 6: Organellar Physiology and Metabolism

S 40 S-6-1 Role of lysosomal Ca^{2+} in mitophagy
Kihyoun Park, Heyjin Lim, Myung-shik Lee
Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea

S 41 S-6-2 Regulation of PDK activity on mitochondrial quality control & metabolic flexibility
Themis Thoudam, In-Kyu Lee
Department of Biomedical Science, Graduate School, BK21 Plus KNU Biomedical Convergence Program, Kyungpook National University, Daegu, Korea

S 41 S-6-3 Transfer of isolated mitochondria: uptake mechanism and therapeutic application
Young Cheol Kang, Youngmi Kim Pak
Department of Physiology, Department of Neuroscience, School of Medicine, Kyung Hee University, Seoul, Korea

S 41 S-6-4 Cellular mechanism of over-exercise
Nammi Park, Jubert Marquez, Tae Hee Ko, JeongRim Ko, Dae Yun Seo, Jae Boum Youm, Hyoungkyu Kim, Jin Han
Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Busan, Korea

S 41 S-6-5 PGC-1α functions as a co-suppressor of XBP1s to regulate glucose metabolism
Jaemin Lee
DGIST, Daegu, Korea

Symposium 7: Neuronal Regulation

S 42 S-7-1 The neural mechanisms underlying cardiovascular autonomic dysfunction in rodent models of cirrhosis and portal hypertension
Seong-Woo Jeong
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 42 S-7-2 Neuronal regulation of the gastrointestinal defense mechanisms
Young-Ho Jin
Department of Physiology, College of Medicine, Kyung Hee University, Seoul, Korea

S 42 S-7-3 Reactivation of critical period-like plasticity at adult TC input in neocortex
Seungsoo Chung
Brain Korea 21 Project for Medical Science, Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

S 42 S-7-4 Neurourologic research resources
Khae-Hawn Kim
Department of Urology, College of Medicine, Gachon University, Gil Medical Center, Incheon, Korea

Symposium 8: Stem cells and Differentiation

S 43 S-8-1 Direct reprogramming for in vivo therapy and disease modeling
Jongpil Kim
Department of Chemistry, Department of Biomedical Engineering, Dongguk University College of Science, Seoul, Korea

S 43 S-8-2 Discovery of new regulators in HSCs and hematological malignancies
Dongjun Lee
Department of Medical Science, Pusan National University School of Medicine, Busan, Korea

S 43 S-8-3 Direct conversion of fibroblast into endothelial cells
Jung-Kyu Han
Department of Internal Medicine, Seoul National University, Seoul, Korea

S 44 S-8-4 Highly accurate prediction of CRISPR-Cpf1 activity
Hyongbum Henry Kim
Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

S 44 S-8-5 Organoid technologies; current limitations and challenges
Jongman Yoo
Department of Microbiology, and Organoid Research Center, School of Medicine, CHA University, Seongnam, Korea

Symposium 9: Skin and Epidermis Research

S 44 S-9-1 Calcium – the central regulator of the extrinsic skin aging
Joo Hyun Nam[1,2]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang, Korea

S 44 S-9-2 TRPV channels and post-burn pruritus
Hye One Kim
Department of Dermatology, College of Medicine, Hallym University, Seoul, Korea

S 45 S-9-3 Keratinocytes in house dust mite-induced atopic skin inflammation
Yong Hyun Jang
Department of Dermatology, School of medicine, Kyungpook National University, Daegu, Korea

S 45 S-9-4 A novel synthetic Piper amide derivative NED-180 inhibits hyperpigmentation by activating the PI3K and ERK pathways and by regulating Ca^{2+} influx via TRPM1 channels
Hwang E[1], Kim S[2], Kim SY[3]
[1]Department of of Oriental Medicinal Material and Processing, College of Life Science, Kyung Hee University, Yongin, [2]College of Pharmacy, Seoul National University, Seoul, [3]College of Pharmacy, Gachon University, Incheon, Korea

S 45 S-9-5 Calcium ion on skin barrier
Eung Ho Choi
Department of Dermatology, Yonse University Wonju College of Medicine, Wonju, Korea

S 45 S-9-6 Clinical application of ion channels in skin
Woo Kyung Kim[1,2]
[1]Channelopathy Research Center (CRC), Dongguk University College of Medicine, [2]Department of Internal Medicine Graduate School of Medicine, Dongguk University, Goyang, Korea

Yudang Academic Award

S 46 APE1/Ref-1 as a therapeutic target molecule
Byeong Hwa Jeon
Research Institute of Medical Science, Department of Physiology, College of Medicine, Chungnam National University, Daejeon, Korea

Poster Presentation

P1: Basic Neurophysiology and Pain

S 47 P1-01 Bee venom decreases hot water-induced pain in mice
Dong-Wook Kang, Jae-Gyun Choi, Cuk-Seong Kim, Sang Do Lee, Byeong Hwa Jeon, Jin Bong Park, Hyun-Woo Kim*
Department of Physiology and Medical Science, Institute of Brain Research, College of Medicine, Chungnam National University, Daejeon, Korea

S 47 P1-02 Endogenous TRPV4 expression of a hybrid neuronal cell line and its utilization for ligand screening
Seung-In Choi, Sungjae Yoo, Geunyeol Choi, Ji Yeon Lim, Minseok Kim, Pyung Sun Cho, Sun Wook Hwang
Department of Biomedical Sciences and Department of Physiology, Korea University College of Medicine, Seoul, Korea

S 47 P1-03 The interaction between N-methyl-d-aspartate receptors (NMDAR) GluN2B and postsynaptic density protein 95 (PSD-95) contributes in the neuropathic pain
Youngkyung Kim[1,2], Young Wook Yoon[1,2]
[1]Department of Physiology, [2]Neuroscience Research Institute, Korea University College of Medicine, Seoul, Korea

S 47 P1-04 Amelioration of gait impairments by extra-synaptic NMDA receptors antagonist in MPTP-induced Parkinson's model mice
Ramesh Sharma[1,2,3], Chiranjivi Neupane[1,2,3], Jin Bong Park[1,2,3]
[1]Department of Medical Sciences, School of Medicine, [2]Department of BK21plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, School of Medicine and Brain Research Institute, Chungnam National University, Daejeon, Korea

S 48 P1-05 Pheripheral and central TRPV1 expression following dental pulp inflammation
Imene Sallem[1], Il-Young Jung[1], Bae Hwan Lee[2], Myeounghoon Cha[2]
[1]Department of Conservative Dentistry and Oral Science Research Center, Yonsei University College of Dentistry, [2]Department of Physiology, Yonsei University College of Medicine

S 48 P1-06 (PO-A-1) *In vivo* voltage-sensitive dye imaging of the insular cortex after mTOR inhibition in nerve-injured rats
Kyeongmin Kim, Myeounghoon Cha, Songyeon Choi, Bae Hwan Lee
Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

S 48 P1-07 (PO-A-2) Pain alleviation via inhibition of mTOR pathway in the insular cortex
Songyeon Choi, Myeounghoon Cha, Kyeongmin Kim, Motomasa Tanioka, Bae Hwan Lee
Department of Physiology, Yonsei University College of Medicine, Seoul, Korea

S 49 P1-08 Neuroprotective effects of lipid emulsion in kainic acid-induced neural injury in the rat hippocampus
Motomasa Tanioka, Kyungmin Kim, Songyeon Choi, Bae Hwan Lee
Department of Physiology and Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea

S 49 P1-09 Pharmacological characterization of low doses of ibuprofen and dexamethasone attenuate trigeminal neuropathic pain in rats
Song-hee Kang[1], Min-Kyoung Park[2], Jo-Young Son[1], Jin-Sook Ju[1], Min-Kyung Lee[3], Dong-Kuk Ahn[1]
[1]Department of Oral Physiology, School of Dentistry, Kyungpook National University, Department of Dental Hygiene, [2]Kyung-Woon University, [3]Dong-Eui University

S 49 P1-10 (PO-A-3) Acute fasting induced-analgesia
Jeong-Yun Lee[1], Grace J Lee[1], Youngnam Kang[2,3], Seog Bae Oh[1,2]
[1]Department of Brain and Cognitive Sciences, College of Natural Sciences, [2]Dental Research Institute and Department of Neurobiology & Physiology, School of Dentistry, Seoul National University, Seoul, Korea, [3]Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka, Japan

S 50 P1-11 Understanding the mechanism of odor-specific memory formation in Caenorhabditis elegans
Hee Kyung Lee[1], Jae Im Choi[2], Hae Su Kim[2], So Young Park[2], Jin I Lee[2], Kyoung-hye Yoon[1]
[1]Mitohormesis Research Center, Department of Physiology, Wonju College of Medicine, [2]Division of Biological Science and Technology, Yonsei University, Wonju, Korea

S 50 P1-12 Interaction of nNOS with PSD-95 modulated by D-serine leads to the induction of mechanical allodynia in a mouse model of neuropathic pain
Sheu-Ran Choi, Hoon-Seong Choi, Ho-Jae Han, Jang-Hern Lee
Department of Veterinary Physiology, BK21 PLUS Program for Creative Veterinary Science Research, Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Korea

S 50 P1-13 Response of dorsal root ganglion neurons innervating intervertebral disc to transient receptor potential vanilloid 1 agonist (capsaicin) and ankyrin 1 agonist (allyl isothiocyanate)
Eui Ho Park, Sun Wook Moon, Hye Rim Suh, Hee Chul Han
Department of Physiology, College of Medicine and Neuroscience Research Institute, Korea University, Seoul, Korea

S 51 P1-14 Layer-specific activation of synaptic inputs onto layer 2/3 pyramidal neurons in the prefrontal cortex of rat
Kwang-Hyun Cho[1], Kayoung Joo[1], Dongchul Shin[1], Hyun-Jong Jang[1,2], Duck-Joo Rhie[1,2]
[1]Department of Physiology, College of Medicine, [2]Catholic Neuroscience Institute, The Catholic University of Korea, Seoul, Korea

S 51 P1-15 Dental primary afferent (DPA) neuron types revealed by single-cell RNA sequencing
Pa Reum Lee[1], Seog Bae Oh[1,2]
[1]Department of Brain and Cognitive Science, College of Natural Science, [2]Dental Research Institute and Department of Neurobio. & Physiology, School of Dentistry, Seoul National University, Seoul, Korea

S 51 P1-16 Constitutive activities of TRPC3 and NALCN channels drive pacemaking in SNc 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, Suwon, Korea, [2]IIB-INTECH, Univ Nacional de San Martin, Prov Buenos Aires, Argentina

S 52 P1-17 Neuroprotective effects of 2-arachidonoylglycerol on hippocampal neuropathology following pilocarpine-induced status epilepticus
Mi-Hye Kim[1,2], Geun-Pyo Hong[1,2], Yeong Ran Hwang[1], Hee Jung Kim[1]*
[1]Department of Physiology, College of Medicine, [2]Department of Medical Laser, Graduate School, Dankook University, Cheonan, Korea

S 52 P1-18 (PO-A-4) The role of Intrinsic plasticity of cerebellar Purkinje cell in cerebellum-dependent motor learning
Dong Cheol Jang[1,2], Hyun Geun Shim[2,3], Sang Jeong Kim[2,3,4]
[1]Department of Brain and Cognitive Science, College of Natural Science, Seoul National Universiy, [2]Department of Physiology, [3]Department of Biomedical Science, [4]Neuroscience Research Institute, Seoul National University College of Medicine, Seoul, Korea

S 52 P1-19 Licochalcone A attenuates status epilepticus-induced synaptic degeneration
Geum Pyo Hong[1,2], Yeong Ran Hwang[1], Mi-Hye Kim[1,2], Hee Jung Kim[1]*
[1]Department of Physiology, College of Medicine, [2]Department of Medical Laser, Graduate School, Dankook University, Cheonan, Korea

S 52 P1-20 The effects of fear conditioning on spontaneous firing of cerebellar Purkinje cells
Jaegeon Lee, Dong Cheol Jang, Soonho Shin, Sang Jeong Kim
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

P2: Neuronal Pathophysiology

Neurologic and Psychiatric Diseases, Neuronal Ischemia

S 53 P2-01 Suppression of FoxO1 by leptin enhances tyrosine hydroxylase and leads to anxiolytic behavior
Seul Ki Kim[1#], Dong Hwee Son[1#], Khanh V. Doan[2], Dong Joo Yang[1], Ji Su Sun[1], Yun-Hee Choi[1], Dong Min Shin[1]*, Ki Woo Kim[1]*
[1]Department of Oral Biology, BK21 PLUS, Yonsei University College of Dentistry, Seoul, Korea, [2]Department of Pharmacology, School of Medicine, Tan Tao University, Tan Duc E.City, Vietnam. [#]Authors equally contributed.

S 53 P2-02 Targeted downregulation of JMJD2A ameliorates Tau-induced Alzheimer's defects in Drosophila melanogaster
Sung Yeon Park[1,3], Yang-Sook Chun[1,2,3]*
[1]Ischemic/Hypoxic Disease Institute, [2]Department of Biomedical Sciences and [3]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 53 P2-03 The possibility of ADHD model; Cortisol-induced hyperactive behaviors and memory deficiency in young rats
Hye-Ji Kim, Sang-Eun Kwak, Na-Hye Hwang, Su-Yong Eun, Sung-Cherl Jung
Department of Physiology, School of Medicine, Jeju National University, Jeju, Korea

S 53 P2-04 Early life stress provokes anxiety and aggressive-like behavior and elevation of GABAergic activity in the ventral hippocampus in adolescence mice
Anjana Silwal Adhikari[1], Sang Yep Shin[1], You Jean Kim[2], Dae-Yong Song[2], Sun Seek Min[1]
[1]Department of Physiology and Biophysics, [2]Anatomy and Neuroscience, Eulji University College of Medicine, Daejeon, Korea

S 54 P2-05 Effect of *in vitro* hyperglycemia on excitability of the rat dorsal root ganglia (DRG) neurons
Jiyeon Kwak
Department of Biophysics and Physiology, Inha University School of Medicine, Incheon, Korea

S 54 P2-06 The activity-dependent modulation of the cerebellar Purkinje cell output requires synergies between synaptic and intrinsic plasticity
Hyun Geun Shim[1,2], Sang Jeong Kim[1,2,3]
[1]Department of Physiology, [2]Department of Biomedical Science, [3]Neuroscience Research Center, Seoul National University College of Medicine, Seoul, Korea

S 54 P2-07 Noradrenergic modulation of cerebellar glial activity during nociception
Seung Ha Kim[1,2], Sun Kwang Kim[3], Sang Jeong Kim[1,2]
[1]Department of physiology, [2]Department of biomedical science, Seoul National University College of medicine, [3]Department of physiology College of Korean medicine, Kyung Hee University, Seoul, Korea

S 54 P2-08 Effects of intranasal oxytocin on impairments in hippocampal plasticity and recognition memory following uncontrollable stress
Yoon-Jung Kim[1], Seong-Hae Park[1], Jung-Cheol Park[2], Jung-Soo Han[2], Se-Young Choi[1]
[1]Department of Physiology, Dental Research Institute, Seoul National University School of Dentistry, [2]Department of Biological Sciences, Konkuk University, Seoul, Korea

P3: Electrophysiology and Ca^{2+} signaling

Ion channels and transporters, Intracellular Ca^{2+} signaling, Pharmacology

S 55 P3-01 Regulation of PKD2L1 channel by direct interaction with calmodulin
Eunice Yon June Park, Youngjoo Baik, Misun Kwak, Insuk So
Department of Physiology, Seoul National University, College of Medicine, Seoul, Korea

S 55 P3-02 Tricyclic antidepressant clomipramine blocks voltage-gated K^+ current in rabbit coronary arterial smooth muscle cells
Jin Ryeol An[1], Sung Hun Na[2], Won Sun Park[1]
[1]Department of Physiology, Kangwon National University School of Medicine, [2]Department of Obstetrics and Gynecology, Kangwon National University Hospital, Kangwon National University School of Medicine, Chuncheon, Korea

S 55 P3-03 Inhibition of voltage-gated K^+ current by tricyclic antidepressant desipramine on rabbit coronary arterial smooth muscle cells
Jin Ryeol An, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, Korea

S 55 P3-04 Changes of ATP-sensitive K^+ channel expression in human umbilical smooth muscle during gestational diabetes mellitus
Mi Seon Seo[1], Sung Hun Na[2], Won Sun Park[1]
[1]Department of Physiology, Kangwon National University School of Medicine, [2]Department of Obstetrics and Gynecology, Kangwon National University Hospital, Kangwon National University School of Medicine, Chuncheon, Korea

S 56 P3-05 DPP-4 inhibitor, vildagliptin induces vasorelaxation via activation of Kv channel and SERCA pump in aortic smooth muscle
Mi Seon Seo, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, Korea

S 56 P3-06 Characterization of voltage-dependent Ca^{2+} channels of human cardiac myofibroblasts and the effect of nitric oxide through cGMP-dependent mechanism
Hyemi Bae, Jeongyoon Choi, Young-Won Kim, Donghee Lee, Yelim Seo, Seong-Tae Kim, Jae-Hong Ko, Hyoweon Bang, Inja Lim
Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

S 56 P3-07 Effect of carbon monoxide on Ca^{2+}-activated K^+ currents of human cardiac fibroblasts through the protein kinase pathways
Hyemi Bae[1], Jeongyoon Choi, Young-Won Kim, Donghee Lee, Yelim Seo, Seong-Tae Kim, Jae-Hong Ko, Hyoweon Bang, Inja Lim
[1]Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

S 56 P3-08 Regulation of transient receptor potential canonical (TRPC)4 by the phospholipase C pathway
Juyeon Ko, Jongyun Myeong, Misun Kwak, Insuk So
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 57 P3-09

Hydrogen peroxide constricts rat arteries by Na^+-permeable non-selective cation channels

Sang Woong Park[1†], Hyun Ji Park[2†], Soon-Kyu Yoou[1], Myeongsin Kang[1], Jae Gon Kim[2], Kyung Chul Shin[2], Dong Jun Sung[3], Wonjong Yu[4], Youngjin Lee[5], Sung Hea Kim[6], Young Min Bae[2]

[1]Department of Emergency Medical Services, Eulji University, Seongnam, [2]Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, [3]Department of Sports and Health Studies, College of Biomedical and Health Science, Konkuk University, Chungju, [4]Department of Physical Therapy, Eulji University, Seongnam, [5]Department of Radiological Science, Gachon University, Incheon, [6]Department of Cardiology, Konkuk University School of Medicine, Chungju, Korea

S 57 P3-10

Cariprazine inhibits hERG 1A and heteromeric hERG 1A/3.1 potassium channels

Hong Joon Lee[1], Bok Hee Choi[2], Jin-Sung Choi[3], Sang June Hahn[1]*

[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Department of Pharmacology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju. [3]College of Pharmacy, Integrated Research Institute of Pharmaceutical, The Catholic University of Korea, Korea

S 57 P3-11

Altered GABAergic tone in STING KO mice

Chiranjivi Neupane[1,2,3], Ramesh Sharma[1,2,3], Jin Bong Park[1,2,3]

[1]Department of Medical Sciences, [2]Department of BK21plus CNU Integrative Biomedical Education Initiative, [3]Department of physiology, School of Medicine and Brain Research Institute, Chungnam National University, Daejeon, Korea

S 58 P3-12

Structure-function relationship of TRPC3 carboxyl coiled coil domain

Tharaka Darshana Wijerathne, Ji Hyun Kim, Min Ji Kim, Kyu Pil Lee

Department of Physiology, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea

S 58 P3-13

pH- and calcium-dependent inhibition of hSlo3 by quercetin

Tharaka Darshana Wijerathne, Ji Hyun Kim, Min Ji Kim, Kyu Pil Lee

Department of Physiology, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea

S 58 P3-14

Ketamine contracts rat arteries by facilitating the activation of serotonin 5-HT_{2A} receptors – clinical implications for the PCP derivatives-induced diseases

Jae Gon Kim[1], Sang Woong Park[2], Hyunju Noh[1], Bok Hee Choi[3], Haiyue Lin[1], Sung Hoon Kim[4], Young Min Bae[1]

[1]Department of Physiology, Konkuk University School of Medicine, Chungju, [2]Department of Emergency Medical Services, Eulji University, Seongnam, [3]Department of Pharmacology, and Institute for Medical Science, Chonbuk National University Medical School, Jeonju, [4]Department of Neurology, Kangwon National University School of Medicine, Chuncheon, Korea

S 59 P3-15

Measurement of ion concentration in the unstirred boundary layer with open patch-clamp pipette – implications for control of ion channels by fluid flow

Kyung Chul Shin[1], Jae Gon Kim[1], Sang Woong Park[2], Bokyung Kim[1], Doyoung Byun[3], Young Min Bae[1]

[1]Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, [2]Department of Emergency Medical Services, Eulji University, Seongnam, [3]Department of Mechanical Engineering, Sungkyunkwan University, Suwon, Korea

S 59 P3-16

Plasma membrane trafficking of PC2 regulated by TRPC4, TRPC5, Gαo, Gαi3 and Gαs

Young Joo Baik, Misun Kwak, Insuk So

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

S 59 P3-17

Characterization of Piezo2 ion channel, a mechanical stimulus receptor in MCC-13 cells

Kyung Chul Shin[1], Sang woong Park[2], Jae gon Kim[1], Hyun Ji Park[1], Young Min Bae[1]

[1]Department of Physiology, KU Open Innovation Center, Research Institute of Medical Science, Konkuk University School of Medicine, Chungju, [2]Department of Emergency Medical Services, Eulji University, Seongnam, Korea

S 59 P3-18 (PO-A-5)

Translocatable voltage-gated Ca^{2+} channel β subunits in α1-β complexes reveal competitive replacement yet no spontaneous dissociation

Jun-Hee Yeon[1], Chen-Gyu Park[1], Bertile Hille[2], Byung-Chang Suh[1]

[1]Department of Brain & Cognitive Sciences, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Korea, [2]Department of Physiology and Biophysics, University of Washington, Seattle, USA

S 60 P3-19

Suppression of hERG K^+ current and cardiac action potential prolongation by 4-hydroxynonenal via dual mechanisms

Seong Woo Choi[1,2], Si Won Choi[1], Young Keul Jeon[1], Sung-Hwan Moon[2], Yin-Hua Zhang[1], Sung Joon Kim[1]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Stem Cell Biology, Konkuk University School of Medicine, Chungju, Korea

S 60 P3-20

The Englerin A-sensing three charged residues for TRPC5 channel activation

SeungJoo Jeong[1], Minji Kim[2], Ki Chul Park[3], Eunice Yon June Park[1], Jinsung Kim[1], Jinhong Wie[4], Art E. Cho[3], Ju-hong Jeon[1], Insuk So[1]

[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Chungnam National University, College of Veterinary Medicine, Daejeon, [3]Department of Bioinformatics, Korea University, Sejong, Korea, [4]Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

S 60 P3-21

TMEM16F/ANO6, a Ca^{2+}-activated anion channel, is negatively regulated by the actin cytoskeleton and intracellular MgATP

Joo Hyun Nam[2,3], Haiyue Lin[1], Sung Joon Kim[1]

[1]Department of Physiology, College of Medicine, Seoul National University, Seoul , [2]Department of Physiology, Dongguk University College of Medicine, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang, Korea

S 61 P3-22 Temperature-dependent increase in the calcium sensitivity and acceleration of activation of ANO6 chloride channel variants
Joo Hyun Nam[2,3], Haiyue Lin[1], Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology, Dongguk University College of Medicine, Gyeongju, [3]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang, Korea

S 61 P3-23 PTPN6 regulates the cell-surface expression of TRPM4 channels in HEK293 cells
Dong Kun Lee[1], Eun A Kim[1], Eun Hye Byun[1], Dawon Kang[1], Jaehee Han[1], Jae Yong Park[2], Eunmi Hwang[3,4], Seong-Geun Hong[1]
[1]Department of Physiology, School of Medicine and Institution of Health Sciences, GNU, Jinju, [2]School of Biosystem and Biomedical Science, College of Health Science, KU, Seoul, [3]Center for Functional Connectomics, KIST, Seoul, [4]KHU-KIST Department of Converging Science and Technology, KHU, Seoul, Korea

S 61 P3-24 Echinochrome A inhibits cardiac SERCA2A by regulating phosphorylation of phospholamban Ser16 and Thr17
Ji Young Moon, Hyoung Kyu Kim, Jae Boum Youm, In Sung Song, Seung Hun Jeong, Sung Ryul Lee, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center, Department of Physiology, Inje University, Busan, Korea

S 61 P3-25 Differential changes of flow-induced vasodilation mechanisms in coronary arteries from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY)
Ming Zhe Yin, Hae Jin Kim, Sung Eun Kim, Yin Hua Zhang, Sung Joon Kim
Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

S 62 P3-26 Connexin-43-hemichannel-mediated ATP efflux triggers arrhythmogenic Ca^{2+} waves via P2X purinoceptor current in atrial myocytes
Min-Jeong Son, Joon-Chul Kim, Qui Anh Le, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, Korea

S 62 P3-27 Calcium/calmodulin dependent inhibition of TRPC6 is governed by the NT and CT interaction of TRPC6 channels
Tharaka Darshana Wijerathne, Ji Hyun Kim, Min Ji Kim, Kyu Pil Lee
Department of Physiology, College of Veterinary Medicine, Chungnam National University, Daejeon, Korea

S 62 P3-28 Poly-phenol enriched green tea extract rescues cognitive impairment by restoring hippocampal synaptic plasticity in post-menopausal depression
Sukjin Ko,[1] Ji-Hyun Jeong,[1] Ji Woong Ahn,[1] Young-Hwan Kim,[2] Seungsoo Chung[1]*
[1]Brain Korea 21 Plus Project for Medical Science, Department of Physiology, Yonsei University College of Medicine, Seoul, [2]BnH Research Co., LTD., Goyang, Korea

S 63 P3-29 (PO-A-6) Contribution of transient receptor potential channels to store-operated calcium entry in autonomic neuron-satellite glia unit
Sohyun Kim, Seung Jun Kang, Seong-Woo Jeong
Department of Physiology, Brain Research Group, Yonsei University Wonju College of Medicine, Wonju, Korea

S 63 P3-30 The N-terminus of voltage-gated Ca^{2+} (Ca_V) channel β3 subunit sensitizes PIP_2 dependence of $Ca_V2.2$ channel gating
Seong-Hyeon Byeon, Byung-chang Suh
Department of Brain and Cognitive Sciences, DGIST, Daegu, Korea

S 63 P3-31 Guanabenz inhibits HCN current in MTN neurons via a2A adrenergic receptor-independent pathway
Jonghwa Won[1], Youngnam Kang[2,3], Seog Bae Oh[1,2]*
[1]Department of Brain and Cognitive Sciences, College of Natural Sciences, [2]Dental Research Institute and Department of Neurobiology & Physiology, School of Dentistry, Seoul National University, Seoul, Korea, [3]Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Osaka, Japan

S 64 P3-32 Evaluating physiological interaction between the electrogenic Na/HCO_3 transporter NBCe1-B and its cytosolic binding partner IRBIT
Seong-Ki Lee, Walter F. Boron
Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH, USA

S 64 P3-33 Neurotensin modulates spontaneous firing of nigral dopamine neurons through region-dependent two distinct pathways
Suyun Hahn, Myoung Kyu Park
Department of Physiology, Sungkyunkwan University School of Medicine, Suwon, Korea

S 64 P3-34 Calpain inhibition mediates NMDAR responsiveness through KCNQ channels in midbrain dopamine neurons
Shin Hye Kim, Sun Hee Jeon, Hyung Seo Park, Se Hoon Kim
Department of Physiology, College of Medicine, Konyang University, Daejeon, Korea

S 64 P3-35 Endogenous ROS induced by menadione accumulates intracellular calcium in mouse pancreatic acinar cells
Hyung Seo Park, Kyung Jin Choi, Jin Wook Hwang
Department of Physiology, College of Medicine, Konyang University, Daejeon, Korea

S 65 P3-36 Proximal C-terminal controls the sensitivity to fluoxetine in TREK-2 channel
Dawon Kang, Eun-Jin Kim, Dong Kun Lee, Seong-Geun Hong, Jaehee Han
Department of Physiology, College of Medicine and Institute of Health Sciences, Gyeongsang National University, Jinju, Korea

S 65 P3-37 Decrease of inward rectifier and voltage-dependent K^+ currents of the right coronary artery smooth muscle cells in pulmonary arterial hypertensive rats
Sung Eun Kim, Ming Zhe Yin, Hae Jin Kim, Yin Hua Zhang, Sung Joon Kim
Department of Physiology and Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea.

S 65 P3-38 Alterations of shear-activated cation channels during chronic mechanical stress-induced atrial remodeling
Min-Jeong Son, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, Korea

S 66 P3-39 Effects of PCB 77 on Kv1.3 channel and Kv1.5 channel
Jonghui Kim[1], Su-Hyun Jo[1,2]
[1]Interdisciplinary Graduate Program for BIT Medical Convergence, [2]Department of Physiology, School of Medicine, Kangwon National University, Chuncheon, Korea

S 66 P3-40 Effects of polychlorinated biphenyl 77 on PKC activation and Kv1.5 channel current
Jonghui Kim[1], Su-Hyun Jo[1,2]
[1]Interdisciplinary Graduate Program for BIT Medical Convergence, [2]Department of Physiology, School of Medicine, Kangwon National University, Chuncheon , Korea

S 66 P3-41 Effects of paroxetine on kinetics of Kv1.3
Soobeen Hwang[1], Su-Hyun Jo[1,2]
[1]Interdisciplinary Graduate Program for BIT Medical Convergence, [2]Department of Physiology, School of Medicine, Kangwon National University, Chuncheon, Korea

S 66 P3-42 Concentration-dependent effects of alprenolol on human Kv1.3 channel
Soobeen Hwang[1], Su-Hyun Jo[1,2]
[1]Interdisciplinary Graduate Program for BIT Medical Convergence, [2]Department of Physiology, School of Medicine, Kangwon National University, Chuncheon, Korea

S 67 P3-43 A novel high-frequency variant of TRPV3 p.A628T in East Asians showing faster activation and higher Ca^{2+} influx by repetitive applications of chemical agonists
Siwon Choi[1,3#], Seong Woo Choi[7#], Jeesoo Chae[2,5,6], Jong-Il Kim[2,5,6], Sung Joon Kim[1,3,4]
[1]Department of Physiology, [2]Department of Biochemistry and Molecular Biology, [3]Department of Biomedical Sciences, [4]Ischemic/Hypoxic Disease Institute, [5]Genomic Medicine Institute, [6]Cancer Research Institute , Seoul National University College of Medicine, [7]Department of Stem Cell Biology, Konkuk University School of Medicine, Seoul, Korea. #Equally contributed

S 67 P3-44 Combination of transcranial alternating current stimulation and fermented Scutellaria baicalensis ameliorates motor recovery and cortical neural excitability following focal stroke
Min Sun Kim[1], Ho Koo[1], Byung Rim Park[1], Myung Ae Choi[1], Se Jin Moon[1], Jae Hyo Kim[2]
[1]Department of Physiology, Wonkwang University School of Medicine, and Brain Science Institute, [2]Department of Meridian & Acupoint, College of Korean Medicine, Wonkwang University, Iksan, Korea

S 67 P3-45 Polyamine-mediated inward rectification of TRPC4 channel
Jinsung Kim, Insuk So
Department of Physiology, College of Medicine, Seoul National University, Seoul, Korea

P4: Muscle Physiology

Cardiac, Skeletal, Smooth muscles

S 67 P4-01 STIM2 regulates both intracellular Ca^{2+} distribution and Ca^{2+} movement in skeletal myotubes
Mi Ri Oh[1], Keon Jin Lee[1], Jun Hee Choi[1], Ji Hun Kim[1], Mei Huang[1], Jin Ock Kim[2], Do Han Kim[2], Chung-Hyun Cho[3], Eun Hui Lee[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]School of Life Sciences, GIST, Gwangju, [3]Department of Pharmacology, College of Medicine, Seoul National University, Seoul, Korea

S 68 P4-02 Transient receptor potential C4 like channel is involved in stretch-induced spontaneous uterine contraction of pregnant rat
Young Hwan Kim[1,3], Young Han Kim[2], Duck-Sun Ahn[1], Seungsoo Chung[1]
[1]Department of Physiology, Brain Korea 21 Plus Project for Medical Science, [2]Department of Obstetrics and Gynecology, Yonsei University College of Medicine, [3]Division of Research and Development, BnH Research co., Ltd

S 68 P4-03 Role of inositol 1,4,5-trisphosphate receptor type 1 in ATP-induced nuclear Ca^{2+} signal and hypertrophy in atrial myocytes
Qui Anh Le, Joon-Chul Kim, Min-Jeong Son, Sun-Hee Woo
Laboratory of Physiology, College of Pharmacy, Chungnam National University, Daejeon, Korea

S 68 P4-04 Mitochondrial dysfunction reduces the activity of KIR2.1 K^+ channel in myoblasts via impaired oxidative phosphorylation
Hyun Jong Kim[1,2], JooHan Woo[3], Joo Hyun Nam[1,2]
[1]Department of Physiology, Dongguk University College of Medicine, Gyeongju, [2]Channelopathy Research Center (CRC), Dongguk University College of Medicine, Goyang, [3] Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea

S 69 P4-05 Difference in calcium sensitivity between right ventricle and left ventricle due to the low expression level of calcium binding protein in right ventricle
Young-Keul Jeon[1], Ji Hyun Jang[1], Hae Jin Kim[1], Su Han Cho[1], Jun-Bean Park[2], Yong Jin Kim[2], Sun-Hee Woo[3], Yin Hua Zhang[1], Sung Joon Kim[1]
[1]Department of Physiology, [2]Department of Internal Medicine/Division of Cardiology, Seoul National University College of Medicine, [3]Chungnam National University College of Pharmacy, Korea

S 69 P4-06 Abnormal calcium signaling and contractions in right ventricular myocytes from pulmonary hypertension model rats
Young-Keul Jeon[1], Ji Hyun Jang[1], Hae Jin Kim[1], Su Han Cho[1], Jun-Bean Park[2], Yong Jin Kim[2], Sun-Hee Woo[3], Yin Hua Zhang[1], Sung Joon Kim[1]
[1]Department of Physiology, [2]Department of Internal Medicine/Division of Cardiology, Seoul National University College of Medicine, [3]Chungnam National University College of Pharmacy, Korea

S 69 P4-07 Shear stress induces ATP release from ventricular myocytes via connexin hemichannel
Qui Anh Le, Joon-Chul Kim, Min-Jeong Son, Sun-Hee Woo
College of Pharmacy, Chungnam National University, Daejeon, Korea

S 69 P4-08 Sabinene inhibits tumor necrosis factor-α-induced atrophy in mouse C2C12 skeletal myotubes
Yunkyoung Ryu[1], Long Cui[1], Seung Hyo Jung[1], Junghwan Kim[2], Kyung Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, [2]Department of Physical Therapy, College of Public Health & Welfare, Yongin University, Yongin, Korea

S 70 P4-09 Fenofibric acid suppresses migration and proliferation via mitogen-activated protein kinase signaling pathway in vascular smooth muscle cells in response to platelet-derived growth factor-BB
Long Cui[1], Yunkyoung Ryu[1], Seung Hyo Jung[1], Hwan Myung Lee[2], Kyung Jong Won[1], Bokyung Kim[1]
[1]Department of Physiology, School of Medicine, Konkuk University, Seoul, [2]Department of Cosmetic Science, College of Life and Health, Hoseo University, Asan, Korea

S 70 P4-10 Fetuin-B activates TGF-β receptor II-mediated signaling pathway in vascular smooth muscle cells: A possible mechanism of vascular plaque rupture
Seung Hyo Jung, Long Cui, Yunkyoung Ryu, Su Jung Kim, Seung-Bo Park, Hengzhe Jin, Kyung Jong Won, Bokyung Kim
Department of Physiology, School of Medicine, Konkuk University, Seoul, Korea

P5: Organ physiology

Heart, Lung, Liver, Kidney, etc

S 70 P5-01 Stimulation of autophagy improves vascular function in the mesenteric arteries of type 2 diabetic mice
Youngin Kwon, Soo-Kyoung Choi, Seonhee Byeon, Young-Ho Lee
Department of Physiology, College of Medicine, Brain Korea 21 PLUS Project for Medical Sciences, Yonsei University, Seoul, Korea

S 70 P5-02 αKlotho protects diabetic nephropathy via stabilizing podocyte Ca^{2+} signaling
Ji-Hee Kim[1-4], Kyu-Hee Hwang[1-4], Jin Kwon[5], So Jin Kwak[3], NaLai Kim[3], In Deok Kong[1-4], Kyu-Sang Park[1-4], Seung-Kuy Cha[1-4]
[1]Departments of Physiology, [2]Global Medical Science and [3]Medicine, and [4]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, [5]Division of Biological Science and Technology, Yonsei University, Wonju, Korea

S 71 P5-03 Oxidative stress triggers hepatic stellate cell activation and fibrosis through TRPC6/MRTF-A signaling pathway
Kyu-Hee Hwang[1-4], Ji-Hee Kim[1-4], Soo-Jin Kim[1-4] , Kwon Jin[5], Ji Hoon Kim[3], Minjoo Cho[3], In Deok Kong[1-4], Kyu-Sang Park[1], Seung-Kuy Cha[1-4]
[1]Departments of Physiology, [2]Global Medical Science and [3]Medicine, and [4]Mitohormesis Research Center, Yonsei University Wonju College of Medicine, [5]Division of Biological Science and Technology, Yonsei University, Wonju, Korea

S 71 P5-04 Ethylenethiourea induces nephrotoxicity in male mice
Hye Yeon Park, Seung Hee Choi, Hyeon Woo Jeon, Seon Woo Park, Seong-Chun Kwon, Byong-Gon Park
Department of Physiology, and Institute for Clinical and Translational Research, College of Medicine, Catholic Kwandong University, Gangneung, Korea

S 71 P5-05 Inhibition of ERK1/2-mTORC1 axis ameliorates proteinuria and fibrogenic action of TGF-β in adriamycin-induced glomerulosclerosis
Soo-Jin Kim[1,2], Ranjan Das[1], Nhung Thi Nguyen[1,2], Luong Dai Ly[1,2], Ji-Hee Kim[1,2], Kyu-Hee Hwang[1,2], Da Dat Ly[1,2], Eunha Chang[1,2], Hyeong Ju Kwon[3], Seung-Kuy Cha[1,2], Kyu-Sang Park[1,2]
[1]Department of Physiology, [2]Mitohormesis Research Center, [3]Department of Pathology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 72 P5-06 Understanding of the role of SREBP-1c neddylation in hepatic lipogenesis and validation of a neddylation inhibitor as a therapeutic for hepatic steatosis
Uk-Il Ju[1], Do-Won Jeong[1], Jong-Wan Park[1,2], Yang-Sook Chun[1,2,3]
[1]Department of Biomedical Sciences, [2]Ischemic/Hypoxic Disease Institute, [3]Department of Physiology, Seoul National University College of Medicine, Seoul, Korea

P6: Endocrine and Energy Metabolism

Endocrine, Metabolism, Mitochondria

S 72 P6-01 Role of JHDM in the regulation of hepatic steatosis
Do-Won Jeong, Yang-Sook Chun
Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea

S 72 P6-02 Hydrogen sulfide augmented hypoxia-induced ANP secretion via HIP1a and PPAR-γ pathway
Weijian Li[1], Lamei Yu[1,2], Byung Mun Park[1], Suhn Hee Kim[1]
[1]Department of Physiology, Research Institute for Endocrine Sciences, Chonbuk National University Medical School, Jeonju, Korea, [2]Department of Physiology, Binzhou Medical University, China

S 73 P6-03 Effect of intensive and repetitive by thermal exposed on the central nervous system sudomotor activity
Jeong-Beom Lee[1], Young-Ki Min[1], Min-Seon Kim[1], Jeong-Ho Kim[2], Yun Su Eun[2], Tae-Hwan Pak[2], Hye-Jin Lee[3], Mi-Young Lee[3]
[1]Department of Physiology, College of Medicine, [2]A student at the College of Medicine, Soonchunhyang University, Cheonan, [3]Global Graduate School of Healthcare, Soonchunhyang University, Asan, Korea

S 73 P6-04 Study of active sweat gland density and sweat gland output per single gland activated by thermal exposed sudomotor activity in the humans
Jeong-Beom Lee[1], Young-Ki Min[1], Min-Seon Kim[1], Jeong-Ho Kim[2], Yun Su Eun[2], Tae-Hwan Pak[2], Hye-Jin Lee[3], Mi-Young Lee[3]
[1]Department of Physiology, College of Medicine, [2]A student at the College of Medicine, Soonchunhyang University, Cheonan, [3]Global Graduate School of Healthcare, Soonchunhyang University, Asan, Korea

S 73 P6-05 Insulin regulates adrenal steroidogenesis by stabilizing SF-1 activity
Dong Joo Yang[1,2,a], Ann W. Kinyua[2,a], Ji Su Sun[1], Seul Ki Kim[1], Jung Yoon Kang[1], Hye Rim Kang[1], Thuy Nhung Luong[1], Jichang Seong[1], Namju Kang[1], Yun-Hee Choi[1], Dong Min Shin[1], Ki Woo Kim[1]
[1]Department of Oral Biology, BK21 PLUS, Yonsei University College of Dentistry, Seoul, [2]Departments of Pharmacology and Global Medical Science, Wonju College of Medicine, Yonsei University, Wonju, Korea. [a]Co-first author

S 73 P6-06 P110β in the ventromedial hypothalamus regulates glucose and energy metabolism
Ji Su Sun[1]*, Teppei Fujikawa[2,3,4]*, Yun-Hee Choi[1]*, Dong Joo Yang[1], Seul Ki Kim[1], Jeong Yoon Kang[1], Hyae Rim Kang[1], Thuy Nhung Luong[1], Jichang Seong[1], Nam Joo Kang[1], Soo Young Oh[1], Dong Min Shin[1], Ki Woo Kim[1]
[1]Department of Oral Biology, BK21 PLUS project, Yonsei University College of Dentistry, Seoul, Korea, [2]Division of Hypothalamic Research, Department of Internal Medicine and, [3]Department of Pharmacology, UT southwestern Medical Center, Dallas, TX, [4]Department of Cellular and Integrative Physiology, Long School of Medicine, UT Health San Antonio, USA. *Co-first authors.

S 74 P6-07 Investigating the cell-Nonautonomous roles of the nuclear hormone receptor NHR-49 in the nervous system of *Caenorhabditis elegans*
Saebom Kwon, Kyoung-hye Yoon
Department of Physiology, Mitohormesis Research Center, Yonsei University Wonju College of Medicine, Wonju, Korea

S 74 P6-08 Equipotent deteriorating effect of angiotensin A to angiotensin II on cardiac I/R injury
Byung Mun Park, Weijian Li, Suhn Hee Kim
Department of Physiology, Research Institute for Endocrine Sciences, Chonbuk National University Medical School, Jeonju, Korea

P7: Epithelium and Exocrine Physiology

Epithelial transport (Kidney, GI, Lung epithelium), Exocrine gland/duct, Skin

S 74 P7-01 CACC and ENaC mediate LPS-induced disruption of epithelial barrier
Minkyoung Kim, Sang Woo Lee, Junchul Kim, Fengjiao Chang, Kyungpyo Park
Department of Physiology, School of Dentistry, Seoul National University, Seoul, Korea

S 74 P7-02 Crif1 deficiency inhibits the invasive growth of keloid fibroblasts via TGF/SMAD signaling pathway
Sungmin Kim[1,2,3,4], Su-jeong Choi[1,2,3], Harsha Nagar[1,2,3], Shuyu Piao[1,2,3], Seonhee Kim[1,2,3], Ikjun Lee[1,2,3], Byeong Hwa Jeon[1,3], Cuk-Seong Kim[1,2,3], Sang-Ha Oh[4]*
[1]Department of Medical Science, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, School of Medicine, Chungnam National University, [4]Department of Plastic and Reconstructive Surgery, Chungnam National University Hospital, Daejeon, Korea

S 75 P7-03 Comparison for molecular mechanisms of different pruritus state in mice
Seongtae Kim, Young-Won Kim, Donghee Lee, Yelim Seo, Hyoweon Bang, Jae-Hong Ko
Department of Physiology[1], Chung-Ang University College of Medicine

P8: Inflammation and Immune Physiology

S 75 P8-01 NecroX-5 shows an anti-inflammation and mitochondrial biogenesis modulation roles to protect hypoxia-reoxygenation injury in rat hearts
Thi Tuyet Anh Nguyen[1], Hyoung Kyu Kim[1], Thi Thu Vu[1,2], Seung Ryul Lee[1], Jubert Marquez[1], Nari Kim[1], Ko Kyung Soo[1], Byoung Doo Rhee[1], Jin Han[1]
[1]National Research Laboratory for Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center, Dept. of Medicine, BK21 Project Team, Dept. of Physiology, Inje Univ., Busan, Korea, [2]VNU University of Science, Hanoi, Vietnam

S 75 P8-02 Endothelial nitric oxide synthase uncoupling in CR6 interacting factor-1 deficiency endothelial cells is related to tetrahydrobiopterin.
Ikjun Lee[1,2,3], Shuyu Piao[1,2,3], Seonhee Kim[1,2,3], Harsha Nagar[1,2,3], Su-Jeong Choi[1,2,3], Sung-min Kim[1,2,3], Saet-byel Jung[1,4], Byeong Hwa Jeon[1,3], Hee-Jung Song[1,5], Cuk-Seong Kim[1,2,3]*
[1]Department of Medical Science, School of Medicine, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, School of Medicine, Chungnam National University, [4]Department of Endocrinology, [5]Department of Neurology, School of Medicine, Chungnam National University Hospital, Daejeon, Korea

S 76 P8-03 Protective effect of anthocyanin-rich extract from red Chinese cabbage on vascular inflammation in hyperlipidemic apolipoprotein E-deficient mice
Hee Kyoung Joo[1], Sunga Choi[1], Yu Ran Lee[1], Eun Ok Lee[1], Myoung Soo Park[2], Byeong Hwa Jeon[1]
[1]Research Institute for Medical Sciences, Department of Physiology, School of Medicine, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Korea

S 76 P8-04 Inhibitory role of APE1/Ref-1 in phosphate-induced vascular smooth muscle cell calcification and phenotype changes
Eun Ok Lee[1], Yu Ran Lee[1], Hee Kyoung Joo[1], Myoung Soo Park[2], Sunga Choi[1], Byeong Hwa Jeon[1]
[1]Research Institute for Medical Sciences, Department of Physiology, School of Medicine, [2]Preclinical Research Center, Chungnam National University Hospital, Daejeon, Korea

S 76 P8-05 The ABCA1/STAT6 pathway is initiated by apoptotic cells and reinforced by activation of PPARγ/LXRα pathway in macrophages
Ye-Ji Lee, Myeong-Joo Kim, Jihee Lee
Department of Physiology, Tissue Injury Defense Research Center, College of Medicine, Ewha Womans University, Seoul, Korea

S 76 P8-06 Development of new indexes to evaluate acute atopic dermatitis-like inflammation
Jeongyoon Choi, Sunghee Moon, Hyemi Bae, Young-Won Kim, Donghee Lee, Seong-Tae Kim, Yelim Seo, Jae-Hong Ko, Inja Lim, Hyoweon Bang
Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

S 77 P8-07 Pro-inflammatory cytokine induces transient receptor potential vanilloid 1 (TRPV1) activation in dermal fibroblasts
Jeongyoon Choi, Sunghee Moon, Hyemi Bae, Young-Won Kim, Donghee Lee, Seong-Tae Kim, Yelim Seo, Jae-Hong Ko, Inja Lim, Hyoweon Bang
Department of Physiology, College of Medicine, Chung-Ang University, Seoul, Korea

P9: Cellular Physiology and Cancer

Cell cycle, Proliferation, Cell death, Stem Cells, Cancer

S 77 P9-01 The effects of actomyosin contractility on acute myeloid leukemia cells
Fengjiao Chang[1], Jin Man Kim[2], Kyungpyo Park[1]
[1]Department of Physiology, School of Dentistry, Seoul National University and Dental Research Institute, Seoul, [2]Department of Dentistry, School of Medicine, CHA University, CHA Bundang Medical Center, Seongnam, Korea

S 77 P9-02 The Relationship between RhoGDI2 and cell migration in CR6- Interacting Factor 1 deficient HUVECs
Harsha Nagar[1,2,3], Su-Jeong Choi[1,2,3], Shuyu Piao[1,2,3], Seonhee Kim[1,2,3], Ikjun Lee[1,3], Sung-min Kim[1,3], Byeong Hwa Jeon[1,3], Hee-Jung Song[1,4], Cuk-Seong Kim[1,2,3]*
[1]Department of Medical Science, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, [4]Department of Neurology, School of Medicine, Chungnam National University, Daejeon, Korea

S 78 P9-03 Study on pathogenesis of atherosclerosis using IDH2: relationship between IDH2 and mitophagy, mtUPR
Su-Jeong Choi[1,2,3], Harsha Nagar[1,2,3], Shuyu Piao[1,2,3], Seonhee Kim[1,2,3], Ikjun Lee[1,3], Sung-min Kim[1,3], Jeen-Woo Park[4], Byeong Hwa Jeon[1,3], Hee-Jung Song[1,5], Cuk-Seong Kim[1,2,3]*
[1]Department of Medical Science, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, School of Medicine, Chungnam National University, Daejeon, [4]Department of Thoracic and Cardiovascular Surgery, School of Life Sciences, College of Natural Science, Kyungbook National University, Taegu, [5]Department of Neurology, School of Medicine, Chungnam National University Hospital, Daejeon, Korea

S 78 P9-04 (PO-B-1) Determining the deubiquitinating enzymes regulating the adipose derived mesenchymal stem cells senescence
Jongbeom Oh[1], Eunah Kim[1], Dong Hyeon Lee[2], Soonchul Lee[1]
[1]Department of Orthopaedic Surgery, CHA Bundang Medical Center, CHA University, [2]Department of Physiology, CHA University School of Medicine

S 78 P9-05 CR6 interacting factor 1 deficiency induced anticancer effects by inducing mitochondrial dysfunction in breast cancer
Shuyu Piao[1,2,3], Harsha Nagar[1,2,3], Seonhee Kim[1,2,3], Su-Jeong Choi[1,2,3], Ikjun Lee[1,3], Sungmin Kim[1,3], Byeong Hwa Jeon[1,3], Hee-Jung Song[1,4], Cuk-Seong Kim[1,2,3]*
[1]Department of Medical Science, School of Medicine, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, School of Medicine, Chungnam National University, [4]Department of Neurology, School of Medicine, Chungnam National University Hospital, Daejeon, Korea

S 79 P9-06 Nitric oxide modulates lipocalin-2 expression via regulation of lipocalin-2 protein stability 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 79 P9-07 (PO-B-2)

EPHB6 mutation induces cell adhesion-mediated paclitaxel resistance via EPHA2 and CDH11 expression
Sarah Yoon[1], Ji-Hye Choi[1], Sung Joo Kim[1,2], Eun-Ju Lee[1], Masaud Shah[3], Sangdun Choi[3], Hyun Goo Woo[1,2]*
[1]Department of Physiology, Ajou University School of Medicine, [2]Department of Biomedical Science, Graduate School, [3]Department of Molecular Science and Technology, Ajou University, Suwon, Korea

S 79 P9-08

CR6-interacting factor 1 deficiency increases premature senescence by impairment of anti-oxidant system in endothelial cells
Seonhee Kim[1,2,3], Shuyu Piao[1,2,3], Harsha Nagar[1,2,3], Su-jeong Choi[1,2,3], Ik jun Lee[1,2,3], Sungmin Kim[1,2,3], Byeong Hwa Jeon[1,3], Hee-Jung Song[1,4], Cuk-seong Kim[1,2,3]*
[1]Department of Medical Science, [2]Department of BK21Plus CNU Integrative Biomedical Education Initiative, [3]Department of Physiology, [4]Department of Neurology, School of Medicine, Chungnam National University, Daejeon, Korea

S 79 P9-09

Discovery of smoking-specific mutations using machine learning in lung cancer
Han-Jun Cho[1], Soonchul Lee[2], Dong Hyeon Lee[1]
[1]Department of Physiology, CHA University School of Medicine, [2]Department of Orthopaedic Surgery, CHA Bundang Medical Center, Seongnam, Korea

S 80 P9-10

Association of specific gene mutations derived from machine learning with survival in lung adenocarcinoma
Han-Jun Cho[1], Soonchul Lee[2], Dong Hyeon Lee[1]
[1]Department of Physiology, CHA University School of Medicine, [2]Department of Orthopaedic Surgery, CHA Bundang Medical Center, Seongnam, Korea

S 80 P9-11 (PO-B-3)

Cardiovascular drug, echinochrome A enhances cardiac differentiation from embryonic stem cell via PKCiota inhibition
Hyoung Kyu Kim[1,2], S. Woo Cho[1,3], H. Jin Heo[1], S. Hun Jeong[1], M. Kim[1], K. Soo Ko[1], B. Doo Rhee[1], N.P. Mishchenko[4], E.A. Vasileva[4], S.A. Fedoreyev[4], V.A. Stonik[4], J. Han[1]
[1]National Research Lab. for Mitochondrial Signaling, Dept of Physiology, Dept of Health Sciences and Technology, BK21 Plus Project Team, Cardiovascular and Metabolic Disease Center, [2]Dept of Integrated Biomedical Science, Inje Univ. College of Medicine, Busan, [3]Division of Cardiology, Dept of Internal Medicine, Inje Univ. College of Medicine, Seoul Paik Hospital, Seoul, Korea, [4]G.B. Elyakov Pacific Inst. of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Science, Vladivostok, Russia

S 80 P9-12

Role of mito-K_{ATP} channel in formation of the de-energized mitochondrial membrane potential
Quynh Mai Ho[1], Jeong Hoon Lee[1], Duong Duc Pham[1], Ki Hwan Hong[1], Sung Jin Kim [1], Yeon Joo Jung[1], Ho Sun Lee[1], Chae Hun Leem[1,2]
[1]Department of Physiology University of Ulsan College of Medicine, [2]ASAN Medical Center, Seoul, Korea

S 81 P9-13 (PO-B-4)

Influence of pharmacological inhibition of AKT by novel inhibitor HS1793 in relapsed multiple myeloma
Amy Kim[1], In-Sung Song[1], Yu Jeong Jeong[2], Seung Hun Jeong[1], Hyoung Kyu Kim[1], Nam-Chul Ha[3], MyungGeun Shin[4], Kyung Soo Ko[1], Byoung Doo Rhee[1], Sungbo Shim[5], Sung-Wuk Jang[2], Jin Han[1]
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S 81 P9-14

A noble finding of miRNAs in neurogenic differentiation of human adipose tissue derived mesenchymal stem cells
Sujeong Jang, Han-Seong Jeong, Sukho Park, Jong-Seong Park, Sah-Hoon Park
Department of Physiology, Chonnam National University Medical School, Jellanamdo, Korea

S 81 P9-15

Differentiation of human bone marrow-derived stem cells into neuron-like cells by histone deacetylase inhibitors
Han-Seong Jeong, Sujeong Jang, Sukho Park, Sah-Hoon Park, Jong-Seong Park
Department of Physiology, Chonnam National University Medical School, Korea

S 82 P9-16

Secretory acetylated APE1/Ref-1 requirement for suppression of tumor growth in triple-negative breast cancer in vivo
Yu Ran Lee[1], Myoung Soo Park[2], Hee Kyoung Joo[1], Eun Ok Lee[1], Jeryong Kim[3], Sunga Choi[1], Byeong Hwa Jeon[1,2]
[1]Research Institute of Medical Sciences, Department of Physiology, School of Medicine, Chungnam National University, [2]Preclinical Research Center, Chungnam National University Hospital, [3]Department of Surgery, School of Medicine, Chungnam National University, Daejeon, Korea

S 82 P9-17

Discovery of common triple basic residues in the middle C-terminal of TREK K^+ channels (KCNK2 and KCNK10) responsible for the activation by low-level PIP_2
Joohan Woo[1], Young Keul Jeon[1], Yin-Hua Zhang[1], Joo Hyun Nam[2], Dong Hoon Shin[3] , Sung Joon Kim[1]
[1]Department of Physiology, Seoul National University College of Medicine, Seoul, [2]Department of Physiology & Ion Channel Disease Research Center, Dongguk University College of Medicine, Gyeongju, [3]Department of Pharmacology, Yonsei University College of Medicine, Seoul, Korea

S 82 P9-18

RhBMP-2 diminished the growth of pancreatic cancer cells via activation of hippo pathway
Yu Chuan Liu, Soo Mi Kim*
Department of Physiology, Institute for Medical Sciences, Chonbuk National University Medical School, Jeon Ju, Korea

S 82 P9-19

HN1 contributes to migration, invasion, and tumorigenesis of colorectal cancer by regulation of autophagy
Yu Chuan Liu, Soo Mi Kim*
Department of Physiology, Institute for Medical Sciences, Chonbuk National University Medical School, Jeon Ju, Korea

S 83 P9-20

SREBP1, targeted by HN1, modulates tumorigenesis of hepatocellular carcinoma
Hua Jin, Soo Mi Kim*
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 83 P9-21 Role of JAK3 in migration and differentiation of nestin-positive progenitor cells in the spinal cord
Soo Yeon Lee[1,2], A-Young Kim[1,2], Soo Hwan Lee[1], Eun Joo Baik[1,2]
[1]Department of Physiology, [2]Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

S 83 P9-22 Sirtuin 6 inhibits liver cancer progression by modulating UPA and MMP9
Cong Shan Li, Soo Mi Kim*
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 84 P9-23 JAK3 as a determinant in migration of GABAergic interneurons
A Young Kim[1,2], Soo Yeon Lee[1,2], Eun Joo Baik[1,2]
[1]Department of Physiology, [2]Chronic Inflammatory Disease Research Center, Ajou University School of Medicine, Suwon, Korea

S 84 P9-24 In vivo treatment with Gas6 inhibits EMT in primary murine alveolar type II cells and lung fibrosis
Ji-Hye Jung, Ye-Ji Lee, So-Jung Park, Tae-Hyun Kim, Jihee Lee
Department of Physiology, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans University, Seoul, Korea

S 84 P9-25 Incidence and management of adverse events associated with panobinostat in gastric cancer cells
Da-Yeah Kim, Soo Mi Kim
Department of Physiology, Institute for Medical Science, Chonbuk National University Medical School, Jeonju, Korea

S 84 P9-26 Anoctamin1 does not function as ion channel in head and neck squamous cell carcinoma due to lack of surface expression
Young Keul Jeon, Joo Han Woo, Ji Hyun Jang, Seong Woo Choi, Hai Yue Lin, Yin Ming Zhe, Sung Joon Kim
Department of Physiology, Seoul National University, College of Medicine

S 85 P9-27 Inactivation of Akt pathway by ursolic acid plus paclitaxel suppressed growth of esophageal cancer cells
Ruo Yu Meng, Soo Mi Kim*
Department of Physiology, Institute for Medical Sciences, Chonbuk National University Medical School, Jeonju, Korea

S 85 P9-28 Expression of TonEBP/NFAT5 is associated with migration in NSCLC Cells
Taehee Kim, Hee ju Song, Sang Do Lee
Department of Physiology, Department of thoracic surgery, Chungnam National University School of Medicine, Daejeon, Korea

S 85 P9-29 Cytokines secreted from macrophage induce cisplatin resistance and migration in A549 cells
Taehee Kim, YHST Wickramasinghe, Sang Do Lee
Department of Physiology, Department of thoracic surgery, Chungnam National University School of Medicine, Daejeon, Korea

S 85 P9-30 The role of TREK1 in cancer cell epithelial-mesenchymal transition
Yangmi Kim
Department of Physiology, College of Medicine, Chungbuk National University, Cheongju, Korea

S 86 P9-31 Molecular target of nobiletin in rotenone-induced mitochondrial dysfunction and apoptosis
Khulan Amarsanaa, Ji Hyung Lee, Sung-Cherl Jung, Su-Yong Eun
Department of Physiology, Jeju National University School of Medicine, Jeju, Korea

S 86 P9-32 (PO-B-5) Impairment of NHE6 recruitment to synaptic vesicle by SCAMP5 deficiency decreases quantal size at glutamatergic synapses
Unghwi Lee[1], Daehun Park[1], Soohyun Kim[1], Sunghoe Chang[1,2,3,*]
[1]Department of Physiology and Biomedical Sciences, [2]Neuroscience Research Institute, Medical Research Center, [3]Biomembrane Plasticity Research Center, Seoul National University College of Medicine, Seoul, Korea

S 86 P9-33 TASK-5 two-pore domain K^+ channel controls sensitivity to H_2O_2 in MCF-7 and MDA-MB-231 breast cancer cells
Eui-Jung Shin[1], Xiaoming Liu[1], Ji Hyeon Ryu[1], Jae-Young Nam[2], Adrian S. Siregar[1], Marie Merci Nyiramana[1], Eun-Jin Kim[1], Dong Keun Lee[1], Seong-Geun Hong[1], Jaehee Han[1], Dawon Kang[1,2]
[1]Departments of Physiology, [2]Departments of Medicine, Institute of Health Sciences and College of Medicine, Gyeongsang National University, Jinju, Korea

S 86 P9-34 Inhibition of autophagy promoted Rk1 induced apoptosis of neuroblastoma through the inhibition of autophagosome-lysosome fusion
Jung Mi Oh, Sungkun Chun
Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea

S 87 P9-35 Downregulation of survivin inhibits epithelial mesenchymal transition (EMT) and promotes RK1-induced apoptosis in neuroblastoma cells
Jung-Mi Oh, Seo-Hyun Yu, Sungkun Chun
Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea

S 87 P9-36 Survivin knockdown increased anti-cancer effect of CK in human malignant neuroblastoma cells
Jung-Mi Oh, Seo-Hyun Yu, Jangrez Khan, Rabia Bibi, Sungkun Chun
Department of Physiology, Chonbuk National University Medical School, Jeonju, Korea

P10: Exercise and Integrative Physiology

Exercise, Environment, Traditional/Alternative medicine

S 87 P10-01 Administration of Banhasasim-tang (BHSST) ameliorates irritable bowel syndrome-like symptoms through TRPA1 or NaV 1.7 channels in a zymosan-induced mouse model
Byung Joo Kim[1], Min Ji Kwon[1], Sung-Young Kim[2], Joo Hyun Nam[3]
[1]Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, [2]Daewoong CO. LTD, [3]Department of Physiology, Dongguk University College of Medicine

S 87 P10-02 Linalyl acetate mitigates the pulmonary endothelial dysfunction in a rat model of COPD with hypertension
You Kyoung Shin, Yu Shan Hsieh, Soonho Kwon, A Young Han, Geun Hee Seol*
Department of Basic Nursing Science, School of Nursing, Korea University, Seoul, Korea

S 88 P10-03 Lancemaside A from *Codonopsis lanceolata* prevents hypertension by inhibiting NADPH oxidase 2-mediated oxidative stress in hypertensive rats
You Kyoung Shin, A Young Han, Yu Shan Hsieh, Soonho Kwon, Geun Hee Seol*
Department of Basic Nursing Science, School of Nursing, Korea University, Seoul, Korea

S 88 P10-04 Exercise training attenuates long-term high-fat diet-induced impairment of mitochondrial structure and function in mice skeletal muscle.
Jun-Won Heo[1,2], Su-Sie Yoo[1,2], Mi-Hyun No[1,2], Dong-Ho Park[1,2], Ju-Hee Kang[2,3], Dae-Yun Seo[4], Jin Han[4], Tae-Woon Kim[5], Hyo-Bum Kwak[1,2,*]
[1]Department of Kinesiology, [2]WCSL, [3]Department of Pharmacology and Medicinal Toxicology Research Center, Inha University, Busan, [4]National Research Laboratory for Mitochondrial Signaling, Department of Physiology, Cardiovascular and Metabolic Disease Center, Inje University, [5]Department of Physiology, Kyung Hee University, Seoul, Korea

S 88 P10-05 Seasonal effect on resting energy expenditure is age and percent body fat dependent
Duong Duc Pham, Jeong Hoon Lee, Ki Hwan Hong, Youn Joo Jung, Sung Jin Kim, Ho Sun Lee, Chae Hun Leem
Department of Physiology, Ulsan College of Medicine, Seoul, Korea

S 89 P10-06 Aerobic exercise training decreases cereblon and increases AMPK signaling in the skeletal muscle of STZ-induced diabetic rats
Jeong Rim Ko, Dae Yun Seo, Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, Department of Health Sciences and Technology, BK21 Plus Project Team, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

S 89 P10-07 Resistance exercise improves mitochondrial function to rescue OLETF rats hearts
Joon Yong Noh[1], Tae Hee Ko[1], Seung Hun Jeong[1], Hyoung Kyu Kim[1], Jubert C. Marquez[1], SungRyul Lee[1], Jae Boum Youm[1], Dae Yun Seo[1], Byoung Doo Rhee[2], Kyung Soo Ko[2], Nari Kim[1], Jin Han[1]*
[1]Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, [2]Department of Internal Medicine, Sanggye Paik Hospital, Cardiovascular and Metabolic Disease Center, College of Medicine, Inje University, Seoul, Korea

S 89 P10-08 Nano-LC-ESI-MS/MS reveals circadian modulation of the cardiac proteome underpins differential adaptation to morning or evening exercise training
Pham Trong Kha[1]*, Dae Yun Seo[1], Louise Anne Dizon[1], Sung Ryul Lee[1], Hyo-Bum Kwak[2], Jae Boum Youm[1], Won Suk Yang[3], Tae Hee Ko[1], Robin A McGregor[1], Jin Han[1]
[1]National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Busan, [2]Department of Kinesiology, Inha University, Incheon, [3]Medicinal Bioconvergence Research Center, College of Pharmacy, Seoul National University, Seoul, Korea

S 89 P10-09 Plasma catecholamine and physical activity levels in Korean elderly people with orthostatic hypotension
Nahyun Kim[1], Jooyeon Park[1], In Deok Kong[2]
[1]College of Nursing, Keimyung University, Daegu, [2]Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, Korea

S 90 P10-10 Effect of exercise training on vascular reactivity in high fat diet induced hypertensive rats
Rany Vorn[1,2], Hae Young Yoo[1]
[1]Chung-Ang University Red Cross College of Nursing, Seoul, [2]Chung-Ang University Graduate School, Seoul, Korea

P11: Physiomes and Systems Biology

Computational Modeling, Bioinformatics

S 90 P11-01 Deep neural network-based classifiers to detect experimental seizures
Hyun-Jong Jang[1,2]
[1]Department of Physiology, College of Medicine, [2]Catholic Neuroscience Institute, The Catholic University of Korea, Seoul, Korea

S 90 P11-02 Teaching cardiac excitation-contraction coupling using a mathematical computer simulation of human ventricular myocyte
Young-Keul Jeon[1], Jae Boum Youn[2], Chae Hun Leem[3], Sung Joon Kim[1,4]
[1]Department of Physiology, [4]Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, [2]Cardiovascular and Metabolic Disease Center, Department of Physiology, College of Medicine, Inje University, [3]Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea

P12: Others

Drugs, Phytochemicals, Miscellaneous

S 91 P12-01 Development of multi-functional theragnostic magneto-ceria nanoparticles for cancer radiotherapy
Sang-woo Lee[1], Sang-Ihn Han[2], Taegwan Hyun[2], Kyungpyo Park[1]
[1]Department of Physiology, School of Dentistry, [2]Institute of Basic Science, Seoul National University, Seoul, Korea

S 91 P12-02 Corylifol C inhibits osteoclastogenesis by inhibition of ROS and induces downregulation of c-Src in osteoclast
Jung Yun Kang[1], Dong Min Shin
[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, Korea

S 91 P12-03 Sestrin 2 regulates osteoclast differentiation through interaction with p62 and TRAF6
Namju Kang[1], Sue Young Oh, Dong Min Shin
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S 92 P12-04 Repurposed drugs for angiogenesis inhibitors using database analysis system
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S 92 P12-05 FAK-integrin mediated cell migration by far-infrared in rat
Yelim Seo[1], Donghee Lee[1], Young-Won Kim[1], Seongtae Kim[1], Hyemi Bae[1], Jeongyoon Choi[1], Inja Lim[1], Hyoweon Bang[1], Jae-Hong Ko[1], Jung-Ha Kim[2]
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S 92 P12-06 Stimulation of platelet-derived growth factor mediated cell migration by far-infrared radiation in rat
Donghee Lee[1], Yelim Seo[1], Young-Won Kim[1], Seongtae Kim[1], Hyemi Bae[1], Jeongyoon Choi[1], Inja Lim[1], Hyoweon Bang[1], Jae-Hong Ko[1], Jung-Ha Kim[2]
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S 92 P12-07 Physiological role of the murine bitter taste receptor *Tas2r108*
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S 93 P12-08 *Flos Magnoliae* and its chemical constituent linoleic acid suppress CD4+ T lymphocyte activation *via* store-operated calcium entry
Yu Ran Nam[1,2], Hyun Jong Kim[1,2], Joo Hyun Nam[2], Woo Kyung Kim[2,3]
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S 93 P12-09 *Flos Magnoliae* modulates chloride secretion *via* ANO1 inhibition in airway epithelial cells
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S 93 P12-10 Cardioprotection in ischemic/reperfusion heart injury conferred by natural pyridine nucleoside NPS A
Jubert Marquez[2,3#], Seung Hun Jeong[1,2,3#], Min Kim[1,2#], Tae Hee Ko[2,3], Hyoung Kyu Kim[1,2,3], Yeon Hee Noh[2,3], Dong Hyun Kim[4], Larisa K. Shubina[5], Tatyana N. Makarieva[5], Dmitry V. Yashunsky[6], Alexey G. Gerbst [6], Nikolay E. Nifantiev[6], Valentin A. Stonik[5], Jin Han[1,2,3*]
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S 94 P12-11 Modulation of *FNDC5* transcription by glucocorticoid receptor
Jessa Flores[1], Hyoung Kyu Kim[1,2], Yu Jeong Jeong[1], In-Sung Song[1,3], Yeon Hee Noh[1], Kyo Won Seo[1], Min Kim[1], Jin Han[1]
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S 94 P12-12 Mitochondrial pyruvate dehydrogenase phosphatase 1 regulates the early differentiation of cardiomyocytes from mouse embryonic stem cells
Hyeonju Jo, Hyoung Kyu Kim, Jae boum Youm, Sung Woo Cho, In-Sung Song, Sun Young Lee, Tae Hee Ko, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
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S 94 P12-13 Osteoporosis prevention effect of *Parthenocissus tricuspidata* extract
Yea-Jin Lee[1], Su Ji Lee[2] , Man Seok Bang[1], Hee won Jung[2], Sang Cheol Lee[2], Jang In Shin[3], Chung-Hun Oh[3]
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S 94 P12-14

The effect on osteogenesis of *paeonia lactiflora* extract in MC3T3-E1 cells

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S 95 P12-15

Effect on osteoclast and osteoblast differentiation from *peanut sprout* extract

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S 95 P12-16

The acute hypotension differently affects neuronal activities in medial vestibular nucleus of rats with time according to various types of their cells

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W-1-1

Novel methods for clearing and labeling the tissues for 3D high-resolution microscopy

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Recent development of the number of different tissue clearing and labeling methods facilitates the three-dimensional imaging of large tissues. Labeling thick tissues with antibody typically relies on slow diffusional process, thus hampering efficient and rapid penetration of macromolecules into deep tissues for labeling. Recent methods that applied centrifugal pressure or stochastic electrotransport have addressed this problem, but they are still laborious and require specialized equipment. Here, we present a novel method for rapid and efficient penetration of antibody into deep biological tissues. This method effectively disperses antibody molecules into a cleared sample, and we could stain large sample efficiently and rapidly (up to 1 mm deep sample within 4 h) with a limited amount of antibody. We successfully applied this method to formalin-fixed postmortem human brain tissues and could stain proteins deep down to 500 μm. We also developed a novel clearing methods to minimize deformation artifacts that are due to the harsh treatment and transient sample swelling during CUBIC or CLARITY process. Our method is optimized to achieve minimal change in sample size and no loss of cellular materials for high-resolution fluorescence imaging. A combination of this method and super-resolution microscopy is now in progress.

Key Words: Tissueclearing, Antibodystaining, Brain, Super-resolutionmicroscopy

W-1-2

Electrophysiological approach and methods for research in cardiac arrhythmias

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The mechanism of cardiac arrhythmias is very complex. Cardiac arrhythmias may arise from abnormalities in impulse formation, impulse conduction, or a combination of both. Thus, various electrophysiological methods have been applied for clinical laboratory as well as basic experiments. Electrocardiography (ECG) is most commonly used diagnostic tool which document the amplified electrical signal from heart using multiple leads. Because it is difficult to record ambulatory ECG in animal, telemetry monitoring is used using implantable loop recorder. To get data about arrhythmogenecity, presence of inducibilty should be tested. Invasive electrophysiologic study (EP study) is somewhat technically difficult in rodent experiments although it is popularly used in clinical electrophysiology. When programmed electrical stimulation is delivered at the right atrium ~ His bundle ~ right ventricle via jugular venous approach using octapolar catheter (8Fr), inducibilty which reflect arrhythmogenecity can be tested. High-rate pacing in atrium or atrial appendage causes heart failure, which can also create atrial fibrillation (AF) animal model. 3-dimensional mapping system is used to investigate the electrophysiological mechanism of tachyarrhythmias in large animal, which also give us anatomical information. Optical mapping is a technique to assess the conduction property within the heart or tissue. And, calcium transient which may suggest intracellular calcium dynamics, which plays an important role in excitation-contraction coupling in myocyte, can be measured. Most cardiac arrhythmias may caused by dysregulation of ion channels (especially in channelopathies that predispose to sudden cardiac death), leading to abnormality of depolarization and repolarization in myocyte. Wholecell patch-clamp technique is a mainstay in studying the mechanisms and evaluating the arrhythmogenecity for cardiac arrhythmia. Recently, feasibiliy and usefulness of super-resolution microscopy has been demonstrated in research in cardiac ion channels, which can be performed conjunction with patch-clamp technique. These technical advances in cardiac electrophysiology will make a great contribution to pave the march towards precision medicine in cardiovascular disorders

Key Words: Arrhythmia, Electrophysiology, Electrocardiography, Ion channel

W-1-3

Nikon microscopes for life science

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Optical microscopes have been frequently used in life science research. Nikon has been offering several solutions with the products of Multi-Photon, Confocal microscope, SIM-super resolution and STORM super resolution microscopes. In this session, such products are to be introduced and the applications are to be discussed.

W-2-1

High throughput screening for drug discovery of protease-activated receptor 2 modulators

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Protease-activated receptor 2 (PAR2) is a G-protein-coupled receptor (GPCR) expressed in various cell types and tissues, and activated by serine proteases such as trypsin and tryptase. PAR2 has been shown to shown to play important roles in numerous physiological events including inflammation, pain, cell migration and cell proliferation. PAR2 is a candidate gene for inflammatory diseases, pain and cancer. For the identification of novel small-molecule antagonists of PAR2, we established a robust cell-based high throughput screening (HTS) assay. A halide sensitive mutant YFP was stably transfected with HT29 cells expressing PAR2 and calcium-activated chloride channels (CaCCs). Activation of PAR2 increases intracellular calcium levels and activates CaCCs having iodide permeability. We measured the PAR2 activity by monitoring changes in intensity of the iodide sensitive YFP fluorescence. Briefly, HT29-PAR2-YFP cells were plated in 96 well plates and after confluence was achieved, the cells were washed with PBS and incubated with compounds for 10 minutes. To measure the effect of the compounds on PAR2, an extracellular solution containing 70 mM iodide was applied with trypsin and then YFP fluorescence profiles were analyzed. Screening of ~60,000 small molecules and ~2,000 natural products revealed several novel potent PAR2 antagonists. This work provides a robust novel HTS assay for screening PAR2 modulators and this assay system can be used with other GPCRs.

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Key Words: HTS, PAR2, GPCR, Antagonist, Modulator

W-2-2

Considerations in the preclinical studies for drug development

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Developing a drug from the discovery phase to its successful drug approval costs an average of $3billion dollars and 10-15 years. Successful rate of Phase I to drug approval is about 10%. Therefore, performing *in vitro* and *in vivo* toxicity and efficacy studies is crucial in order to select the high potential drugs in preclinical phases. Safety of a novel therapeutic agent must be rigorously analyzed and proven to prevent adverse effect in clinical phase. Thus, safety studies under GLP conditions and following regulatory guidelines must be performed to advance towards IND approval.Selections of animal species and dose levels are key considerations in safety studies. Nonhuman primates are often the only relevant species that can be used to assess the safety of a biotherapeutics. Biomarkers of toxicity are key considerations. A clear and efficient path to regulatory acceptance is needed so that breakthroughs in the biomarker toolkit for preclinical drug safety assessment can be utilized to aid in the drug development process

Key Words: Preclinical, Toxicity, IND, GLP

W-2-3

Chondroitin sulfate-based nanoparticles for tumor penetration

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Adequate access of anti-cancer drugs to tumor lesion is crucial for effective chemotherapy. Unfavorable tumor microenvironments hamper the homogeneous distribution of the drug molecules across the entire tumor tissue particularly in the hypoxic region distant from functioning blood vessels. In this work, it was hypothesized that the tumor-targetable chondroitin sulfate (CS)-based nanoparticle (NP) system with hypoxic region-targeting moiety added may achieve enhanced tumor targeting and penetration of anticancer agents. As a proof-of-concept, self-assembled NPs that consist of phenylboronic acid (PBA)-modified amphiphilic CS derivatives were designed, and their improved anti-cancer efficacy was demonstrated. Briefly, deoxycholic acid (DOCA) was conjugated to the CS backbone via ethylenediamine linker, followed by the introduction of (3-aminomethylphenyl)boronic acid (AMPB) to CS-DOCA. The successful synthesis of the graft copolymers was verified using proton nuclear magnetic resonance spectroscopy. The amphiphilic CS-DOCA and CS-DOCA-AMPB conjugates were loaded with doxorubicin (DOX), resulting in self-assembled NPs with an average diameter of ≈200 nm, narrow size distribution, negative zeta potential, and spherical morphology. With the relatively high drug entrapment efficiency of around 80%, the developed NPs exhibited an increased DOX release at acidic pH (pHs 5.5 and 6.8) compared to at pH 7.4. Further experiments using confocal laser scanning microscopy and flow cytometry indicated that the developed NPs have an enhanced cellular uptake and penetration into spheroids, and enhanced cytotoxic effects, likely via the CS-CD44 and PBA-sialic acid interactions. Using near-infrared fluorescence imaging technology, improved tumor targeting and drug penetration of the developed NPs were observed in mouse xenograft models, which potentially leads to improved anti-tumor efficacy and reduced systemic toxicity of DOX. In summary, the overall results proved the improved tumor targeting and penetration of CS-DOCA-AMPB NPs, suggesting their potential application to the treatment of various solid cancers.

Key Words: Chondroitin sulfate, Phenylboronic acid, Tumor penetration, Tumor targeting

W-2-4

New drug development of SP-8203(otaprimastat) in ischemic stroke

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SP-8203 is a new neuroprotective compound under development by Shin Poong Pharm. Co., Ltd. in the treatment of acute ischemic stroke.
In cerebral ischemia, neurons and glia are damaged by various mechanisms including excitotoxicity, oxidative stress, and inflammatory responses. Thus, simultaneous blockade of multiple cytotoxic pathways would be a therapeutic strategy for the treatment of ischemic injury.
Promising pharmacological approaches for treating acute ischemic stroke have included thrombolytic therapy and neuroprotective agents to prevent or reduce the intensity of ischemic cascade leading to a loss of viable brain tissue. The only thrombolytic agent is recombinant tissue plasminogen activator (rtPA) which is currently approved for the treatment for patients with acute ischemic stroke. Therapeutic benefit has been shown in a select group of patients where rtPA is administered within 4.5 hours of stroke onset, with earlier treatment associated with bigger proportional benefits. However as rt-PA is also associated with the potential of intracranial hemorrhage which consequently limits its use. Recent studies suggest increased risk of fatal hemorrhage during the first few days of administration of rtPA irrespective of treatment delay, age or stroke severity of patients
The central goal of acute stroke treatment is to improve neurological outcomes at 90 days. This demands both protection of the brain neurons and glial cells, and preservation of microvascular structure and function, given that the size and growth of the ischemic area is dependent on the extent of compromised collateral flow. Furthermore inhibition of brain edema and preservation of blood-brain barrier integrity are critical to protect brain tissue that lay outside the original ischemic areas.
SP-8203 is a quinazoline-2,4-dione derivative with multiple potent neuroprotective mechanisms of action including anti-inflammatory activities and antioxidant characterized. Taken together, our data indicate that SP-8203 is an innovative multi-target-directed neuroprotective agent which can be used in conjunction with rtPA. The combined use of SP-8203 with rtPA protects ischemia- and rtPA-evoked brain damage simultaneously. Therefore, SP-8203 has high potential to be a 'First-in-Class' multi-target directed neuroprotective agent for ischemic stroke which satisfies current unmet needs.

Key Words: Ischemic stroke, SP-8203, rt-PA, Neuroprotective agent

SM-1

EKG training using three-dimensional heart model

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EKG는 심장의 전기생리학적 변화를 비침습적으로 볼 수 있는 유용한 장치이다. 그러나 EKG의 총12 channel에서 나오는 복잡한 신호들로부터 심장의 전기적 패턴 및 이에 따른 역학적 변화를 유추할 수 있기 위해서는 오랜 기간의 교육과 실습이 필요하다. 이러한 EKG training에 있어, 가상의 컴퓨터 시뮬레이션을 활용하고자 하는 시도들이 있어 왔다. 현재, 컴퓨터의 고성능화 및 심장의 3차원 모델 기술의 발전에 따라 실제 심장생리의 변화와 EKG양상을 시뮬레이션을 통하여 상당히 정교하게 재현할 수 있게 되었다. 본 강연에서는, 심장세포들의 흥분-수축과 이에 따른 심장전기변화가 EKG양상을 어떻게 변화시킬 수 있는지를 기전적으로 설명할 수 있는 심장 3차원 컴퓨터 시뮬레이션 모델을 소개한다. 또한 심장의 병리적 현상 시, EKG 변화를 컴퓨터 시뮬레이션 모델로서 직접 재현함으로써 피교육자의EKG 해석 능력을 증대시키기 위한 training도구로서 유용함을 실증한다.

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SM-2

Simulation-based general electrophysiology

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전기생리학은 인체 생체전기의 발생과 이를 활용한 조직의 생리를 이해하는 데 필요한 필수적인 생리교육내용이다. 이러한 이유로 전기생리학은 생리학 교육을 받는 학생들이 초기 과정에 접하게 된다. 전기생리학은 그 기반에 전기학, 물리화학, 일반화학 및 수학 등 기초분야의 학문지식이 필요하나 최근 의학과정에서 예과과정의 축소등으로 말미암아 이러한 기초분야의 지식체계가 제대로 갖춰지지 않은 상태에서 전기생리학을 접하게 됨으로써 실제 학생들을 지도/교육하는 데 많은 어려움이 있게 되었다. 또한 전기생리학의 교육 내용의 상당 부분이 이론적 배경으로 지도하게 되어 실제 생리적 현상에 대해 통합적으로 깊이 있는 이해를 도모하기가 쉽지 않다. 시뮬레이션은 실제 학생들이 신경세포를 대상으로 여러가지 상황을 적용하여 시험해 볼 수 있는 수단을 제공할 수 있다. 이를 활용하여 신경세포의 전기현상에 대한 기초이론부터 신호전달의 수단인 활동전압의 발생에 이르기까지 세포의 내외환경, 세포막의 전기적 특성, 이온농도 및 통로의 동역학등이 어떻게 관여하는지 시뮬레이션을 적용시켜 기존 이론적 강의수단으로부터 충분히 제공할 수 없었던 통합적 이해를 학생들이 도모할 수 있게 되었다. 본 시뮬레이션은 세포내외 이온농도에 의한 막전압형성, 온도변화에 의한 활동전압의 변화, 역치의 형성원리, 활동전압 발생 시 관여하는 이온통로들의 전도도 변화, 이온농도 변화에 의한 활동전압의 변화, Na^+통로의 비활성화에 의한 불응기 및 순응현상 등 신경세포 전기를 배우면서 획득해야 하는 인체의 생리적 반응에 대한 기전적 이해를 시뮬레이션을 통해 제공할 수 있다. 또한 신경세포의 활동전압 전도과정을 보여줌으로써 활동전압전도속도에 영향을 미치는 여러 인자들에 대한 기전적 이해를 도모할 수 있다. 마지막으로 세포내외 이온환경이 세포내 단백질의 존재에 의해 어떻게 조성되는지 이러한 조성이 가지는 문제와 해결 방법을 세포는 어떻게 확보하게 되었는지 이론과 시뮬레이션을 통한 실습을 통해 일반전기생리를 충분히 이해할 수 있는 기반을 제공할 수 있을 것으로 기대한다.

SM-3

Integrative understanding of human circulatory functions in students practicum of physiology using quantitative circulation physiology program (QCP-2005)

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QCP-2005는 생리학자들에게 잘 알려진 교과서의 저자이자 순환생리학의 선구자인 Arther Guyton이 재직했던 The University of Mississippi에서 만들어낸 인체생리의 통합적 전산모델이다 (Abram et al., 2007). 4000개 이상의 다양한 생리학적 변수들의 상호작용을 수학적 모델로 구현한 QCP-2005는 학부교육과 실습에 유용하다 (Rodríguez-Barbero et al., 2008). 서울대학교 의과대학 생리학교실은 의학과 1학년 학생들을 대상으로 하는 '인체생리와 조직학' 강좌의 실습에 해당 프로그램을 활용하고 있다. 2017년에 처음 도입 당시에는 1회 3시간 실습에, 2018년에는 2회 6시간의 실습에 적용하였다. 자세변동 및 운동 등에 일어나는 혈압조절반사를 통합적으로 이해하는데 유용할 뿐만 아니라, 실혈, 장-단기 수분섭취 및 식이 변화, 온도와 기압의 변화에 따른 순환기, 호흡기, 신장과 내분비 기능 변화를 함께 관찰하는 것이 가능하였다. 학생들 스스로 새로운 질환조건 및 생리적 극한 상황에 대한 반응을 구성하면서 생리적 반응 결과를 해석하는 과정에서 적극적 참여와 함께 좋은 피드백을 얻을 수 있었다. 실습 뿐만 아니라 강의 수업 중에 실시간 활용에도 유용할 것이며, 앞으로 많은 활용과 확산을 기대한다.

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SM-4

Simulation-based lecture on muscle physiology using EDISON software

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Muscle physiology covers a wide scope of aspects including synaptic transmission in neuro-muscular junction, excitation-contraction coupling, cross-bridge cycle, and energy metabolism. Emerging mathematical models of muscle contraction motivated me to begin simulation-based lectures on muscle physiology. 3-types of muscle were selected to develop the EDISON (EDucation-research -industry Integration through Simulation On the Net) software: Cardiac muscle, skeletal muscle, and smooth muscle. Specifically, mathematical model of human ventricular myocytes (Himeno et al., 2015), mouse soleus and extensor digitorum longus (Shorten et al., 2007), and rat cerebrovascular arteries (Yang et al., 2003) were employed. Effects of ion channel modulation and changes in ion concentrations on cardiac action potential and contractility are well reproduced in the EDISON software for cardiac muscle. Action potential abnormalities including delayed after depolarization (DAD) and EAD (early after depolarization) by pathologic changes in ion channels are also successfully reproduced. Development of summation and tetanus from single twitch by increased rate of stimulation is reproduced in the EDISON software for skeletal and smooth muscle. In the EDISON software of smooth muscle, role of myosin light chain kinase and phosphatase could be examined. Users can also simulate the latch state by varying rate constants in the multi-state kinetic model of Ca-Calmodulin dependent myosin phosphorylation. In the EDISON software of skeletal muscle, users can simulate fatigue effect. In conclusion, users could verify various aspects of muscle physiology and test the effects of aberrant conditions on the muscle action potential and contractility by using the EDISON

software.

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Key Words: Muscle physiology, Simulation, Mathematical model

YS-1

Brain mechanisms of neuropathic pain and interaction between chronic pain, depression and empathic distress

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Chronic pain often accompanies negative mood symptoms such as depression and anxiety, and increases emotional contagion and empathic distress. The interaction between negative affections implies the existence of common or interacting neural pathways. However, the neural circuits relevant to the interactions and underlying mechanisms are not fully understood. I studied the mechanisms of chronic pain and ensuing negative affections in an animal model of neuropathic pain. The various alterations in neural circuits of pain animals were investigated using positron emission tomography (PET), electrophysiological recording, pharmacological and genetic manipulation and animal behavior analysis. Metabotropic glutamate receptor 5 was a particular target, as this molecule plays a pivotal role in the plastic changes in neural circuits. Using the techniques, brain-level mechanisms of the amplified aversion were investigated and therapeutic and diagnostic methods were explored.

YS-2

Tissue-to-tissue communication of mitochondrial function in the model organism C. elegans

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The spectrum of pathophysiological conditions that result from mitochondrial dysfunction reveals the many essential roles it plays in the cell. While most conditions can be attributed to impaired mitochondrial dysfunction within the affected cell or tissue, sometimes the effects of mitochondrial dysfunction in one tissue is seen in another. Examples of tissue-to-tissue communication of mitochondrial function is also seen in worms and flies, revealing an evolutionarily conserved mechanism of communicating and coordinating mitochondrial function among different tissues to regulate whole-body metabolism.

To study this phenomenon in further detail, we are studying the neuron-specific role of a ubiquitously expressed nuclear hormone receptor in C. elegans. NHR-49 is one of the 284 nuclear hormone receptors expressed in C. elegans and an important regulator of lipid metabolism. Deletion of *nhr-49* results in impaired fasting response, shortened lifespan, as well as mitochondrial fragmentation. However previous reports showed that restoring NHR-49 in the neurons is sufficient to prolong lifespan and restore mitochondrial morphology in distant tissues. To find out how this communication occurs, we are currently employing genetics studies to elucidate the neuronal circuitry and neurotransmitters involved.

YS-3

SHANKs deficiency impairs hyperalgesia in neuropathic and inflammatory pain models

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Abnormal pain sensitivity is commonly associated with autism spectrum disorders (ASDs) and affects the life quality of ASD individual. Deficiency of postsynaptic density molecules such as *SHANKs* (SH3 and multiple ankyrin repeat domains proteins) and *LRRTMs* (leucine-rich repeat transmembrane

neuronal proteins) was implicated in ASD. Here we report that *Shank3* knockout results in impaired heat hyperalgesia in inflammatory and neuropathic pain models. SHANK3 interacts with transient receptor potential subtype V1 (TRPV1) via Proline-rich region and regulates TRPV1 surface expression. Furthermore, partial knockdown of SHANK3 expression in human DRG neurons abrogates TRPV1 function. Our findings reveal a peripheral and presynaptic mechanism of *SHANK3*, which may underlie pain deficits in SHANK3-related ASDs.

Key Words: Heat hyperalgesia, SHANK3, TRPV1, Autism spectrum disorders (ASDs)

Plenary Lecture

Aging and chronic kidney disease: phosphate connection

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In 1997, an obscure mouse mutant was reported that displayed myriad of aging-like symptoms including shortened life span, frailty, vascular calcification, aging lung, osteopenia, cardiac hypertrophy, cognition impairment, and atrophy of multiple organs (gonads, skin, fat, muscle, thymus, etc.). The gene defective in the mutant was named after a Greek goddess Klotho who spins the thread of life. The klotho gene encoded a single-pass transmembrane protein of unknown function and was expressed predominantly in renal tubular cells. In 2006, Klotho protein was identified as the obligate co-receptor for fibroblast growth factor-23 (FGF23). FGF23 is a peptide hormone secreted from the bone upon phosphate intake and acts on Klotho complexed with FGF receptors expressed on renal tubular cells to increase urinary phosphate excretion. Thus, the fundamental pathophysiology of the klotho mouse is phosphate retention due to impaired urinary phosphate excretion. In fact, restoration of phosphate balance by placing klotho mice on low phosphate diet ameliorated most of their aging-like symptoms, leading us to a notion that phosphate accelerates aging. In humans, phosphate retention and aging-like symptoms are universally observed in patients with advanced chronic kidney disease (CKD). A striking resemblance between CKD patients and klotho mice has rendered CKD a clinical model of premature aging. In this lecture, the mechanism by which phosphate accelerates aging and novel strategy for the treatment of CKD will be discussed.

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Key Words: Klotho, FGF23, Phosphate, Chronic kidney disease (CKD)

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1985	M.D. (University of Tokyo)
1991	Ph.D. (University of Tokyo)

Positions and Employment

1985-86	Intern, Tokyo University Hospital, Tokyo, Japan.
1986-87	Intern, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.
1987-88	Resident in Internal Medicine (Cardiology), Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.
1988-91	Clinical Fellow, the 3rd Department of Internal Medicine, University of Tokyo.
1991-94	Post-doctoral Fellow, Division of Molecular Genetics, National Institute of Neuroscience, NCNP, Tokyo, Japan.
1994-98	Domestic Research Fellow, Division of Molecular Genetics, National Institute of Neuroscience, NCNP, Tokyo, Japan.
1998-06	Assistant Professor, Department of Pathology, University of Texas Southwestern Medical Center (UT Southwestern).
2006-12	Associate Professor, Department of Pathology, UT Southwestern.
2008-	Member, Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, UT Southwestern.
2012-13	Professor, Department of Pathology, UT Southwestern.
2013-	Professor, Center for Molecular Medicine, Division of Anti-aging Medicine, Jichi Medical University. Adjunct Professor, Department of Pathology, UT Southwestern Medical Center.
2014-	Vice Director, Center for Molecular Medicine, Jichi Medical University.
2018-	Volunteer Faculty, Department of Internal Medicine, UT Southwestern Medical Center.

Honors

1992	Young Investigator's Award, Japanese Circulation Society.
1997	Irvine H. Page Arteriosclerosis Research Awards for Young Investigators (Finalist), American Heart Association
1998	Southwestern Medical Foundation Scholar in Biomedical Research
1998	Erwin von Bälz Preis (Boelinger Ingerheim)
1999	President's Research Council Distinguished Young Researcher Award at UT Southwestern
1999	Pew Scholars Program in the Biomedical Science
2000	Ornish Award in Alzheimer's Disease Research at UT Southwestern
2008	Jack W. Coburn Endowed Lectureship, American Society of Nephrology
2008	Kern and Marnie Wildenthal President's Research Council Professorship in Medical Science
2012	The Frederic C. Bartter Professorship in Vitamin D Research
2017	Investigator's Award, The Kidney Foundation, Japan

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S-1-1

Cortical circuits for the multi-sensory integration: role of inhibition

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Sensory perception in the real world requires proper integration of different modality inputs. The process of multisensory integration is not uniform. It varies from individual to individual and changes at different behavioral states of the animal. What factors affect the multisensory integration? Here I present our recent findings on neural circuit mechanisms for audiovisual integration in the cortex. We found that the posterior parietal cortex (PPC) receives converging inputs from the primary visual and auditory cortices and plays a critical role in audio-visual integration in mice. In resolving conflicts between audition and vision, parvalbumin-positive inhibitory neurons in the PPC mediate auditory dominance over visual perception in mice. We further found that locomotion modulates this circuit and in turn modifies the multisensory perceptual behaviors in mice. Our results demonstrate inhibition in the higher association cortex is important for active integration of audition and vision in mammals and leads to unique and subjective experience of perception.

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Key Words: Multisensory integration, Perception, Audition, Vision, Posterior parietal cortex

S-1-2

Cellular and molecular mechanisms of epilepsy in focal brain malformations

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Focal malformation of cortical development (FMCD) is a neurodevelopmental disorder, frequently associated with intractable seizures. Postzygotic somatic mutations in the components of PI3K-AKT-mTOR pathway have been found in brains with FMCD. Previous studies have shown that ectopic overexpression of AKT3 or MTOR genes with FMCD mutations in developing mouse brain caused seizures. However, the cellular and molecular mechanisms underlying how neurons with FMCD mutations play roles in the propagation of hyperactivity to other regions of brain remain obscure. We found that the neurons expressing the pathologic mutations showed cellular changes including elevated spontaneous depolarization with exuberant growth of soma, dendrites and spines similar to neuronal changes seen in other epileptic syndromes. Higher percentage of these spines were positive for the excitatory presynaptic marker VGLUT1. To understand the mechanisms of seizure propagation, we have generated mouse model expressing FMCD-associated mutation in one hemisphere by in utero electroporation. The mice develop spontaneous absence seizures as early as 1-month-old age. Interestingly hemispheric pattern of seizure show difference among individuals. We now aim to characterize transcriptional profiles at single cell level that will reveal molecular mechanisms underlying hemispheric difference.

Key Words: Cortical development, AKT3, Epilepsy

S-1-3

Lithium-responsive and layer-specific prefrontal dysfunction in a mouse model of mania

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The presence of manic episodes, characterized by increased activity, energy, and mood, is a major diagnostic criterion for bipolar disorder. Although structural and functional abnormalities of the prefrontal cortex are observed in bipolar disorder patients, specific prefrontal neurons and their properties associated with pathophysiology and drug response in mania remain unknown. Here, we report that adult mice heterozygous for *cytoplasmic FMR1-interacting protein 2 (Cyfip2$^{+/-}$)*, a gene encoding a synaptic actin-regulatory protein, exhibit mania-like behaviors ameliorated by the mood stabilizer lithium. By combining molecular, ultrastructural, and electrophysiological analyses, we found prefrontal dysfunction in adult *Cyfip2$^{+/-}$* mice specifically in layer 5, but not layer 2, neurons. Furthermore, among the identified prefrontal defects in *Cyfip2$^{+/-}$* mice, lithium selectively rescued the hyperexcitability of layer 5 neurons. Beyond providing a new mouse model of mania, these results suggest that prefrontal layer 5 neurons could be associated with both pathophysiology and lithium-mediated amelioration of mania-like behaviors.

S-1-4

Synaptic dysfunction of mild intellectual disability

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Intellectual disability is a mild mental retardation showing impaired intellectual ability with IQ below 70. Recent studies have revealed a series of synaptic proteins as factors related to the mild mental retardation. Cereblon is one of mild mental retardation related factor which is one of ATP-dependent Lon protease. The nonsense multation (R419X) of cereblon gene located in the human chromosome 3 (3p26.3) is thought to cause the intellectual disability in the patients with IQ 50-70. However the detailed mechanism of cereblon in synapse is mostly unclear yet. Here we studied the role of Cereblon in the synaptic structure and function using multiple model systems including mouse hippocampal slices and Drosophila neuromuscular junction. The knockout mouse lacking the expression of cereblon shows generally normal gross brain anatomy as well as spine density and length. However cereblon KO animal shows clear impairment in cognitive function analyzed by Y-maze test, passive avoidance test and novel object recognition test. The synaptic function in cereblon KO animal are intact in Schaffer-collateral synapse in hippocampus with normal input-output relationship, LTP, LTD, long-lasting LTP, and mGluR-induced LTD. Interestingly the paired-pulse ratio was altered in KO animal, implying the changes in presynaptic release function. To further confirm the function of cereblon and to identify its signaling mechanism, we monitored the synaptic function of Drosophila cereblon KO animal. The changes in paired-pulse ratio were also monitored in KO animal. Interestingly the mutants also showed the increased evoked excitatory postsynatpic currents and size of releasable neurotransmitter pool as well. These results suggest that the changes in presynaptic neurotransmitter release could be involved in the pathology of mild mental retardation in the patients with cereblon mutation.

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Key Words: BK channels, CRBN, Intellectual disability, Neurotransmitter release, Presynaptic

S-1-5

Cell type-specific signaling networks in learning disabilities

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Mutations in Ras signaling molecules cause diverse neurodevelopmental disorders, collectively called Rasopathy. Rasopathies are highly associated with diverse cognitive problems including learning disabilities. Interestingly, previous studies have suggested that distinct cell types in the nervous system are affected among Rasopathies including Noonan syndrome (NS) and neurofibromatosis 1 (NF1), but molecular mechanisms underlying the cell type specificity are unclear. In this talk, I will talk about the molecular mechanism underlying cell type specific pathophysiology in Rasopathies with main focus on NS. To define the cell type responsible for the deficits in NS and other Rasopathies, we used single cell-type RNA-seq analyses combined with electrophysiological, biochemical, and behavioral analyses. Although Ras signaling is a ubiquitous signaling pathway, our study highlights that Ras-Erk signaling networks are significantly different in different neuronal cell types. Accordingly, mutations in specific Ras regulators in distinct Rasopathies can cause deficits restricted to specific cell types. Our study expands our understanding on RAS-ERK signaling in synaptic plasticity and memory, and also provides a plausible explanation for how different RASopathies including NS and NF1 affect distinct cell types in the nervous system. Furthermore, our results would provide insights into developing mechanism-based personalized treatments for cognitive deficits in RASopathies.

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Key Words: Ras signaling, LTP, Spatial memory

S-2-1

Blockade of Kv1.5 by PCP derivatives and its clinical implications

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MK801 and ketamine, which are phencyclidine (PCP) derivative N-methyl-d-aspartate receptor (NMDAr) blockers, reportedly enhance the function of 5-hydroxytryptamine (HT)-2A receptors (5-HT_{2A}Rs). Both are believed to directly affect the pathogenesis of schizophrenia as well as hypertension. 5-HT_{2A}R signaling involves the inhibition of Kv conductance. In this study, we explored the interaction of these drugs with Kv1.5, which plays important roles in 5-HT_{2A}R signaling and in regulating the excitability of the cardiovascular and nervous system, and the potential role of this interaction in the enhancement of the 5-HT_{2A}R-mediated response. Using isometric organ bath experiments with arterial rings and conventional whole-cell patch-clamp recording of Chinese hamster ovary (CHO) cells ectopically over-expressing Kv1.5, we examined the effect of ketamine and MK801 on 5-HT_{2A}R-mediated vasocontraction and Kv1.5 channels. Both ketamine and MK801 potentiated 5-HT_{2A}R-mediated vasocontraction. This potentiation of 5-HT_{2A}R function occurred in a membrane potential-dependent manner, indicating the involvement of ion channel(s). Both ketamine and MK801 rapidly and directly inhibited Kv1.5 channels from the extracellular side independently of NMDArs. The potencies of MK801 in facilitating the 5-HT_{2A}R-mediated response and blocking Kv1.5 were higher than those of ketamine. Our data demonstrated the direct inhibition of Kv1.5 channels by MK801/ketamine

and indicated that this inhibition may potentiate the functions of 5-HT_{2A}Rs. We suggest that 5-HT_{2A}R-Kv1.5 may serve as a receptor-effector module in response to 5-HT and is a promising target in the pathogenesis of MK801-/ketamine-induced disease states such as hypertension and schizophrenia.

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Key Words: Kv1.5, MK801, 5-HT2A receptor, Schizophrenia, Ketamine, Hypertension

S-2-2

Identification of C-terminal domains regulating TREK K^+ channels

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TWIK-Related two-pore domain K+ channels (TREKs) are activated by acidic intracellular pH (pHi), membrane stretch and arachidonic acid. In contrast, physiological $PI(4,5)P_2$ seems to inhibit TREKs; removing intracellular ATP activate TREKs via lowering intrinsic $PI(4,5)P_2$ determined by counterbalancing lipid phosphatase and PIP4/5 kinase. However, excessive scavenging of $PI(4,5)P_2$ abolishes TREK activity, implying dual roles of $PI(4,5)P_2$. We have investigated the anionic and cationic residues of proximal region of C-terminal (pCt) for the pHi and ATP-sensitivity in human TREK-2 (hTREK-2) by using site-directed Ala substitution and inside-out patch clamp recording ($I_{TREK\text{-}2,i\text{-}o}$). A neutralization of Glu (E332A) showed fully activated state without further activation by acidic pHi nor inhibition by ATP. Neutralization of cationic Lys (K330A) also showed tonic activation without inhibition by ATP nor the activation by acidic pHi. In contrast, neutralization of triple Arg (R355-7A) showed very low basal activity even without ATP while preserving the activation by acidic pHi and arachidonic acid. The activation by anionic phospholipid with smaller headgroup (phosphatidic acid) was not observed in R355-7A. Neutralization of more distally located poly-cationic residues (R377-9A) had no effect. Combined neutralization of the Lys (K330A/R355-7A) or Glu (E332A/R355-7A) did not rescue the suppressed TREK-2 activity. Interestingly, acidic pHi could activate K330A/R355-7A while not E332A/R355-7A. Although the activation by acidic pHi is intact in R355-7A, the lack of tonic activation in E332A/R355-7A indicate that the single Glu (E332) might not be the single site of pHi-sensitivity in hTREK-2. Similar responses of the corresponding mutants in hTREK-1 to the above conditions were confirmed. Taken together, we propose a novel model of dual regulation by $PI(4,5)P_2$ via electrostatic interaction with independent cationic amino acids in pCt of TREKs.

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Key Words: Two-pore domain K^+ channel, TREK, $PI(4,5)P_2$, Electrostatic interaction, Patch clamp

S-2-3

Regulation of endothelial Ca^{2+}-activated K^+ channels in health and vascular diseases

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Ca^{2+}-activated K^+ channels, $K_{Ca}2.3$ and $K_{Ca}3.1$ (K_{Ca}s), play an important role in endothelial control of vascular contractility. Activation of these K^+ channels induces endothelial hyperpolarization, which spreads to vascular smooth muscle cells (VSMCs) through gap junctions, and thereby inducing hyperpolarization of VSMCs. In addition, K^+ efflux through the channels also induces hyperpolarization of VSMCs. Furthermore, endothelial hyperpolarization elevates intracellular Ca^{2+} levels via facilitating Ca^{2+} influx through Ca2+ entry channels, thereby stimulating NO production in endothelial cells (ECs). Hyperpolarization and NO relax VSMCs, thereby modulating vascular contractility. Since the magnitude of endothelial hyperpolarization is affected by levels of these K^+ channels in cell membrane, endothelial K_{Ca}s levels were examined in health (aging and normal pregnancy) and vascular diseases (preeclampsia and Fabry disease). Endothelial K_{Ca}s were upregulated in health, and K_{Ca}s upregulation potentiated L-NAME-resistant and K_{Ca}s activation-induced endothelium-dependent relaxation (EDR). H_2O_2 levels were enhanced via elevating SOD1 levels and reducing catalase and glutathione peroxidase 1 levels, thereby enhancing K_{Ca}s levels via a H_2O_2/fyn/ERK-mediated pathway. On the contrary, endothelial K_{Ca}s were downregulated in vascular diseases and K_{Ca}s downregulation in activity and expression caused endothelial dysfunction. Superoxide levels were enhanced via elevating NADPH oxidase 2 (NOX2) and NOX4 levels and reducing SOD1 levels, thereby downregulating K_{Ca}s. Sphingolipids, VEGF, ox-LDL, sFlt-1, estrogen and progesterone act as modulators of K_{Ca}s levels in health and vascular diseases via altering redox state. These findings suggest that enhanced K_{Ca}s activity may compensate for decreased NO signaling during vascular aging, or contribute to hemodynamic adaptation in normal pregnancy. In contrast, reduced K_{Ca}s activity may contribute to the development of vascular diseases such as preeclampsia and Fabry disease.

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Key Words: Ca^{2+}-activated K^+ channels, Endothelial cells, Redox state, Aging, Pregnancy, Fabry disease

S-2-4

KCNQ channel methylation in control of neuronal excitability

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Abnormally intense bursts of electrical activity from many neurons at once can cause seizures such as those experienced by people with epilepsy. Since M channel acts as a powerful brake in the brain, its openers have emerged as a potential new class of anti-epileptic drugs. A better understanding of how M channels work, and how their opening by PIP_2 lipid signals is regulated, could help to develop more effective therapies for epilepsy. To date, however, the mechanisms of M channels modulation have been mostly

characterized to be inhibitory via Gq-coupled receptors, Ca^{2+}/CaM, and protein kinase C. Here we demonstrate that protein arginine methyltransferase 1 (Prmt1) methylates the KCNQ, the molecular correlates of M channel in neuron, and that this methylation is essential for suppressing seizures. Mice born without the Prmt1 protein developed epileptic seizures and the KCNQ/M channels in their neurons featured a reduced level of methylation. However, increasing the amount of PIP2 in these neurons restored their excitability back to normal levels. The methylation of KCNQ/M channel proteins increases their affinity for PIP2, which is critical to open KCNQ/M channels. We propose that these "opening" controllers balance the action of known "closers" of KCNQ/M channels to maintain neurons in a healthy condition.

S-2-5

KCNQ4 potassium channelopathy and hearing loss

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KCNQ4 encodes a voltage-gated K+ channel (Kv7.4), and is highly expressed in the hair cells of the cochlea and plays a pivotal role in maintaining cochlear K+ homoeostasis. Mutations in KCNQ4 potassium channel are etiologically linked to deafness nonsyndromic autosomal dominant 2 (DFNA2), which is an autosomal dominant nonsyndromic hearing loss (NSHL) disorder characterized by postlingual progressive hearing loss. Using whole-exome sequencing (WES) of Korean NSHL families, we identified novel missense mutations and in-frame deletion mutations in KCNQ4. In heterologous KCNQ4 mutants expressed in HEK293T cells, these mutant proteins did not show defects in protein trafficking to the plasma membrane or in interactions with wild-type (WT) KCNQ4 channels. Whole-cell patch-clamp analysis demonstrated that mutant channels lost ion conductance and were completely unresponsive to KCNQ activators (retigabine, zinc pyrithione, and ML213). When the mutants were assembled with WT KCNQ4, some concatemer channels exhibited conductance and responsiveness to KCNQ activators. However, other KCNQ4 concatemers did not show ion conductance and response to the activators, suggesting that these mutations caused complete loss-of-function with a strong dominant-negative effect on functional WT KCNQ4 channels. Taken together, causative mutations in KCNQ4 should be examined in individuals with progressive NSHL. The main pathological mechanism may be related to loss of KCNQ4 K^+ channel activity, not defects in protein trafficking.

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Key Words: Channelopathy, KCNQ4, Hearing loss, Mutation

S-2-6

BK_{Ca} channel drug discovery targeting overactive bladder

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The large-conductance calcium-activated potassium channel (BK_{Ca} channel) plays critical roles in smooth muscle relaxation. In urinary bladder smooth muscle, BK_{Ca} channel activity underlies the maintenance of the resting membrane potential and repolarization of the spontaneous action potential triggering the phasic relaxation. In order to identify novel BK_{Ca} channel activators, we previously established a cell-based fluorescence assay using a hyperactive mutant channel (Lee *et al*., 2013). By screening a library of natural compounds, we initially identified a flavanone from *Sophora flavescens* as a potent activator of BK_{Ca} channels and characterized its activation mechanism. We also showed that the compound potently relaxed acetylcholine-induced contraction of rat bladder smooth muscle and thus decreased the micturition frequency of rats with overactive bladder symptoms (Lee *et al*., 2016). Using the cell-based assay, we have screened >20,000 synthetic compounds and identified several hit compounds greatly potentiating BK_{Ca} channel activity. We are currently optimizing the novel hit compounds to further improve their activity and to provide better druggable properties. Thus, we demonstrated the therapeutic potentials of novel BK_{Ca} channel activators of natural and synthetic origins for developing anti-overactive bladder medications and food supplements.

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Key Words: Calcium-activated potassium channel, Activators, Overactive bladder syndrome, Urinary incontinence, Cell-based assay

S-3-1

Single nucleotide polymorphisms and world-class Korean athletes

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There are strong genetic components to athletic performance and its response to exercise training[1-4]. The main goal of this research was to find the genetic determinism of athlete status (ie., cardiopulmonary endurance, power and speed) and to describe some novel and important DNA polymorphisms that may underlie differences in the potential to be an elite athlete. Genome-wide association studies (GWASs) were undertaken on six cohorts of 91 elite endurance athletes (world-class endurance, power, and speed athletes as well as domestic level endurance, power, and speed athletes) and 1,525 Korean controls (1,024 male and 501 female). To validate obtained results, we further performed case-control studies by comparing the frequencies of the most significant SNPs (with $P < 10^{-5}$ and two or more SNPs within 100Kb) between athletes by motor abilities (world-class 15 endurance athletes, 18 power athletes and 10 speed athlete) and opposite cohorts (domestic level 18 endurance athletes, 18 power athletes, 12 speed athlete, and 104 Japanese controls). The SNPs with two or more SNPs within 100 kb of less than p <.0001 were 20 endurance-related, 25 maximal-strength-related, and 20 speed-related SNPs. Regarding replication, the markers derived from the world-class endurance (39 markers) group, the maximal strength (43 markers) group and the speed (45 markers) group were significantly different in the majority of SNPs (~94%) compared to the Japanese control group. On the other hand, about 42% of the markers on the average showed a significant difference in the group comparison by the domestic level athletic ability (endurance, power and speed). In conclusions, genetic testing has already been widely used in medical field and can also be used as a tool of talent identification in various fields. Despite the increased use of such testing, there is a lack of evidence that genetic testing is useful in predicting athletic ability, and careful consideration should be given to the ethical issues surrounding the testing in general population. Future studies will require assessment and validation of biological functions related to motor function for SNPs derived from this study.

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Key Words: Athletic performance, Endurance, Power, Speed, Single nucleotide polymorphisms

S-3-2

Inter-individual variation in the changes in insulin sensitivity in response to regular exercise

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It has been well demonstrated that regular physical activity is inversely associated with risk factors for cardiovascular and type 2 diabetes mellitus (T2DM). Previous studies have shown that regular exercise performed for >150 minutes per week is associated with significant reductions in total fat, in particular abdominal fat, and improvements in insulin resistance in both children and adults. However, there is substantial individual differences in the ability to improve one's fitness, T2DM risk factors, body composition in response to regular exercise. In this presentation, we will discuss the inter-individual variation in the changes in risk factors for T2DM and the potential factors that may influence the changes in cardiometabolic risk factors in response to regular exercise in obese children and adults.

Key Words: Regular exercise, Obesity, fitness, Inter-individual variation

S-3-3

Beneficial role of HIT exercise on hippocampal plasticity

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Voluntary wheel running(WR) has beneficial effects on hippocampal cognitive functions if done abundantly. However it is still uncertain whether resistance wheel running would be the same. Voluntary resistance wheel running with a load(RWR) as high intensity training(HIT) is a suitable model, since it allows increased work levels and resultant fast-twitch muscular adaptation. Here we examined whether RWR has beneficial effects on hippocampal functions with increased BDNF signaling. Moreover, we have utilized the high-throughput DNA microarray approach to gain deep insight into underlying molecular mechanisms. We used 10-week-old male Wistar rats and divided randomly into sedentary (Sed), WR, and RWR (a load of 30% of b.w.) groups. To access spatial learning and memory, rats were tested in the Morris water maze task. The protein levels of BDNF and its signaling molecules (TrkB, p-CREB) were detected by Western blotting. Whole genome (4x44K) high-density oligonucleotide microarrays were used to monitor the expression level of gene transcripts in the hippocampus. We found that even the average running distance were significantly reduced, the average work levels were drastically increased for the RWR group. Both WR and RWR improved spatial learning, while only RWR group enhanced spatial memory compared with Sed. RWR increased hippocampal BDNF, TrkB and p-CREB protein levels, while WR increased only BDNF. Also, we found inflammatory cytokines and chemokines that might help to counteract neuronal dysfunction and vulnerability. These results suggest providing new evidence that HIT exercise, even with short distance, is beneficial for enhancing brain functions associated with hippocampal plasticity.

Key Words: Resistance wheel running(RWR), Hippocampus, Brain-derived neurotrophic factor(BDNF), High intensity exercise, DNA microarray

S-3-4

Investigation of vitamin D level and its role in inactive submariner

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Lack of DNA repair pathway is characterized by an increased susceptibility to metabolic syndrome and cancers. Recently, several studies reported that obese person have a mutation or polymorphism of DNA repair gene in cell signal pathway. Interestingly, 25(OH)D levels of vitamin D are typically lower in obese individuals who are more likely to develop diabetes mellitus and metabolic syndrome. How DNA repair pathway prevents metabolic disease and the vitamin D level affects to obesity are still unknown. Here we study two groups in obese group (≥25.0kg/m^2) and normal group (18.5~22.9kg/m^2) in Korean submariner with an intake of vitamin D or placebo on double-blind study. Subjects of each group were fifteen people and sixteen, respectively. The study was to compare and analysis Korean submariner's plasma vitamin D levels and metabolic syndrome status. We conducted a study of assessing the plasma vitamin D levels in collected their blood, dietary record, physical activity record in each group for analysis. Obtained data were analyzed using descriptive statistics, Chi-square test, t-test and Pearson correlation coefficient (SPSS 22.0). We hope this study provides a basis evidence for the intervention of obesity prevention program for male submariner.

S-3-5

Regulation of hepatic stellate cells by the factors derived from contracting skeletal muscle cells

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Recent clinical studies reported that regular exercise can improve hepatic steatosis and hepatic stiffness in patients with non-alcoholic fatty liver disease or non-alcoholic steatohepatitis. However, the molecular mechanisms of how physical exercise can physiologically modulate hepatic fibrosis remains unclear. Activation of quiescent hepatic stellate cells (HSCs) upon extracellular signals during liver injury leads to hepatic fibrosis accompanying cellular responses such as trans-differentiation into myofibroblast-like cells and overproduction of extracellular matrix. Exercise can induce the expression of the various myokines, which have been shown to mediate several beneficial effects including anti-fibrotic potentials. Hereby, we investigated the effects of exercise-induced myokines on HSCs activation using an in vitro exercise model. Electrical pulse stimulation (EPS) was applied on differentiated C2C12 myotubes to mimic exercise and enhance the expression of contraction-induced myokines. To investigate the cell non-autonomous effect of exercise-induced myokines on liver fibrosis, primary mouse HSCs were incubated with a mixture of normal culture media and conditioned media (CM) from C2C12 cells applied with or without EPS (EPS-CM or Control-CM, respectively). EPS-CM led to a downregulation of α-smooth muscle actin and collagen expression in the HSCs whereas the Control-CM seemed to be partially effective in downregulation process. In addition, the response to EPS-CM was accompanied by attenuated TGF-β1 expression and reduced Smad3 phosphorylation. These results suggest that exercise-induced myokines may contribute to suppressing hepatic fibrosis by attenuating HSCs activation.

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Key Words: Skeletal muscle cell, Electrical pulse stimulation, Myokine, Hepatic stellate cell, Liver fibrosis

S-3-6 Young Physiologist Award

The role of echinochrome A for exercise capacity

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Echinochrome A (Echi A) improves mitochondrial function in the heart; however, its effects on skeletal muscle are still unclear. We hypothesized that Echi A administration during short-term exercise may improve exercise capacity. Twenty-four male Sprague-Dawley rats were randomly divided into the following groups: control group (CG), Echi A-treated group (EG), aerobic exercise group (AG), and aerobic exercise treated with Echi A group (AEG) (n = 6 per group). Echi A was administered intra-peritoneally (0.1 mg/kg of Echi A in 300 μL phosphate-buffered saline) daily 30 min before each exercise training. The AG and AEG groups performed treadmill running (20 m/min, 60 min/day) five days/week for two weeks. The exercise capacity was significantly higher in the AG and AEG groups compared to other groups. Interestingly, the exercise capacity increased more effectively in the AEG group. The body weight in the EG tended to be slightly lower than that in the other groups. There were no significant changes in the plasma lipids among the groups. However, the gastrocnemius muscle mitochondria content was greater in the EG and AEG groups. These findings show that Echi A administration after short-term endurance training enhances exercise capacity, which was associated with an increase in skeletal muscle mitochondrial content.

Key Words: Echinochrome A, Aerobic exercise, Mitochondrial function, Skeletal muscle

S-4-1

Ultrastructural basis for craniofacial sensory processing in the brainstem (Expression of glycine receptor alpha 3 in the primary sensory neurons

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Many studies have reported that primary somatosensory afferents and somata of the trigemial mesencephalic neurons show synaptic contact with glycine-immunopositive axon terminals. However, it has been doubted for the presence of functional glycinergic synapse in the primary afferents and Vmes neurons because glycine receptor (GlyR) was not observed in them by electrophysiological and immunohistochemical studies.
Here, we report, for the first time, that trigeminal primary afferent terminals (PATs) express GlyRa3 but not gephyrin (indicating homomeric GlyR), whereas dendrites express both GlyRa3 and gephyrin (indicating heteromeric GlyR). GlyRa3-immunoreactivity in PATs was found far from the presynaptic site and in dendrites at subsynaptic sites. These findings suggest that trigeminal PATs receive presynaptic modulation via homomeric, extrasynaptic GlyRa3, and that different subtypes of GlyR may be involved in pre-and post-synaptic inhibition. In addition, we also report that somata of Vmes neurons express homomeric GlyR at extrasynaptic site, suggesting that it may be involved in the modulation of Vmes neuron excitability .

Key Words: Glycine receptor, Primary afferents, Presynatic inhibition, Trigeminal, Immunohistochemistry

S-4-2

Role of mechanosensitive ion channels in tooth pain

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The teeth have a distinctive nociceptive mechanism by which tooth pain is induced not only by noxious stimuli but also by even weak air-puff stimulus when dentin is exposed. The hydrodynamic theory has been proposed to explain dentinal hypersensitivity, which attributes dental pain to fluid movement within dentinal tubules and thus cellular mechanical transducers for the detection of fluid movement. We investigated molecular transducers expressed by dental primary afferent (DPA) neurons or odontoblast to explain how the teeth transduce noxious pain in response to innocuous mechanical stimuli. We identified that piezo2 contributes to mechanical responsiveness of DPA neurons, mostly functional in IB4(-), capsaicin-insensitive DPA neurons, which are distinctive from conventional nociceptors found in the other tissue of our body. We also found that adult rat odontoblasts are indeed mechanically sensitive and TRPM7 may be critical for the mechano-transduction in odontoblasts. Activation of TRPM7 increased intracellular calcium and TRPM7 is more preferentially expressed in odontoblast process rather than cell soma. However, the exact role of TRPM7 in dental nociception remains to be elucidated.

Key Words: Tooth pain, Hydrodynamic theory, Piezo2 channel, TRPM7

S-4-3

Maresin 1 inhibits TRPV1 in temporomandibular joint (TMJ)-related trigeminal nociceptive neurons and TMJ inflammation-induced synaptic plasticity in the trigeminal nucleus

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In the trigeminal system, disruption of acute resolution processing may lead to uncontrolled inflammation and chronic pain associated with the temporomandibular joint (TMJ). Currently, there are no effective treatments for TMJ pain. Recently, it has been recognized that maresin 1, an endogenous pro-resolution lipid mediator that is derived from the addition of docosahexaenoic acid to macrophages, is a potent analgesic for somatic inflammatory pain without noticeable side effects in mice and a potent endogenous inhibitor of transient receptor potential vanilloid 1 (TRPV1) in the somatic system. However, the molecular mechanisms underlying the analgesic actions of maresin 1 on TMJ pain are unclear in the trigeminal system. Here, by performing TMJ injection of a retrograde labeling tracer DiI (a fluorescent dye), I showed that maresin 1 potently inhibits capsaicin-induced TRPV1 currents and neuronal activity via Gαi-coupled G-protein coupled receptors in TMJ-related trigeminal nociceptive neurons. Further, maresin 1 blocked TRPV1 agonist-evoked increases in spontaneous excitatory post-synaptic current (sEPSC) frequency and abolished TMJ inflammation-induced sEPSC increases (frequency and amplitude) in the trigeminal nucleus. These results demonstrate the potent actions of maresin 1 in regulating TRPV1 in TMJ-related trigeminal nociceptive neurons and TMJ inflammation-induced synaptic plasticity in the trigeminal nucleus. Therefore, these new findings suggest that maresin 1 may serve as a novel endogenous inhibitor for treating TMJ-inflammatory pain in the orofacial region.

Key Words: Temporomandibular joint (TMJ), Maresin 1, Trigeminal ganglion neuron, Transient receptor potential vanilloid 1 (TRPV1), Synaptic plasticity

S-4-4

Experimental animal models for trigeminal neuralgia

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Trigeminal neuralgia is a severe chronic pain syndrome characterized by intense stabbing or electrical shock-like paroxysmal pain. Although vascular compression is an important factor in the etiology of trigeminal neuralgia, there are no satisfactory animal models in order to confirm the etiology and identification of pathological mechanisms. We report here on a novel method for producing trigeminal neuralgia like pain behavior in rats. Under anesthesia, rats were mounted on a stereotaxic frame. Agar was injected into the left trigeminal nerve root for compression. We also injected LPA (1 nmol, 3 μl) into the left trigeminal nerve root. Compression of the trigeminal nerve root produced prolonged mechanical allodynia and hyperalgesia in the cutaneous territory of the affected nerve. Microinjection of LPA produced severe demyelination of axonal portion in the trigeminal nerve root and dramatic increases in the responses to mechanical stimulation. Intraperitoneal administration of carbamazepine attenuation of mechanical allodynia in rats with trigeminal neuralgia. We further showed several underlying mechanisms which is participated in the development of trigeminal neuralgia. Finally, subcutaneous single injection of botulinum toxin produced prolonged antinociceptive effects in rats with trigeminal neuralgia. Our current analysis provides that this new animal model seems to be of great value in studying the underlying mechanisms of trigeminal neuralgia. Also these results suggest a potential therapeutic strategy for treatment of trigeminal neuralgia.

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Key Words: Trigeminal neuralgia, Rat, Compression, Trigeminal nerve root, Demyelination

S-4-5

Possible involvement of oral dysfunction in inducing stress disorder

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Primary sensory neurons in the mesencephalic trigeminal nucleus (MTN) play a central role in regulating the isometric contraction of jaw-closing muscles, which are crucially involved in the slow jaw closing phase of the masticatory cycle. Temporomandibular joint disorder (TMJD) is caused by malocclusion or bruxism/clenching that is caused by psychological stress. This masticatory impairment is likely to be accompanied by malfunction of MTN neurons because of its crucial involvement in mastication. MTN neurons are uniquely located in the brain stem and receive synaptic inputs, thereby functioning in the two different modes; one as the primary sensory neurons or the other as premotor neurons. GluR synaptic currents in MTN neurons were effectively controlled by the interaction between HCN and GluR channels that share a Na+ microdomain in spines. Activation of adrenergic alpha2A receptor mainly by Locus Coeruleus (LC) shut HCN channels by downregulating cAMP production in MTN neurons, otherwise HCN can effectively suppress GluR activity. Thus, LC can prevent MTN neurons from acting as the primary sensory neuron, dysregulating mastication. LC and MTN neurons have ontogenetically and tropically very close relationship. Once psychological stress is caused by masticatory dysfunction, a vicious spiral would be easily initiated between LC and MTN neurons.

S-5-1

Structural and functional significances of the atrial T-tubules

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Atrial fibrillation (AF) is the most common sustained arrhythmia associated with a high morbidity, which shows chamber-specific and spatiotemporal organization patterns. However, it is underrated whether different structural and electrophysiological properties found in atrial myocytes give intrinsic substrate for spatiotemporal AF organization. We isolated rat atrial myocytes from left (LA) and right atrium (RA), and compared their T-tubule development, electrophysiological properties, E-C coupling, and arrhythmic fragility in response to sympathetic stimulation. SICM and confocal imaging of atrial myocytes revealed a heterogeneous degree of T-tubule development which can be classified into 3-types; reticular T-tubules, sparse T-tubule and no T-tubules. The RA myocytes were smaller and have poorly developed T-tubule network than the LA myocytes. Compared to LA myocytes, RA myocytes showed much shorter action potential duration (APD) and higher densities of transient outward potassium channel and L-type Ca^{2+} channel (LTCC). In response to β-adrenergic stimulation (isoproterenol with caffeine), RA myocytes showed much higher frequency of arrhythmias, which is evaluated by higher occurrence of spontaneous Ca^{2+} events, delayed after depolarization (DAD), triggered activities and contractions (TA and TC), and self-sustained contractions. Higher arrhythmic incidence observed in RA myocytes accorded well with higher LTCC increase by isoproterenol stimulation, suggesting that β-adrenergic stimulation-induced $[Ca^{2+}]_i$ overload occurs more severely in poorly T-tubulated RA myocytes. After acute removal of T-tubules, both LA and RA myocytes showed increased arrhythmias in response to β-adrenergic stimulation. Nonetheless, arrhythmic fragility was maintained higher in RA over LA myocytes after detubulation. Our study suggests that poorly T-tubulated RA myocytes are prone to arrhythmic insults, probably due to weaker Ca^{2+} handling capacity that causes $[Ca^{2+}]_i$ overload to trigger arrhythmias under the strong β-adrenergic stimulation.

Acknowledgement: This work was supported by the National Research Foundation of Korea(NRF) Grant funded by the Korean Government(MSIP) (2016R1D1A1B03934748).

Key Words: Arrhythmia, Atrial fibrillation, Atrium, Isoproterenol, T-tubules

S-5-2

Ca^{2+} signaling triggered by shear-autocrine P2X receptor pathway in rat atrial myocytes

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The atrium is exposed to high shear stress during heart failure and valvular diseases. We aimed to understand atrial shear-induced Ca^{2+} signaling and its underlying mechanisms. Pressurized micro-flow was applied to single rat atrial myocytes, and Ca^{2+} signal, membrane potential, and ATP release were assessed using confocal imaging, patch clamp technique, and luciferin-luciferase assay, respectively. Shear stress (~16 dyn/cm^2) induced transversely-propagated Ca^{2+} waves (~0.1 events/s) from the periphery to the center of cells ("T-wave"; ~145 μm/s). Pharmacological interventions and simultaneous recording of membrane potential and Ca^{2+} demonstrated that shear-induced T-waves resulted from action potential (AP)-triggered Ca^{2+} release from the sarcoplasmic reticulum. T-waves were not sensitive to inhibitors of known shear signaling mechanisms except connexin hemichannels and ATP release. Shear stress caused ATP release from these myocytes (~1.1×10^{-17} moles/unit membrane, μm^2); ATP release was increased by enhancement of connexin hemichannels and suppressed by inhibition of the hemichannels, but not affected by inhibitors of other ATP release path-

ways. Blockade of P2X receptor, but not pannexin or the Na^+-Ca^{2+} exchanger, eliminated shear-induced T-wave initiation. Our data suggest that shear stress triggers APs and concomitant Ca^{2+} signaling via activation of P2X receptors by connexin hemichannel-mediated ATP release in atrial myocytes.

Key Words: Shear stress, AP-triggered Ca^{2+} wave, Atrial myocytes, ATP release, P2X receptor

S-5-3

Atrial fibrillation and atrial electrophysiology

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Atrial fibrillation (AF) remains the most common adult rhythm disorder, and it associated with a substantial rate of morbidity and economic burden. The incidence of AF is expected to continue to rise with the aging of the population. AF involves a wide spectrum of arrhythmias from lone AF to paroxysmal to chronic AF. It is likely that AF comprises a spectrum of disease with no single mechanism adequate enough to comprehensively explain AF and its variability. Mechanism of fibrillation is explained by multiple wavelets and focal activation theories. Electrical, contractile and mechanical remodeling is involved in AF progression. Atrial remodeling may also increase in atrial fibrosis which can slow conduction velocity and can shorten the refractory period in atria with long-standing AF. Mechanical remodeling manifests as decreased atrial contractility and increased atrial compliance which leads to a stretch of the atrial myocardium. The importance of intracellular Ca2+ handling abnormalities has been highlighted, both for the induction of triggered ectopic activity and for the activation of Ca^{2+}-related cell signaling that mediates profibrillatory remodeling.
Modulating factors such as genetic factors, age, obesity, sleep apnea, inflammation, autonomic factors and atrial and pulmonary vein stretch only partially account for the increase in AF. Although significant progress in understanding the mechanism of this arrhythmia has been accomplished, the pathophysiology of AF is complex and likely has many possible mechanisms which may be interrelated.

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Key Words: Atrial fibrillation, Arrhythmia mechanism, Remodeling, Risk factor

S-5-4

Atrial natriuretic peptide in cardiovascular biology and diseases

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Atrial natriuretic peptide (ANP), a cardiovascular hormone mainly secreted by heart atria in response to atrial stretching, causes diuresis, natriuresis and vasorelaxation and plays a major role in the homeostasis of blood pressure as well as of water and salt balance. More recently, ANP and related peptides have been implicated in lipid metabolism and metabolic diseases. Defects in the ANP pathway including variants in the human NPPA gene (encoding the ANP precursor) are associated with hypertension, stroke, coronary artery disease, heart failure and obesity. Gene-targeted and transgenic mouse models have advanced our understanding of the importance of ANP, brain natriuretic peptide (BNP), and their receptors in disease states at the molecular level. Importantly, ANP and BNP are also used as critical markers of cardiac events; however, their therapeutic potentials for the diagnosis and treatment of hypertension, heart failure, and stroke have just begun to be realized.
Studies on ANP, BNP, and their receptor have greatly increased our knowledge of the control of hypertension and cardiovascular disorders. Several recent studies have helped to define the cellular mechanism contributing to the regulation of ANP secretion including stretch-activated ion channels, KATP channel, endothelin, angiotensin, and calcium. The release of ANP in disease states such as myocardial infarction and heart failure appears to be related to both mechanical and cellular events. Cellular, biochemical, and molecular studies have helped to delineate the receptor function and signaling mechanisms of ANP receptor. A number of steps in the cellular transduction of the ANP signal remain to be resolved.

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Key Words: Atrial natriuretic peptide, Hypertension, Obesity, Receptor, Secretion

S-6-1

Role of lysosomal Ca^{2+} in mitophagy

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Autophagy is critical in the maintenance of pancreatic b-cell function. However, the role of mitophagy in pancreatic beta cell function has not been clearly shown. Recently, calcineurin and lysosomal Ca^{2+} have been reported to play a regulatory role in autophagy. We studied the role of lysosomal Ca^{2+} in mitophagy after mitochondrial stressor treatment. We also studied whether calcineurin inhibitors can affect mitophagy of pancreatic b-cells and thereby pancreatic b-cell function. Mitochondrial stressors such as rotenone- or oligomycin/antimycin A combination induced mitophagy in INS-1 insulinoma cells as identified by mito-Keima staining or LC3/Tom20 colocalization studies. Mitochondrial stressors also induced nuclear translocation of Tfeb family members such as Tfeb, Tfe3 or MiTF. FK506, a calcineurin inhibitor, suppressed mitochondrial stress-induced mitophagy through inhibition of Tfeb nuclear translocation. Mitochondrial stressors induced perilysosomal Ca^{2+} release and subsequent reduction of lysosomal Ca^{2+} content. Probably because of lysosomal Ca2 release, cytosolic Ca^{2+} content was increased by mitochondrial stressors. The increase in cytosolic Ca^{2+} content was found to be important in mitophagy induction by mitochondrial stressors since mitophagy after treatment with mitochondrial stressors was inhibited by Ca^{2+} chelation. Lysosomal Ca^{2+} exit channel involved in lysosomal Ca^{2+} release after mitochondrial stressors appeared to be TRPML1 because lysosomal Ca^{2+} release after mitochondrial stressors was inhibited by an antagonist of TRPML1 channel (ML-SI3 and mitophagy induction by mitochondrial stressor was reduced by TRPML1 knockdown.

Lysosoomal Ca^{2+} channel activation by mitochondrial stressors was inhibited by antioxidant, suggesting the role of mitochondrial ROS in lysosomal Ca^{2+} emptying by mitochondrial stressors. Probably through reduction of mitophagy, FK506 retarded recovery of mitochondrial potential after treatment with mitochondrial stressors. Mitochondrial oxygen consumption was also reduced by FK506, supporting reduced mitochondrial function by FK506. Mitochondrial Cox activity of pancreatic islets was reduced by prolonged FK506 administration. At least partly due to impaired mitochondrial function, insulin release from INS-1 cells was reduced by FK506 in vitro. FK506 also reduced insulin release and induced impaired glucose tolerance in vivo. The number of GFP-RFP puncta in pancreatic islets was reduced by FK506 administration. These results suggest importance of lysosomal Ca^{2+} in mitophagy which is crucial in the maintenance of pancreatic b-cell function and insulin release.

S-6-2

Regulation of PDK activity on mitochondrial quality control & metabolic flexibility

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Mitochondria-associated ER membrane (MAM), a structural link between the mitochondria and the endoplasmic reticulum (ER), forms a channel consisting of IP3R1, GRP75, and VDAC1 that transports calcium from the ER to the mitochondria. Moderate calcium transport by MAM stimulates oxidative metabolism and ATP synthesis. Excessive transport causes calcium overload and mitochondrial dysfunction. In this study, we have demonstrated that the induction of pyruvate dehydrogenase kinase 4 (PDK4) in skeletal muscle in obesity promotes MAM formation. PDK4 inhibition prevented MAM-induced mitochondria dysfunction and ER stress whereas *Pdk4*$^{-/-}$ mice exhibits reduced MAM formation and is protected against diet-induced skeletal muscle insulin resistance. Finally, forced formation and stabilization of MAMs with a synthetic ER-mitochondria linker prevented the beneficial effects of PDK4 deficiency on insulin sensitivity. Overall, our findings demonstrate a critical mediatory role of PDK4 on mitochondrial quality control via enhancement of MAM formation.

S-6-3

Transfer of isolated mitochondria: uptake mechanism and therapeutic application

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In symbiosis theory, ancient eukaryotes gained mitochondria from prokaryotes 1.5 billion years ago. Mitochondria supply energy to the host cell and still contain their own DNA (mtDNA). Abnormal mitochondria are linked to a variety of heritable and acquired diseases, aging and lifespan. Recently, the mitochondria transplant has become as a new strategic concept to overcome mitochondrial dysfunction in diseases, primarily in the experimental ischemic heart model. However, the mechanism and scope of mitochondrial transfer has not been understood yet. In this study, we investigated the conditions and mechanism by which mitochondria isolated from normal cells were transferred to recipient cells and tissues. Isolated mitochondria were rapidly taken-up by recipient cells in a concentration-dependent and time-dependent manner regardless of floating or adherent cells. The transferred mitochondria were exactly merged with endogenous mitochondria of recipient cells and observed inside cells for up to 60 hours. Membrane-impermeable protease-treated mitochondria were also transferred into the cells. Cytochalasin D, a macro-pinocytosis inhibitor, completely inhibited the uptake of donor mitochondria, which were encapsulated in the plasma membrane of recipient cell. This suggests that recipient cells can engulf mitochondria by macro-pinocytosis without receptor. The transferred mitochondria rescued the mitochondrial damages of recipient cells: the mtDNA-depleted ρ0 cells or mitochondrial toxin-treated neuronal cells. When isolated mitochondria were stereotaxically injected into substantia nigra pars compacta (SNpc) of MPTP((1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-injected mice, the bradykinesia and dopaminergic neurons were rescued to normal level. These findings suggest that mitochondrial transfer would be a novel therapeutic tool for mitochondrial diseases such as Parkinson's disease by replacing the damaged mitochondria in tissues of patient.

S-6-4

Cellular mechanism of over-exercise

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AMPK is known to be activated during exercise. The aim of study is to figure out the effect of AMPK modulatory protein in heart and its mitochondrial function. Eight weeks of Control and AMPK modulatory protein KO models were examined their body weight, heart rate and heart/body ratio. *In vivo* cardiac functions of animals were assessed by echocardiography. To evaluate mitochondrial function of those animals, cardiac mitochondria were isolated then examined their ATP contents and ATP production rate, ROS production rate, oxygen consumption rate (OCR) and membrane potential (ΔΨm). As results, the body weight, heart weight and heart/body ratio were not significantly different between them. Echocardiography showed enhanced cardiac contractility in KO mice based on increased ejection fraction (%) and fractional shortening (%). In their mitochondria, basal ATP contents and substrate/ADP stimulated ATP production rate were significantly higher in KO mice than Control. In addition, basal H2O2 level and rotenone induced ROS production rates were significantly lower in KO mice than Control. The KO mice showed higher single cell contractility with higher Ca^{2+} transient amplitude in isolated left ventricular cardiac myocytes. Our results suggested that AMPK modulatory protein is an important mitochondrial functional regulator which link cytosol to mitochondrial energy metabolic signaling.

S-6-5

PGC-1α functions as a co-suppressor of XBP1s to regulate glucose metabolism

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Peroxisome proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α) promotes hepatic gluconeogenesis by activating HNF4α and FoxO1. PGC-1α expression in the liver is highly elevated in obese and diabetic conditions, leading to increased hepatic glucose production. Since its discovery, PGC-1α has been acknowledged solely as a direct co-activator for the transcriptional factors. Here, we show that PGC-1α physically interacts with the spliced form of X-box binding protein 1 (XBP1s), which plays an anti-gluconeogenic role in the liver by suppressing FoxO1 activity. The physical interaction between PGC-1α and XBP1s leads to suppression of XBP1s activity rather than its activation. Upregulating PGC-1α expression in the liver of lean mice lessens XBP1s protein levels and reducing PGC-1α levels in obese and diabetic mouse liver restores XBP1s protein induction. Taken together, our findings reveal a novel function of PGC-1α as a suppressor of XBP1s, suggesting that hepatic PGC-1α promotes gluconeogenesis through multiple pathways as a co-activator for HNF4α and FoxO1 and also as a suppressor for anti-gluconeogenic transcription factor XBP1s.

S-7-1

The neural mechanisms underlying cardiovascular autonomic dysfunction in rodent models of cirrhosis and portal hypertension

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Autonomic regulation of the cardiovascular functions plays central roles in maintenance of optimal blood flow to every organ of our body to meet its metabolic demand under normal and stressed conditions, and is achieved primarily through a reflexive control of the sympathetic and parasympathetic nervous systems. Cirrhosis represents the final pathological pathway in the process of many chronic liver diseases of different causes, and is associated with cardiovascular dysfunction including portal hypertension and hyperdynamic circulation. In cirrhotic patients, cardiovascular autonomic dysfunction (CAD) is prevalent irrespective of etiology and contributes to an increased risk of morbidity and mortality. The onset and progression of CAD is manifested by an impairment of arterial baroreflex and decreased heart rate variability which reflect an imbalance between sympathetic and parasympathetic activities. To date, however, the neural mechanisms that underpin the cirrhosis-induced CAD remain unsolved. Thus, we hypothesized that cirrhosis-induced CAD is attributable to the functional plasticity of peripheral neural components within the arterial baroreflex arc. To address this hypothesis, biliary and non-biliary cirrhotic rats were generated by common bile duct ligation and the intraperitoneal injection of thioacetamide, respectively. In this talk, I will describe the ionic mechanisms underlying cirrhosis-induced changes in the excitability of cardiac afferent and efferent neurons. In addition, I will show some evidence suggesting that portal hypertension is associated with development of cirrhosis-induced CAD and functional plasticity of cardiac afferent and efferent neurons. Our findings may suggest some plausible strategies for the prevention and treatment of cirrhosis-induced CAD.

S-7-2

Neuronal regulation of the gastrointestinal defense mechanisms

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Vomiting (emesis) is a defensive reflex response to avoid absorption of the toxic substances through coordinated work of multiple internal organs. Toxins in the gastrointestinal (GI) tract recognized by vagal afferent nerve and that was transferred to nucleus tractus solitarii (NTS) and chemoreceptor trigger zone (CTZ) in the brain stem. Meanwhile, toxins absorbed in the blood recognized by CTZ and integrated in the NTS. Thus, the CTZ integrates both the afferent signals from the gastrointestinal tract and the chemical signals from the blood. In this process, several neuromodulators are known to execute important roles, however, their mechanism of action on vomiting pathway is not well understood.
Nausea and vomiting (*NV*) are the *most* common side effects of the cytotoxic chemotherapy and often interrupt chemotherapy and negatively impact the outcome. Diverse kind of antiemetic drugs have been used to inhibit chemotherapy-induced nausea and vomiting (CINV) (i.e. serotonin type3 receptor (5-HT_3R) antagonist, dexamethasone and substance P antagonist), however, many cancer patients still suffer by CINV. To further suppress antiemetic drug resistant CINV several natural constituents including ginger, marihuana (*Cannabis sativa*) have been used but these constituents' antiemetic efficacy and mechanism of the action are still unclear.
In this presentation, I will update the current progress to inhibit CINV and introduce our current work for neuronal mechanism underlying CINV.

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S-7-3

Reactivation of critical period-like plasticity at adult TC input in neocortex

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Experience-dependent plasticity in the adult brain has clinical potential for functional rehabilitation following central and peripheral nerve injuries. It remains challenging, however, to identify the sites and mechanisms of such plasticity at the synaptic level. Here, plasticity induced by unilateral infraorbital nerve resection in four week-old rats was mapped using fMRI and synaptic mechanisms were elucidated by slice electrophysiology. The MRI analysis demonstrated increased thalamocortical (TC) input to barrel cortex on stimulation of the spared input. Brain slice electrophysiology revealed TC input strengthening onto layer 4 stellate cells due to an increase in postsynaptic strength and the number of functional synapses. This work shows that the TC input is a site for robust plasticity after the end of the previously defined critical period for this input. Thus, TC inputs may represent a major site for adult plasticity in contrast to the consensus that adult plasticity mainly occurs at cortio-cortical connections. In addition, during development of the adult TC plasticity, long-term potentiation (LTP) could be induced in TC inputs during a limited time-period due to regeneration of silent synapses. Unlikely to developmental critical period, Blockade of NR2B could not affect LTP induction or detection of silent synapses during adult TC plasticity. However, inhibition of NR2B-containing NMDARs during development of the adult TC plasticity nearly completely prevented LTP induction, implying a possible role of NR2B in regeneration of silent synapses during development of the adult TC plasticity. Our study demonstrates that regeneration of silent synapses and subsequent LTP induction play a role as an essential mechanism for the post-critical period plasticity in TC inputs.

S-7-4

Neurourologic research resources

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Physiology, which studies the shape, structure, components and functions of cells and organs, is not only important to researchers doing basic research, but also provides important knowledge to clinicians treating actual patients. In the physiology and cell biology researches, efforts are being made to identify potential new targets for novel therapeutic drugs, at same time the studies are conducted for find the mechanism of action of existing treatments. Accumulation of the results of these basic medical studies is likely to make it possible to provide individualized treatment according to individual differences for each patient. In particular, knowledge of clinical neurophysiology is fundamental to all clinical studies because it helps to understand molecular pathology basics, the consequences of cellular dysfunction, the dysfunctions and interactions of tissues and organs. In addition, collaboration with other areas of research is essential to use these knowledge of the physiological studies in practice. Neurourology has been working on collaborative research to utilize basic medical research results in clinical practice. Through this presentation, I will summarize the results that have been studied for the treatment of lower urinary tract dysfunction.

S-8-1

Direct reprogramming for in vivo therapy and disease modeling

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The discovery of reprogramming by ectopic expression of a defined set of transcription factors, known as direct reprogramming, provided a tractable platform to uncover molecular characteristics of cellular specification, differentiation, and pluripotency. We discuss the controlling cellular identity by cell reprogramming, with an emphasis on nanotechnological approach. Recently, we report that nanoelectronices based in vivo reprogramming efficiently and non-invasively alleviated symptoms in mouse Parkinson's disease models. This study provides a proof of principle for in vivo lineage conversion as a potentially viable and safe therapeutic strategy for the treatment of neurodegenerative disorders. We also discuss the strategies used to generate such disease models using either patient-specific reprogrammed cells, creating new possibilities for the establishment of neurological models for their use in drug screening.

Key Words: Reprogramming, Parkinson disease

S-8-2

Discovery of new regulators in HSCs and hematological malignancies

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We identified UT2 as a transmembrane molecule altered in leukemic cells that emerged from an animal with modifications in specific bone marrow stromal cells. Hypothesizing that the gene altered in the malignant cells that emerge from this niche-induced oncogenesis model might reflect how an abnormal microenvironment leads to cancer; we focused on this gene encoding transmembrane molecule. UT2 interacts directly with RICTOR and thereby inhibits mTOR kinase activity in the RICTOR-containing mTORC2 complex. The molecule was therefore named Upstream-of-mTORC2 (UT2). Increased expression of UT2 prolonged survival in NOTCH-induced T-ALL mouse models. Therefore, these data suggest that UT2 is a distinctive molecular inhibitor of the mTORC2 signaling pathways whose transmembrane location makes it readily accessible for pharmacologic targeting (1, 2).
Further, nutritional status can impact steady-state hematopoiesis, as observed in malnutrition or obesity, as well as be an important determinant in hematopoietic stem cell transplantation. Surprisingly, how information about changing nutrient levels is interpreted by hematopoietic cells has remained a largely unexplored avenue of therapeutically informative research. A major molecular sensor of cellular nutritional status is the mTOR. mTOR kinase is in a multiprotein complex along with the scaffolding protein RAPTOR, termed mTORC1, it can sense multiple upstream energy-status inputs and non-nutritional signals. Once activated mTORC1 phosphorylates substrates that promote anabolic cellular processes. mTORC1 is required for HSC regeneration under transplantation conditions and lineage-choice decisions. The RAG GTPases activate mTORC1 in response to nutritional input, specifically glucose and amino acids. However, the upstream inputs to mTORC1 governing hematopoiesis are not known. To investigate the role of nutrient sensing signaling to mTORC1 in the hematopoietic system, we utilized Mx1Cre-mediated homozygous deletion of the RagA, which a core recruiter of mTORC1 to the lysosome post amino acid (AA) stimulation. RagA mutant's phenocopy loss of the mTORC1 component Raptor, resulted in mild pancytopenia, splenomegaly and monocytoid cell outgrowth. However, RagA loss did not impair HSC activity under stress conditions. While RagA-deficient HSCs were unresponsive to acute AA changes, they displayed compensatory basal upregulation of mTORC1 activity in response to serum factors, which allowed them to grow under stress conditions. Collectively, manipulation of the nutrient sensing arm of the mTOR pathway is therapeutically attractive in several disease states (3).

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Key Words: Hematopoietic stem cell, Hematological malignancies, mTORC2, mTORC1

S-8-3

Direct conversion of fibroblast into endothelial cells

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Introduction: Previously, we reported direct conversion of adult fibroblasts (FBs) into endothelial cells (ECs) using defined factors in mice. Here, we assessed whether this approach can be applied for transdifferentiation of human adult ECs to authentic ECs.
Methods & Results: We tested whether 5 defined factors (Foxo1, Er71, Klf42, Tal1, Lmo2) for mouse induced ECs (iECs) could convert human dermal FBs (HDFs) to ECs. 28 days after infection of lentiviruses expressing each gene to HDFs, ECs defined by VE cadherin expression on FACS were detected (32.1±5.1%). Interestingly, 2 factors were dispensable, and only 3 factors (factor X, Y, Z) were necessary and sufficient to make human iECs (49.4±3.5%). To enhance the efficiency, a lentivirus expressing 3 factors plus GFP altogether were made using 2A system. Unexpectedly, VE cadherin+/GFP+ cells induced by this virus was integrated with VE cadherin-/GFP+ and HDF control together in hierarchical analysis in whole transcriptome sequencing, which meant VE cadherin + cells were not completely converted yet. The proportion of cells expressing another endothelial specific marker, CD31, together (VE cadherin / CD31 double positive (DP) cells) was only 3.0±0.3%. To get complete conversion, several means were tried. Among them, rosiglitazone (mesenchymal epithelial transition inducer) treatment, prolonged incubation after VE cadherin sorting, and suppression of some FBs specific transcription factors (TFs) using siRNA worked. However, 2nd stage infection of other endothelial specific TFs, shear stress, treatment of VEGF, SB431542 (TGFβ inhibitor) or Wnt modulators, time dependent expression using Tet-on system did not. Final protocol could convert 14.7±0.1% of HDFs into DP cells 6 weeks after infection. DP cells showed characteristics of authentic human ECs (Matrigel tube formation, Ac-LDL uptake, lectin binding, NO production, IF staining for EC markers, characteristic EC morphology on optical and electron microscope, and whole transcriptome sequencing). Our iEC protocol showed the most efficient EC conversion rate, compared with the protocols suggested by other groups.
Conclusions: Our study revealed the efficient protocol to directly convert human adult fibroblasts into authentic ECs.

S-8-4

Highly accurate prediction of CRISPR-Cpf1 activity

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Cpf1 is a recently reported effector endonuclease protein of the class 2 CRISPR-Cas system. Here, we developed a method for evaluating Cpf1 activity based on target sequence composition in mammalian cells in a high-throughput manner. A library of >11,000 target sequence and guide RNA pairs was delivered into human cells using lentiviral vectors. Subsequent delivery of Cpf1 into this cell library induced indels at the integrated synthetic target sequences, which allowed en masse evaluation of Cpf1 activity using deep sequencing. Using this approach, we determined protospacer adjacent motif sequences of two Cpf1 nucleases, from Acidaminococcus sp. BV3L6 and Lachnospiraceae bacterium ND2006 (AsCpf1 and LbCpf1, respectively), and target sequence-dependent activity profiles of AsCpf1. Based on these data sets, we developed Seq-deepCpf1, a deep learning-based algorithm trained on a data set of AsCpf1-induced indel frequencies at 15,000 target sequences, which outperformed conventional machine learning-based algorithms. Subsequent fine-tuning of Seq-deepCpf1 using data sets of AsCpf1-induced indel frequencies at endogenous target sites with chromatin accessibility information enabled the development of DeepCpf1. We provide DeepCpf1 as a web tool, which predicts AsCpf1 activities at endogenous target sites with unprecedentedly high accuracy.

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Key Words: Genome editing, CRISRP-Cas, Cpf1, Cas9, nucleases

S-8-5

Organoid technologies; current limitations and challenges

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Organoids are three-dimensional in-vitro-grown cell clusters with near-native microanatomy that arise from self-organizing stem cells. Organoid based models can provide breakthrough platforms for studying pathophysiology, screening drug efficacy, and predicting drug toxicity. In addition, the organoids are capable of regenerative therapeutics that can restore the damaged organ functions when injected into animal models such as inflammatory bowel diseases. However, there are many limitations to the application of organoids. A high cost for organoid expansion, low viability after cryopreservation, inefficient expansion by spontaneous differentiation and using Matrigel as an extracellular matrix are major obstacles in the clinical and industrial applications. Here I present the current limitations for clinical and industrial application of organoids, and introduce our challenges.

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Key Words: Organoids, Disease model, Regenerative medicine

S-9-1

Calcium – the central regulator of the extrinsic skin aging

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Skin is composed of various cell types, and the most representative skin cells are keratinocytes and melanocytes. Although these cells function differently, calcium ions (Ca^{2+}), which are present at different concentrations inside and outside the cell, play a very important role in the maintenance of cell homeostasis. For example, it is well known that extracellular and intracellular Ca^{2+} plays an important role in keratinocyte differentiation. In particular, calcium signaling in keratinocyte promotes cell differentiation into corneocyte by up-regulating various genes involved in keratinocyte differentiation. Various transient receptor potential (TRP) channels play a role during cell differentiation. E.g., TRPV3 is an ion channel important for terminal keratinocyte differentiation. Activation of TRPV3, which induces Ca^{2+} influx into keratinocyte, results in increased transglutaminase activity, which is essential for the formation of cornified envelope in the epidermis. In addition, other studies have shown that activation of TRPV3 is involved in proliferation of oral epithelial cells and promotes wound healing. In the case of melanocytes, a recent study reported that the photopigment rhodopsin is produced by human epidermal melanocytes (HEMs), which are involved in UV phototransduction. UV exposure rapidly induces Ca^{2+} mobilization via the G_q protein-coupled signaling pathway and leads to early Ca^{2+}-dependent melanin synthesis. Among the various calcium channels, the calcium release-activated calcium channel protein 1 (ORAI1) plays an important physiological role in UV-induced melanogenesis. In the current session, I will briefly review and discuss different roles of Ca^{2+} in keratinocyte and melanocyte physiology.

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Key Words: Keratinocyte, Melanocyte, Calcium channel, Calcium ion, TRP, ORAI1

S-9-2

TRPV channels and post-burn pruritus

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Background: Post-burn pruritus is a common distressing sequela of burn wounds. Empirical antipruritic treatment often fails to have a satisfactory outcome because the mechanism has not been fully elucidated.
Transient receptor potential (TRP) channels are related to pathway of pruritus.

Methods: Sixty-five burn patients with (n = 40) or without (n = 25) pruritus were investigated, including skin biopsies. Keratinocytes and fibroblasts from those samples were separated. Immunohistochemical staining for TRPV3 and TRPA1; and immunofluorescence staining for TSLP, TSLPR, loricrin, involucrin, α-SMA, and TGF-β, were performed on samples of burn scars and normal skin. Real-time PCR and western blotting of TRPV3, TRPA1, PAR2 NK1R, TSLP, and TSLPR were done. We also measured intracellular Ca^{2+} levels in keratinocytes from scars with or without pruritus, following TRPV3 activation and blocking, and measured the effects of PAR2 agonist on TRPV3 function. Expressions of TSLP after TRPV3 activation in keratinocytes were measured by western blotting and real-time PCR.

Results: In Immunohistochemical and immunofluorescence staining, TRPV3, TSLP, and TSLPR stained more intensely the epidermis of the burn scars of post-burn-pruritus patients, than that of non-pruritic-burn patients. Real time-PCR showed that mRNA of TRPV3 and TSLP were significantly more abundant in keratinocytes from pruritic burn scars than in keratinocytes from non-pruritic burn scars. In addition, mRNA and protein levels

of PAR2, NK1R, TSLP, and TSLPR were also significantly increased in pruritic burn scars. With TRPV 3 activation, intracellular Ca^{2+} concentrations were more significantly increased in keratinocytes from pruritic burn scars than in those from non-pruritic ones. In keratinocytes from pruritic burn scars, PAR2 activation markedly potentiated opening of TRPV3 channels. TRPV3 activation itself resulted in little increase of Ca^{2+} influx with PAR2 inhibition in keratinocytes. In keratinocytes from all samples, PLC-β, PKA, PKCs, and PKD inhibitor markedly reduced intracellular Ca^{2+} level by TRPV3 activation, as well as by PAR2 activation. TRPV3 activation also increased mRNA and protein expression of TSLP in keratinocytes.
Conclusions: In conclusion, we confirmed that TRPV3 of keratinocytes and PAR2, NK1R, TSLP, and TSLPR were highly expressed in pruritic burn scars. In addition, it seemed that PAR2 sensitized TRPV3 channels with PKA, PKC, PKD signaling pathways. It also seemed that TRPV3 activation induced TSLP expression.

Key Words: Post-burn pruritus, Pruritus, Transient receptor potential, TRPV3

S-9-3

Keratinocytes in house dust mite-induced atopic skin inflammation

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The pathogenesis of atopic dermatitis (AD) is multifactorial and involves a complex immunologic cascade, including skin barrier dysfunction, defects in the cutaneous cell-mediated immune response, IgE dysregulation, genetic susceptibility factors, and environmental factors. Particularly, defects in epidermal skin barriers lead to elevated
sensitivity to atopic aeroallergens including house dust mite (HDM). The HDM, a ubiquitous organism, has been implicated in the etiology and exacerbation of AD. HDM worsens AD severity through the following mechanisms: inherent proteolytic enzyme activity, activation of proteinase-activated receptors-2, and IgE binding, leading to increased inflammation. HDM is a carrier of not only allergenic proteins, but also microbial adjuvant compounds, both of which can stimulate innate signaling pathways and lead to allergy. However, how the innate immunity triggered by HDM contributes to AD by programming and maintaining Th2-bias adaptive immunity and by the recruitment of inflammatory cells is also not investigated. This presentation will cover the studies on the activation of innate immune system in keratinocytes by HDM I have recently performed.

Key Words: Atopic dermatitis, House dust mite, Inflammation, Innate immune system

S-9-4

A novel synthetic Piper amide derivative NED-180 inhibits hyperpigmentation by activating the PI3K and ERK pathways and by regulating Ca^{2+} influx via TRPM1 channels

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Piper amides have a characteristic, unsaturated amide group and exhibit diverse biological activities, including proliferation and differentiation of melanocytes, although the molecular mechanisms underlying its antimelanogenesis effect remain unknown. We screened a selected chemical library of newly synthesized Piper amide derivatives and identified (E)-3-(4-(tert-butyl)phenyl)-N-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acrylamide (NED-180) as one of the most potent compounds in suppressing melanogenesis. In murine melan-a melanocytes, NED-180 downregulated the expression of melanogenic regulatory proteins including tyrosinase, Tyrp1, Dct, and MITF. PI3K/Akt-dependent phosphorylation of GSK3β by NED-180 decreases MITF phosphorylation and inhibits melanogenesis without any effects on cytotoxicity and proliferation. Furthermore, topical application of NED-180 significantly ameliorated UVB-induced skin hyperpigmentation in guinea pigs. Interestingly, data obtained using calcium imaging techniques suggested that NED-180 reduced the TPA-induced activation of TRPM1 (melastatin), which could explain the NED-180-induced inhibition of melanogenesis. All things taken together, NED-180 triggers activation of multiple pathways, such as PI3K and ERK, and inhibits TRPM1/TRPV1, leading to inhibition of melanogenesis.

Key Words: Piper amides, TRPM1, Melanogenesis, Skin-lightening agent

S-9-5

Calcium ion on skin barrier

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Skin barrier resides in the stratum corneum (SC) composed of corneocytes ('bricks') which surrounded by intercellular lipid lamellae ('mortar') and attached by corneodesmosome (CD) ('rivet') and the tight junctions connecting the lateral walls of upper epidermal keratinocytes. Calcium ion(Ca^{2+}) concentration and its gradient in the epidermis are essential in regulating epidermal differentiation, skin barrier formation, and barrier homeostasis.
Formation of skin barrier and maintenance of barrier homeostasis are important to protect mammalians from external insults. Abnormal epidermal differentiation and barrier function are the primary causes or aggravating factors in some skin problems including atopic dermatitis, psoriasis, allergic contact dermatitis and aged skin. Intracellular Ca^{2+} is stored in the endoplasmic reticulum (ER) and formed the epidermal calcium gradient. The ER calcium homeostasis is crucial for regulating keratinocytes differentiation, intercellular junction formation, permeability barrier homeostasis, and antimicrobial barrier. Both mechanisms of the Ca^{2+} release from intracellular stores such as the ER and the Ca^{2+} influx are important in skin barrier function and its homeostasis.
Therefore, understanding of the Ca^{2+} related mechanism in epidermal differentiation and skin barrier homeostasis will be a first step to understand the pathogenesis of many skin diseases and develop the effective therapeutic strategies.

Key Words: Calcium ion, Skin barrier, Epidermis, Homeostasis, Aging

S-9-6

Clinical application of ion channels in skin

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Intracellular Ca^{2+} signaling *via* various calcium channels, such as Orai1, Transient receptor potential (TRP)A1, TRPV1, and TRPV3, has been shown to directly modulate epidermal proliferation, differentiation, barrier homeostasis, and inflammation. Ca^{2+} influx through these channels eventually generates intracellular Ca^{2+} signaling that results in different outcomes dependent on the individual Ca^{2+} channel type, for example, keratinocyte proliferation and migration through Orai1, epidermal barrier formation and keratinocyte differentiation through TRPA1, and keratinocyte cornification through TRPV3. Therefore, a specific agonist/antagonist for each calcium channel is required for maintaining skin barrier homeostasis and for the treatment of dermatological diseases. To identify applicable topical botanically derived chemicals for use in functional cosmetics or agents for dermatological diseases. We prepared 70% MeOH extracts of 30 medicinal herbs, performed bioassay-guided fractionation of the active extracts, and

then isolated and identified the bioactive constituents. By performing the combination of automated and conventional whole-cell patch clamp studies, we found eight medicinal herb fractions for Orai1, four for TRPV1, two for TRPA1, and one for TRPV3 that showed >50% inhibition rates at 30 μg/mL. We also found three fractions with TRPA1 agonist activity. Further, we also identified chemical constituents that inhibit Orai1 (compound V: 95 ± 5% inhibition at 90 μM) and TRPV1 (compound M: 93.9 ± 2.45% inhibition at 90 μM; compound CYP: 61 ± 5% inhibition at 90 μM). Chemical constituents that showed agonist/antagonistic effects on TRPA1 and TRPV3 also will be discussed.

Considering that most regional plants have not been investigated chemically or pharmaceutically, they remain as untapped potential sources of topical agents for cosmetics and drugs. We found major active components and chemical constituents of plant extracts for the modulation of various calcium ion channels, which may have potential clinical applications for abnormal skin barrier functions such as atopic dermatitis, elastosis, and contact dermatitis and so on.

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Key Words: Keratinocytes, Transient receptor potential, Differentiation, Calcium release-activated calcium channel, Natural products

Yudang Academic Award

APE1/Ref-1 as a therapeutic target molecule

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Apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1), also known as APEX1, is the mammalian ortholog of *Escherichia coli* Xth (exonuclease III). APE1/Ref-1 function as an apurinic/apyrimidinic endonuclease in the DNA base repair pathway and modulator of the redox status of several transcription factors such as activator protein-1 (AP-1), nuclear factor kappa B (NF-κB). Knockout of APE1/Ref-1 in mice causes embryonic lethality, suggesting essential for cell viability and genomic stability. Studies have identified a nuclear localization signal, nuclear export system, and a mitochondrial target sequence in APE1/Ref-1 as determinants of APE1/Ref-1 subcellular distribution. Moreover, it was shown that APE1/Ref-1 is secreted in response to hyperacetylation at specific lysine residues.

APE1/Ref-1 is playing a pleiotropic role in controlling cellular responses to oxidative stress. APE1/Ref-1 reduces intracellular production of reactive oxygen species (ROS), and overexpression of APE1/Ref-1 suppresses tumor necrosis factor-α-induced monocyte adhesion to endothelial cells and inhibits hypoxia-induced endothelial-cell apoptosis. Moreover, intracellular APE1/Ref-1 inhibits balloon-injury-induced neointimal formation in rats, suggesting that it exhibits anti-inflammatory functions in the vascular endothelium. Since the new concept of APE1/Ref-1 secretion is established in 2013, several studies have been conducted in the usefulness of serological biomarker for certain diseases such as cancer or vascular inflammatory disorder. The presence of extracellular APE1/Ref-1 was reported in supernatant of hyperacetylated cells and in plasma of endotoxemic rats. However, the binding receptor of secreted APE1/Ref-1 is not clear. Recently, hyperacetylation in vivo animal models caused secretion of acetylated (Ac)-APE1/Ref-1 into the blood, where the factor bound directly to advanced glycation end products (RAGE), suggesting putative binding receptor of APE1/Ref-1. Ac-APE1/Ref-1-stimulated apoptosis was markedly reduced in RAGE-knockdown cell or in vivo xenograft tumors, suggesting therapeutic usefulness of Ac-APE1/Ref-1.

Recombinant human APE1/Ref-1 protein with reducing activity induced a conformational change in TNF-α receptor or Toll-like receptor by thiol-disulfide exchange. Therefore, extracellular non-acetylated APE1/Ref-1 play as thiol-dependent antioxidant system in defense against oxidative stress. Furthermore, recombinant human APE1/Ref-1 inhibited VCAM-1 expression in interleukin-1β-stimulated endothelial cells and inhibited inducible nitric oxide synthase or cyclooxygenase-1 expression in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. These results strongly indicate that anti-inflammatory effects of recombinant APE1/Ref-1 is useful as therapeutic biomolecules against vascular inflammation.

Key Words: APE1/Ref-1, Biomarkers, Vascular inflammation, Therapeutic biomolecules

P1-01

Bee venom decreases hot water-induced pain in mice

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This study was designed to investigate the inhibitory effect of bee venom on the burn injury-induced pain in mice. In order to develop scalding burn injury, right hind foot was immersed in 65℃ of hot water for 2 second. Bee venom (0.01, 0.02 or 0.1 mg/kg in saline) was subcutaneously applied into the ipsilateral knee area once daily for 14 days. Von Frey filament test was performed to assess pain response and change of gait parameters were evaluated by CatWalk automated gait analysis system. Additionally, changes of foot appearance was observed by photograph for 14 days. Burn injury evoked mechanical hypersensitivity, decreased paw related parameters of gait analysis test, and increased tissue damages in ipsilateral foot. Repeated bee venom treatment significantly ameliorated burn injury-induced mechanical allodynia, gait analysis test responses and tissue damages. In conclusion, results of this study suggest that the bee venom may be a good candidate to treat burn–related pain.

Key Words: Bee venom, Burn injury, Pain

P1-02

Endogenous TRPV4 expression of a hybrid neuronal cell line and its utilization for ligand screening

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Immortalized neuronal hybrid cell lines enable rapid exploring questions arising in the neuroscience field. Utility of sensory neuronal cell lines is promising in terms of investigation on the sensory neuronal physiology and pharmacology. However, only a limited number of the sensory neuronal cell lines are currently utilized and their functional sensory properties have been rarely described. N18D3 cell line was developed more than a decade ago and a part of mechanisms whereby oxidative neuronal damages or peripheral neuropathy has been elucidated by studies using this line. However, its sensory function itself has not been assessed although the cell line is of sensory neuronal origin. The present study focuses on its sensory features by examining whether a repertoire of sensor molecules, the sensory TRP ion channels operate in this hybrid cell line. Furthermore, in order to determine pharmacological usefulness of it, we tried to take advantage of this line to discover a novel ligand acting on its functional TRP ion channel.

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Key Words: TRPV4, Ligand, N18D3, Hybrid cell line, Channel

P1-03

The interaction between N-methyl-d-aspartate receptors (NMDAR) GluN2B and postsynaptic density protein 95 (PSD-95) contributes in the neuropathic pain

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N-Methyl-D-aspartate receptor (NMDAR) GluN2B subtype is gaining attention because blocking of its activity is reported to reduce neuropathic pain with minimal side effects. However, the effectiveness of GluN2B antagonist on neuropathic pain following peripheral nerve injury and the signal transduction pathways associated with GluN2B activation are not known. After spinal nerve ligation (SNL), we investigated the temporal changes in GluN2B, its phosphorylation residue at Ser1303 and Tyr1472, calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC) and postsynaptic density protein 95 (PSD-95) in the dorsal spinal cord. Co-immunoprecipitation was used to examine the interaction between p-Ser1303 and CaMKII, PKC and PSD-95, respectively. Mechanical paw withdrawal threshold was measured before and after intrathecal administration of relevant drugs in SNL. Protein expression of GluN2B increased from 6 hours to 4 days and p-Ser1303 expression increased up to 2 weeks. The interaction between p-Ser1303 and CaMKII was robustly enhanced from 6 hours to 4 days, and that between p-Ser1303 and PSD-95 was increased from 4 days to 2 weeks after injury. Both antagonists of GluN2B and CaMKII reduced the interaction between p-Ser1303 and PSD-95, and more effectively reduced mechanical allodynia in the early period than it did in the later period. These results demonstrate that GluN2B antagonist effectively reduced mechanical allodynia in the early period and CaMKII phosphorylates Ser1303, which then interacts with PSD-95. Glutamate signaling through GluN2B enhances the interaction between p-Ser1303 and PSD-95. This interaction may play a role in the long term maintenance of neuropathic pain.

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Key Words: Peripheral nerve injury, Neuropathic pain, GluN2B, Ser1303, PDS-95

P1-04

Amelioration of gait impairments by extra-synaptic NMDA receptors antagonist in MPTP-induced Parkinson's model mice

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Parkinson's disease (PD) is a major neurodegenerative disorder, clinically characterized by onset of motor symptoms including resting tremors, rigidity, slowness of movement and postural instability. Progressive loss of dopaminergic neurons in substantia nigra pars compacta (SNpc) hampered rhythmical walking in PD patients. Studies suggest that gait readouts such as walking duration, step cycle, duty cycle, stance and cadence reflects PD behaviours and loss of dopaminergic neurons in SNpc. Here we confirmed MPTP mouse models mimic many aspects of PD by using computer-assisted CatWalk tests. In MPTP model mice we observed decreased stride lengths of limbs with increased run duration and cadence (step/s) was dropped significantly with compared to control. Also, swing speed of paws was decreased markedly whereas stance, duty cycles and step cycles of paw increased substantially suggesting MPTP model mice spend more time to change their paws during walking, results in slowness of movement.

Furthermore we evaluate pain perception and confirmed that MPTP-induced pain was not cause for the altered gait readouts. Numerous efforts had done to improve gait deficits in PD, here our results demonstrate that extrasynaptic NMDA receptor's (eNMDAR) antagonist, memantine, ameliorates gait deficits in MPTP induced PD model mice.

Key Words: Catwalk, Gait, MPTP, Memantine, Parkinson's disease

P1-05

Pheripheral and central TRPV1 expression following dental pulp inflammation

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Considerable evidence suggests that the pulpal inflammation produces significant changes in peripheral and central nervous system, which induces hyperalgesia. These inflammatory modifications have been strongly correlated with the response of the transient receptor potential vanilloid 1(TRPV1) channel, best known for its role in sensory transmission in the nociceptive neurons of the peripheral nervous system. The protein product c-Fos of the proto-oncogene *c-fos* is also widely applicable marker of nociceptive neuronal activation. However, the activation of TRPV1 and c-Fos following pulpal noxious stimulation has not been investigated in central nervous system yet. The aim of the present study was to verify whether experimentally-induced pulp inflammation activates the expression of TRPV1 and c-Fos, peripherally (trigeminal ganglion neurons) and centrally (spinal trigeminal nucleus).

Acute pulpitis was assigned to Sprague–Dawley rats through pulp exposure and application of complete Freund's adjuvant or saline. Additional animals with no tooth preparation served as a control group. Three days post CFA or saline application, face grooming activity was recorded and analyzed then rats were sacrificed in order to conduct histological and immunohistochemical analyses on teeth, trigeminal ganglion and spinal trigeminal nucleus levels.

At the results, we observed significantly increased nociceptive behaviors in CFA-treated animals simultaneously with histological evidence of a severe pulp inflammation. In trigeminal ganglions belonging to CFA group, c-Fos labelling showed that neuronal activity was significantly higher as compared to saline group ($p<0.05$) along with statistically higher immunoreactivity for TRPV1 ($p<0.05$). In the spinal trigeminal nucleus, the lack of immunoreactivity for c-Fos was indicative for the absence of neuronal activity in the intermediate region (trigeminal nucleus interpolaris) in all animals with a comparable expression of TRPV1 between all groups. In contrast, neurons in the caudal region of the spinal trigeminal nucleus (trigeminal nucleus caudalis) were able to fix c-Fos and TRPV1 markers at a higher rate in CFA group with no statistical difference. The superficial lamina of trigeminal nucleus caudalis showed significant higher expression of TRPV1 in comparison to the deep layers.

In conclusion, the findings of this study indicated that acute pulp inflammation triggered peripherally a robust neuronal activation in trigeminal ganglion with a significant involvement of TRPV1 in nociceptive signal processing. Unlike the central TRPV1 channels which appeared to play a less prominent role as compared their peripheral counterparts.

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Key Words: pulpal inflammation, TRPV1 receptor, tigerminal ganglion, subnucleus interpolaris, subnucleus caudalis

P1-06 (PO-A-1)

In vivo voltage-sensitive dye imaging of the insular cortex after mTOR inhibition in nerve-injured rats

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The insular cortex (IC) is mainly involved in discriminative sensory and motivative emotion. Recent studies have shown that mTOR kinase is closely related to initiation of chronic pain. The mTOR kinase is major regulator of protein synthesis. It could be involved in the regulation of synaptic plasticity and memory formation in the central nervous system. Our previous report showed that inhibition of mTOR affects neuropathic pain control. However, no studies have been reported on the immediate brain activity changes in the IC upon mTOR inhibition. The present study was conducted to investigate spatiotemporal patterns related to mTOR inhibition in the IC after nerve injury using voltage-sensitive dye optical imaging. Under isoflurane anesthesia, the neuropathic pain surgery was conducted to Sprague-Dawley rats, and craniotomy was performed for optical imaging after 7 days. Electrical stimulations were applied to the injured-hind paw, and the responses of neuronal activities were recorded and analyzed. To induce the mTOR inhibition, Torin1 and XL388, which have been known as inhibitors of mTOR complexes 1 and 2, respectively, were used. Also, we used a vehicle for comparison. The vehicle group has significantly increased peak amplitude signals and showed enlarged activation area at 1.25, 2.5, and 5 mA. In contrast, the drug treatment group showed decreased signals and reduced activation area. These results indicate that blocking the mTOR complexes 1 and 2 is strongly associated with attenuation of neuronal activity in the IC. These results suggest that modulating both mTOR complexes 1 and 2 can decrease the neuropathic pain.

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Key Words: Neuropathic pain, mTOR inhibitor, Insular cortex, Optical imaging, Neuroplastic change

P1-07 (PO-A-2)

Pain alleviation via inhibition of mTOR pathway in the insular cortex

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After peripheral nerve injury, neuronal changes in the injured-nerve could induce the neuropathic pain, such as allodynia and hyperalgesia. Signaling via mammalian target of rapamycin (mTOR), a regulation kinase of protein synthesis, has been suggested a possible involvement in development of chronic pain. mTOR forms two distinct protein complexes named mTOR complex 1 (mTORC1) and complex 2 (mTORC2). Our previous report has shown the inhibition of mTOR signaling in the insular cortex (IC) alleviates neuropathic pain after peripheral nerve injury. However, it has not been examined whether mTORC1 or C2 plays a key role of pain modulation in the IC. Especially, the role of complex 2 in pain control has not yet been identified. This study was to investigate the specific role of mTOR in the development of chronic pain through selective inhibition of mTORC1 and C2. Torin1 and XL388, the selective ATP-competitive inhibitor of mTORC1 and C2, respectively, were used to reveal the interaction of the mTORCs in our study. Neuropathic pain was induced by ligation of tibial and sural nerves on adult male Sprague-Dawley rats. Mechanical allodynia was assessed at pre- and post-injection of drugs. Each drug (Torin1 or XL388) was microinjected into the IC on postoperative day 7. Four hours after drug injection, IC tissues were extracted and stored for the analysis. Western blot was carried out in order to ascertain the changes in mTOR and its downstream effectors. The mechanical threshold test showed a remarkable pain decrease in the mTOR

inhibition group. The phosphorylation of the mTORC1 downstream effectors, p-P70S6K and p-4EBP, were significantly increased in the vehicle-treated group. However, they did not change at the drug-treated groups. The mTORC2 downstream effectors, p-Akt and p-PKCα, also increased in the vehicle-treated group while significantly reduced in the mTOR inhibitor-treated groups. In addition, the expression of p-mTOR significantly increased in the vehicle-treated group but, decreased in the drug-treated groups. Changes in the mechanical allodynia and the regulation of p-mTOR and mTORC1 downstream effectors after mTOR inhibition were consistent with our previous results. However, p-Akt and p-PKCα were found to be the lowest in the XL388-treated group than in the Torin1-treated group. These data suggest that mTORC1 is more effective in chronic pain modulation than mTORC2 in the IC.

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Key Words: Neuropathic pain, Insular cortex, mTOR pathway, Western blot, selective inhibition of mTOR

P1-08

Neuroprotective effects of lipid emulsion in kainic acid-induced neural injury in the rat hippocampus

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Numerous anti-epileptic drugs (AEDs) have been successful in attenuating seizures. However, neuroprotective methods for the neural injuries that occur after seizures are still in jeopardy due to the lack of specificity in targeting protective markers. In recent research, lipid emulsion (LE), a well-known parental nutrition has attenuated excitotoxic cell death caused by local anesthetics in myocardial cells. Intravenous LE was reported to provide cytoprotection via various mechanisms involving fatty acid interactions at molecular levels. In our study, we tested the cytoprotective effect of LE on excitotoxic injury in the brain and investigated its protective mechanisms. This study used male Sprague Dawley rats which were divided into 4 groups: vehicle, KA+vehicle, KA+LE 0.01%, and KA+LE 1%. The experimental animals underwent guide cannulae implantation surgeries under pentobarbital anesthesia. Bilateral intrahippocampal injections of KA and LE were given to the experimental groups. Protective effects were observed for both LE-pretreated animals, and animals that were treated with LE after the induction of acute seizures. After the treatment of the experimental groups, the passive avoidance behavioral tests was performed and analyzed. After the behavioral tests, brains were perfused and stained with cresyl violet to measure morphological changes. As a result, LE with the concentration of 1% increased the survival of neurons in the pyramidal tract of the hippocampus. Behavioral analysis also displayed less impairment of cognition in the passive avoidance test compared to the control group. Wnt1 was up-regulated as a potential protective biomarker at molecular levels which may be due to the palmitate bonding provided by LE. As a result, the canonical Wnt signaling pathway can be a potential target for neuroprotection via fatty acid interaction. Our data suggest that the nutritional aspects of phytochemicals may provide neuroprotection. Further studies that accompany phytochemicals with existing AEDs may lead to new treatments and possibly aid patients suffering from cognitive deficits caused by neurodegenerative diseases.

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Key Words: Epilepsy, Lipid emulsion, Neuroprotection, Kainic acid, Wnt signaling

P1-09

Pharmacological characterization of low doses of ibuprofen and dexamethasone attenuate trigeminal neuropathic pain in rats

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To evaluate the effect of combined therapy of dexamethasone and ibuprofen on neuropathic mechanical allodynia in rats with inferior alveolar nerve injury. Sprague-Dawley male rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). Under anesthesia, the left lower second molar was extracted, followed by the placement of a mini-dental implant to intentionally injure the inferior alveolar nerve. Inferior alveolar nerve injury, induced by the mal-positioning of dental implants, produced a significant mechanical allodynia on postoperative day (POD) 1 and persisted until POD 30. Our current findings show that a nerve injury induced by mal-positioned dental implants produces significant mechanical allodynia and then intraperitoneal injection of high doses of ibuprofen (30 mg/kg) or dexamethasone (25, 50 mg/kg) inhibited mechanical allodynia, but low doses of ibuprofen (1, 5, 10 mg/kg) or dexamethasone (2.5 mg/kg) did not attenuate neuropathic mechanical allodynia in rats with inferior alveolar nerve injury. We examined effects of combined treatment with low doses of ibuprofen (5 mg/kg) and dexamethasone (0.01, 0.1, 1 mg/kg) on neuropathic mechanical allodynia on POD 1, 2, 3 (early treatment) respectively. Early combined treatment with ibuprofen (5 mg/kg) and dexamethasone (0.1, 1 mg/kg) significantly inhibited mechanical allodynia. This anti-allodynic effect was recovered within 24 hours after injection. We also examined combined treatment with ibuprofen and dexamethasone on mechanical allodynia on POD 7, 8, 9 (late treatment). Similar to early and late treatment with ibuprofen (5 mg/kg) and dexamethasone (0.1, 1 mg/kg) also significantly inhibited mechanical allodynia. Anti-nociceptive effect of combined treatment of low doses ibuprofen and dexamethasone is compatible to effects of gabapentin treatment (30, 100 mg/kg). We confirmed anti-nociceptive effects of combined therapy on neuropathic mechanical allodynia by analysis of c-fos expression. Inferior alveolar nerve injury produced significantly increases in c-fos immuno-positive cells in the medullary dorsal horn on POD 3. Combined treatment with ibuprofen (5 mg/kg) and dexamethasone (1 mg/kg) significantly inhibited the number of *c-fos* immuno-positive cells on POD 3, respectively. These results suggest that combined treatment with low doses of ibuprofen and dexamethasone, which inhibited development of the trigeminal mechanical allodynia after nerve injury, is a new potential therapeutic target for neuropathic pain control including the orofacial area pain

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Key Words: Neuropathic pain, Ibuprofen, Dexamethasone

P1-10 (PO-A-3)

Acute fasting induced-analgesia

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Pain is considered to be both sensory and emotional experience. Since emotion is affected by homeostasis such as temperature, hunger, satiety and thirsty, the perception of pain is also likely to be regulated by homeostatic control. Feeding behavior is crucial for maintaining homeostasis and it is well known that pain perception is changed in eating disorder patients. Endocannabinoid and opioid system are known to modulate pain as well as feeding homeostasis. However, little is known about the relationship be-

tween feeding homeostasis and pain. In the present study, we examined whether pain perception is affected by change of feeding homeostasis, with focus on endocannabinoid system, especially the cannabinoid receptor type 1 (CB1) and opioid system using formalin-induced acute inflammatory pain model in mice. We found that 24h acute fasting suppressed formalin-induced paw licking behavior in the second phase. Formalin-induced c-Fos expression was also suppressed by 24h acute fasting. Intraperitoneal administration of CB1 receptor antagonist (SR 141716, 10 mg/kg) and opioid receptor antagonist (naloxone, 10 mg/kg) inhibited acute fasting-induced analgesic effect. Taken together, our results suggest that acute changes in feeding homeostasis lead to analgesic effects, which are associated with endocannabinoid and opioid system.

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Key Words: Formalin test, c-Fos, Acute fasting

P1-11

Understanding the mechanism of odor-specific memory formation in Caenorhabditis elegans

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Forming associations between specific odors and their environmental context is important for an animal's survival. Likewise, *C. elegans* can sense a multitude of odors and form simple but context-specific memories. Unlike the mammalian system where each odor-sensing neuron expresses one receptor gene, each *C. elegans* sensory neurons express many receptor genes. In the AWC neuron, prolonged exposure to an odor in the absence of food induces a signaling cascade that converges at the nuclear localization of a cGMP-dependent kinase EGL-4. Despite the overlap in signaling pathway, however, worms can form odor-specific memories within the same neuron. To find out how this occurs, we set out to expand the repertoire of odors to be used for odor studies. We identified over a dozen additional odors that are attractive to worms and identified the sensory neuron responsible for detection. We also established a new odor behavior assay to observe odor memory formation in real-time, as worms travel between two ends of the plate with and without the odor. Interestingly, we found that the timing of odor memory formation varied depending on the odor, and it roughly correlated with the timing of EGL-4 nuclear localization. Our results suggest that there may be subtle differences in the downstream signaling pathway subsequent to odorant receptor activation that leads to different timing of odor memory formation. We are currently using the odors to study cross-adaptation among AWC-sensed odors, as well as identifying odor-receptor pairs.

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Key Words: Odor, Memory, Behavior, Sensory, C. elegans, Smell, Neuron, PKG, Learning, Adaptation

P1-12

Interaction of nNOS with PSD-95 modulated by D-serine leads to the induction of mechanical allodynia in a mouse model of neuropathic pain

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It has been suggested that interaction of neuronal nitric oxide synthase (nNOS) with postsynaptic density-95 (PSD-95) plays an important role in the nociception. Here we examined whether spinal D-serine modulates the interaction of nNOS with PSD-95, and whether this interaction increases the phosphorylation of NMDA receptor GluN1 subunit (pGluN1) in mice with chronic constriction injury (CCI) of sciatic nerve. Sciatic nerve injury increases the interaction of nNOS with PSD-95, and this increase was significantly attenuated by intrathecal administration of the serine racemase inhibitor, LSOS or the D-serine degrading enzyme, DAAO. Administration of the nNOS-PSD-95 interaction disruptor, IC87201 suppressed the CCI-induced increase in both total NO levels and PKC-dependent (Ser896) pGluN1 in the lumbar spinal cord dorsal horn. In addition, sciatic nerve injury elicited a significant translocation of PKC-ε isoform from the cytosol to the membrane fraction in the lumbar spinal cord dorsal horn. Administration of IC87201 significantly inhibited the CCI-induced translocation of PKC-ε, while expression of PKC-α and -ξ in the cytosol and membrane fraction did not change by sciatic nerve injury or injection of IC87201. Furthermore, administration of the PKC-ε inhibitor, εV1-2 attenuated the CCI-induced development of mechanical allodynia as well as increase in pGluN1. Collectively these results demonstrate that spinal nNOS-PSD-95 interactions modulated by D-serine play a key role in the PKC-dependent GluN1 phosphorylation via activation of PKC-ε isoform, ultimately contributing to the development of mechanical allodynia induced by peripheral nerve injury.

Key Words: D-serine, Neuronal nitric oxide synthase, Postsynaptic density-95, GluN1, Phosphorylation, Mechanical allodynia

P1-13

Response of dorsal root ganglion neurons innervating intervertebral disc to transient receptor potential vanilloid 1 agonist (capsaicin) and ankyrin 1 agonist (allyl isothiocyanate)

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Intervertebral disc (IVD) can be a major source of low back pain (LBP). Some studies reported that degenerated IVD releases cytokines, beta-nerve growth factor (β-NGF), and brain-derived neurotrophic factor (BDNF), and that in dorsal root ganglion (DRG) these neurotrophins induce the upregulation of the transient receptor potential vanilloid 1 and ankyrin 1 (TRPV1 and TRPA1, respectively). However, it is not clear whether TRPV1 and TRPA1 can participate in sensory or nociceptive processing associated with IVD. The purpose of this study was to characterize the inward current and calcium influx activated by TRPV1 agonist (capsaicin) and TRPA1 agonist (allyl isothiocyanate, AITC) in DRG neurons innervating lumbar IVD. We used male SD rats (300~350g, Orient Bio., South Korea) and injected DiI (3μl; a lipophilic and fluorescent dye) into L4-5 IVD under anesthesia. Two weeks later, neural cells were extracted from T13-L4 DRG and the dissociated cells were plated onto circular glass coverslips coated with poly D-lysine. Intracellular calcium imaging and whole-cell patch clamp technique were used to check capsaicin and AITC response in DiI-labeled neurons. Intracellular calcium imaging revealed that labeled neurons responded to 1μM capsaicin (45/58; 78%) and 30μM AITC (32/91; 35%). Capsaicin or AITC-induced peak inward current density (pA/pF) of labeled neurons were measured

in dose-dependent manner (responder/tested cells; Capsaicin, 0.03μM, n=2/10, 0.1μM, n=8/12, 0.3μM, n=7/12, 1μM, n=8/11, 3μM, n=7/11, 10μM, n=8/12; AITC, 3μM, n=0/10, 10μM, n=7/14, 30μM, n=8/14, 100μM, n=8/14, 300μM, n=6/14), and a calculated EC50 of dose-response curve (fitted to four-parameter logistic equation) was 0.82μM (capsaicin) and 16.14μM (AITC). The present study implicates that the nociceptive information from IVDs can be multi-segmentally transmitted to lumbar DRG neurons and TRPV1 and TRPA1 in these DRGs can have a critical role in discogenic low back pain.

Key Words: Intervertebral disc (IVD), Whole-cell patch clamp, Calcium imaging, TRPV1 agonist capsaicin, TRPA1 agonist allyl isothiocyanate, peak inward current density

P1-14

Layer-specific activation of synaptic inputs onto layer 2/3 pyramidal neurons in the prefrontal cortex of rat

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The prefrontal cortex especially, orbitofrontal cortex, is implicated in behavioral flexibility, goal-directed decision-making, reversal learning, odor processing and reward. In layer 2/3 pyramidal neurons (L2/3 PyNs) of the prefrontal cortex, distal apical dendrite in layer 1 receives nonspecific thalamocortical (TC) input from the midline, intralaminar, and paralaminar thalamic nuclei whereas the perisomatic dendritic area including basal dendrites in layer 2/3 receive various synaptic inputs from other cortex (corticocortical), striatum, amygdala, and hippocampus. Thus L2/3 PyNs are well suited for the integration of these TC and diverse synaptic inputs. Although the properties of the information flow in these synaptic inputs are critical for understanding of cortical information processing, almost little is known yet due to the structural and functional complexity of the prefrontal cortex. Here, we investigated the layer-specific synaptic activation with local extracellular electrical stimulation and FM1-43 dye unloading in prefrontal cortex of rat. Under whole-cell recording of L2/3 PyNs, tungsten (100 μm diameter) and glass (10-20 μm diameter) electrodes were positioned at layer 2/3 (L2/3) and 1 (L1). The amplitude of the arithmetic sum of individual EPSPs evoked by stimulation of L1 and L2/3 was comparable to EPSPs evoked by combined stimulation of both layers. EPSPs evoked by stimulation of L1 have shown slow kinetics than L2/3. Moreover, paired-pulse ratio was different between L1-EPSPs and L2/3-EPSPs. These results suggest that electrical stimulation of L1 and L2/3 activated independent inputs onto L2/3 PyNs. To confirm the layer-specificity of synaptic activation with electrical extracellular stimulation, FM1-43 dye unloading of the prefrontal cortex was conducted. After load of FM1-43 dye into prefrontal cortical slices by the short bath application of high K^+ (40 mM K^+), electric stimulation (5 Hz) was delivered to either L1 or L2/3 with extracellular stimulus electrodes. FM1-43 in L1 was unloaded only by electrical stimulation of L1 but not by L2/3. Likewise, FM1-43 near the soma was unloaded by electric stimulation of layer L2/3 but not of L1. Thus, these results indicate that local electrical stimulation of L1 and L2/3 specifically activates inputs in distal apical dendritic and perisomatic basal dendritic compartments, respectively. Supported by Basic Science Research Program through the NRF funded by the Ministry of Education, Science and Technology (2016R1A2B2016533).

Key Words: Prefrontal cortex, Orbitofrontal cortex, FM1-43, Input-specific, Layer-specific

P1-15

Dental primary afferent (DPA) neuron types revealed by single-cell RNA sequencing

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Dental primary afferent (DPA) neurons were considered all pain-sensing neurons defined as nociceptive afferents, because tooth pulp produces only pain. However, virtually weak mechanical stimuli such as air puffs evoke pain known as dentin hypersensitivity at exposed dentin. To elucidate this paradox of pain from tooth pulp, we aimed transcriptional profiling of mouse DPA neurons using single-cell RNA sequencing. In this study, DPA neurons were retrograde labeled in vivo by filling a fluorescent tracer 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) into the first upper molars in mice. Two weeks later, cultured DiI-labeled DPA neurons were examined for isolectin B4 (IB4), and collected with micromanipulation using glass patch pipettes. We confirmed that representative ion channels TRPV1, TRPM8, and Piezo2 mRNA expressions and soma size distributions of DPA neurons using single-cell RT-PCR. Next, we conducted profiling 12 individual DiI-labeled DPA neurons by single-cell RNA sequencing and classified heterogeneous DPA neuron types in size-based analysis. Therefore, this study may reveal that the unique characteristics of DPA neurons in the trigeminal system to understand dentin hypersensitivity.

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Key Words: Dental Primary Afferent (DPA), Dentin hypersensitivity, Single-cell RNA sequencing

P1-16

Constitutive activities of TRPC3 and NALCN channels drive pacemaking in SNc dopamine neurons

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Dopamine neurons in the substantia nigra pars compacta (SNc) are slow pacemakers that generate spontaneous action potentials, regularly. Although this pacemaking activity is very important in maintenance of background dopamine levels and proper functioning of basal ganglia, it is not clear what channels play a major role in driving of pacemaking in SNc dopamine neurons. Here we report that two leak cation channels, TRPC3 and NALCN channels are essential for pacemaking in SNc dopamine neurons. In both midbrain slices and acutely dissociated SNc dopamine neurons, we found that pyr10, a selective blocker for TRPC3 channels, completely inhibited spontaneous firing and hyperpolarized membrane potentials. In this condition, somatic current injection like leak currents revived firing activity again, suggesting that TRPC3 channels drive pacemaking as a part of leak channels, that have been regarded as major inward currents in midbrain dopamine neurons during the initial phase of pacemaking cycle. However, spontaneous firing survived in dopamine neurons of TRPC3 knockout (KO) mice and the spontaneous firing rate did not differ between TRPC3 KO and wild type mice. Nevertheless, in the TRPC3 KO mice, pyr10 did not affect spontaneous firing rates at all, indicating that pyr10, in wild type mice, abolished pacemaking by specifically blocking only TRPC3 channels. In TRPC3 KO mice, blockade of NALCN channels stopped spontaneous firing and hyperpolarized membrane potential, which was greater than the changes in wild type mice, indicating that NALCN channels completely compensate TRPC3 channel currents in TRPC3 KO mice. In wild type mice, blockade of NALCN channels completely inhibited spontaneous firing, indicating that NALCN is also essential for pacemaking. Taken together, we could conclude

that TRPC3 and NALCN channels are two major channels that drive pacemaking in SNc dopamine neurons.

Key Words: TRPC3, NALCN, dopamine neuron, pacemaking

P1-17

Neuroprotective effects of 2-arachidonoylglycerol on hippocampal neuropathology following pilocarpine-induced status epilepticus

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Endocannabinoids regulate neuronal excitability by activating presynaptic cannabinoid receptor type I(CB1 receptor) and inhibiting neurotransmitter release in a retrograde manner. Endocannabinoids induce neuroprotection, anti-inflammation, anti-excitotoxicity and regulate calcium homeostasis in the central nervous system (CNS). 2-arachidonoylglycerol(2AG) and anandamide(N-arachidonoylethanolamine, AEA) are the most-characterized endocannabinoids. Endocannabinoids are known to suppress seizure activity in pilocarpine-induced status epilepticus. However, the effects of endocannabinoids on pathological neuronal changes in the hippocampus after status epilepticus are not well understood. In this study, we investigated the effects of 2AG on NMDA-induced neuronal and glial cell pathological changes in cultured rat hippocampal neurons using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide(MTT) assay. In addition, we investigated the effects of 2AG on NMDA-induced synapse loss using an imaging-based assay to detect the intensity of green fluorescence expressing postsynaptic density 95(PSD95). Moreover, we recorded SE onset time and mortality after pilocarpine-induced status epilepticus in mice. We showed that 2AG protected against pilocarpine-induced hippocampal neuropathology *in vitro* and *in vivo*. Also, 2AG suppressed status epilepticus-induced neuroinflammation in mouse hippocampus. Collectively, these results indicate that 2AG has a neuroprotective effect on status epilepticus-induced synapse loss followed by neuronal cell death and glial activation, and these results would contribute to develop an effective drug for the treatment of various neurodegenerative diseases, especially status epilepticus.

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Key Words: Endocannabinoids, Status epilepticus, Synapse loss, Neuronal death, Glial activation

P1-18 (PO-A-4)

The role of Intrinsic plasticity of cerebellar Purkinje cell in cerebellum-dependent motor learning

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Intrinsic plasticity of cerebellar Purkinje cells (PCs) is highlighted the other side of memory engram in the cerebellar local circuits, however, the physiological impact on the cerebellar learning and memory remains elusive. Using a mouse model of memory consolidation deficiency, we found that the intrinsic plasticity of PCs may be involved in motor memory consolidation. Gain-up training of the vestibulo-ocular reflex produced a decrease in the synaptic weight of PCs in both the wild-type and knockout groups. However, intrinsic plasticity was impaired only in the knockout mice. Furthermore, the observed defects in the intrinsic plasticity of PCs led to the formation of improper neural plasticity in the vestibular nucleus (VN) neurons. Our results suggest that the synergistic modulation of intrinsic and synaptic plasticity in PCs is required for the changes in the local connectivity between the cerebellum and VN that contribute to the long-term storage of motor memory.

Key Words: Cerebellum, Intrinsic plasticity, Purkinje cell, Vestibulo-ocular reflex (VOR), Vestibular Nucleus

P1-19

Licochalcone A attenuates status epilepticus-induced synaptic degeneration

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Status epilepticus(SE) is a life-threatening neurologic disorder that evokes neuronal cell death and microglial activation. In fact, reduced synaptic density often occur prior to neurodegeneration. Licochalcone A (Lico A), a flavonoid derived from licorice root (Glycyrrhiza glabra) is known to have anti-inflammatory, anti-cancer, anti-oxidative stress properties. Licochalcone A has been also shown to protect the loss of dopaminergic neurons. However, the synapto-protective effects of licochalcone A on status epilepticus-induced neurodegeneration have not been elucidated. In this study, we evaluated effects of licochalcone A on NMDA-induced neurotoxicity in cultured rat hippocampal neurons using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide(MTT) assay. We also investigated effects of licochalcone A on NMDA-induced synapse loss using an imaging-based assay to quantify synapse number of green fluorescence puncta expressing postsynaptic density 95(PSD95). Moreover, we tested effects of Licochalcone A on SE-induced neurodegeneration in a mouse model of pilocarpine-induced SE. Collectively, these results indicate that licochalcone A has a synapto-protective effect on status epilepticus-induced synapse loss followed by neuronal cell death and could be a potent synapto-protective agent against NMDA-induced neurodegeneration, with therapeutic benefits for patients with seizure disorder and neurodegenerative disorders.

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Key Words: Licochalcone A, Status epilepticus, Synapse, NMDA, PSD-95

P1-20

The effects of fear conditioning on spontaneous firing of cerebellar Purkinje cells

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In recent studies, the cerebellum is considered as one of the brain regions related to non -motor function. Although the previous study suggested that long-term potentiation (LTP) of the synapse between parallel fibers (PFs) and Purkinje cell (PC) in fear conditioning, the role of intrinsic excitability is not clear. In addition, recent *in vitro* studies found that synaptic plasticity of PF-PC synapse is accompanied by the plasticity of intrinsic excitability of PC. Here, we show that synaptic LTP induced by fear conditioning is also accompanied by the plasticity of intrinsic excitability. We recorded PC excitability by ex vivo electrophysiological recording in fear-conditioned adult mice to prove whether the plasticity of intrinsic excitability is occurred by fear conditioning in mature PC. In 1hr after fear conditioning, there was no difference between the conditioned and unconditioned group with whole-cell recording. Remarkably, there was the depression of spontaneous firing in PC of the conditioned mice. This change recovered 24hrs after fear conditioning. These results suggested that the activity of cerebellar PCs is related to the early phase of fear conditioning. We are recording Purkinje cell activity by in vivo electrophysiology to figure out which memory process occurs during auditory fear conditioning.

Key Words: Cerebellum, Purkinje cell, Fear conditioning, Intrinsic excitability, Non-motor function

P2-01

Suppression of FoxO1 by leptin enhances tyrosine hydroxylase and leads to anxiolytic behavior

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Leptin has been linked to psycho-physiological functions. However, the molecular network in leptin-induced mood regulation is poorly understood. Here, we show that administration of leptin induces anxiolytic-like phenotype through the activation of signal transducer and activator of transcription 3 (STAT3) and the inhibition of forkhead box protein O1 (FoxO1) in dopaminergic (DA) neurons of the midbrain. Specifically, STAT3 and FoxO1 directly bind to and exert opposing effects on tyrosine hydroxylase (TH) expression, where STAT3 acts as an enhancer and FoxO1 acts as a prominent repressor. Accordingly, suppression of the prominent suppressor FoxO1 by leptin strongly increased TH expression. Furthermore, specific deletion of FoxO1 in DA neurons (FoxO1 KODAT) led to a profound elevation of TH activity and dopamine contents. Finally, FoxO1 KODAT mice exhibited enhanced leptin sensitivity as well as displayed reduced anxiety- and depression-like behaviors. Altogether, this work establishes a novel molecular mechanism of mood regulation by leptin and suggests TH activation through FoxO1 suppression might be a key for leptin-mediated behavioral manifestation in DA neurons.

Key Words: FoxO1, Leptin, Anxiolytic behavior

P2-02

Targeted downregulation of JMJD2A ameliorates Tau-induced Alzheimer's defects in Drosophila melanogaster

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Human tauopathies such as Alzheimer's disease (AD), represent a group of neurodegenerative diseases which are characterized by abnormal hyper-phosphorylation of the microtubule associated protein (MAP) tau. Transgenic flies neuronally overexpressing mutant human tau, hTauR406W recapitulated many of salient features of Alzheimer's disease. Given that tau promoted neuronal death through heterochromatin loss, global chromatin relaxation, and aberrant transcriptional activation, using transgenic flies neuronally overexpressing hTauR406W, we examined whether the histone methylation change by tissue specific downregulation of histone demethylases can affect tau-induced AD deficits. Through unbiased forward genetic screens of several histone demethylases, we discovered that neuronal specific downregulation of JMJD2A suppressed tau-induced locomotion impairment by reducing the amount of tau and restoring the heterochromatin loss.

Key Words: Alzheimer, Drosophila, JMJD

P2-03

The possibility of ADHD model; Cortisol-induced hyperactive behaviors and memory deficiency in young rats

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The high level of blood cortisol is the key factor to identify major depressive disorder (MDD) which is mediated with the abnormal modulation of brain-derived neurotrophic factor (BNDF) in mammalian brains. However, it is not well known if and how the elevation of cortisol level during neonatal period affects brain functions and induces psychiatric disorders such as MDD after birth. For this issue, we constantly elevated the cortisol level of neonatal rats by injecting corticosterone (20 mg/kg) to maternal rats every day for 21 consecutive pregnant days until delivery. This procedure critically elevated cortisol level in both maternal and postnatal pups. After delivery, pups were bred with their mother rat and isolated from their mother in postnatal 21st day for behavioral tests. Behavioral tests to observe cortisol effects in brain functions were performed by hiring a forced swim test (FST), Morris water maze test (MWT) and open field moving test (OFT). Pups delivered from corticosterone-injected maternal rats (CortiPups) showed significantly different behavioral patterns, compared normal pups (NorPups, saline-injected). In FST performed for 5 min, immobility time of CortiPups was critically shorter than that of NorPups, showing anxiety-mediated hyperactivity. In addition, CortiPups were confirmed to have the critical impairment of learning and memory functions in MWT as they needed longer time to figure out where a hidden platform located. These behavioral patterns of CortiPups seemed to be correlated with those showing in Attention Deficit Hyperactivity Disorder (ADHD) patients. To clarify the impairment of memory function, we also tried electrophysiological experiments to observe neuronal characteristics and memory functions in a cellular level. Using hippocampal slices (p16~21), patch-clamp recordings for observing the long-term potentiation (LTP) patterns and membrane excitability in CA1 neurons were performed. In results, CortiPups showed higher excitable properties of CA1 membranes and incomplete potentiation in LTP pattern. Therefore, it is possible that cortisol may affect learning and memory functions in developmental brains and consequently behaviors are revealed as the abnormal patterns similar with ADHD (GRANT 2016R1D1A1B01010863).

Key Words: Major depressive disorder, ADHD, Cortisol, Long-term potentiation, Learning and Memory

P2-04

Early life stress provokes anxiety and aggressive-like behavior and elevation of GABAergic activity in the ventral hippocampus in adolescence mice

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Postnatal Maternal Separation (MS) stress is known to induce long-lasting alterations in emotional and anxiety-related behaviors. So far, plenty of research had been performed on MS related to behavioral Science. Despite that the consequences of MS on synaptic plasticity, GABAergic activity and PV- and GAD67 of the central nervous system, especially in the hippocampus is not well known yet. In this we examined that neonatal MS (4-hour a day, during 19 days) affect anxiety- and aggressive-like behavior in the adolescence mice using elevated plus maze (EPM) and the resident-intruder test. The results showed that there was anxiety-like behavior in the EPM test and aggressive-like behavior in resident-intruder test. Furthermore, MS provoked elevation of GABAergic activity in vHipp using whole cell patch clamp technique and immunohistochemistry. The result showed that MS mice have elevated levels of PV- and GAD67- positive cells in the vHipp-

DGCs when analyzed using digital photography and semi-quantitative analysis. Therefore, these results manifest that early life stress due to MS may induce major symptom like anxiety and aggressive behaviors in adolescence period and there is a possibility that anxiety and aggressive behavior may be related with a reduced activity of vHipp.

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Key Words: Maternal separation, Anxiety, Aggression, GABA, Hippocampus

P2-05

Effect of *in vitro* hyperglycemia on excitability of the rat dorsal root ganglia (DRG) neurons

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Diabetic neuropathy results from various changes in the environment of peripheral sensory neurons. Diabetic peripheral neuropathy exhibits various sensory symptoms such as loss of nociception or allodynia and hyperalgesia. Diabetic peripheral neuropathy sensory symptoms have been linked to functional, structural and biochemical abnormalities in sensory neurons. Studies using diabetic animal models and *in vitro* high glucose treatment have reported that activities of several ion channels as well as pain related molecules, including cytokines and growth factors, are modified in the sensory neurons. In this study, we investigated whether the excitability of sensory neurons changes when cultured under high glucose condition using a whole-cell patch clamp technique. *In vitro* high glucose (50 mM) did not change morphology and viability of the DRG neurons until the 7th day. Unlike studies using diabetic animal models, resting membrane potential, peak amplitude of action potential, as well as rheobase and threshold were not significantly changed in the high glucose treated DRG neurons. Half width (APD50) was significantly decreased in the high glucose treated DRG neurons. Parameters related to neuronal excitability were not changed by H2O2 (10 mM) or LPS (10 μg/ml) treatment. These results suggest that the hyperalgesia or loss of pain associated with diabetic peripheral neuropathy may be related to a complex changes in molecular level rather than simply a change in basal excitability of peripheral sensory neurons, even *in vitro*.

Key Words: DRG neuron, High glucose, Excitability, Diabetic peripheral neuropathy

P2-06

The activity-dependent modulation of the cerebellar Purkinje cell output requires synergies between synaptic and intrinsic plasticity

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The cerebellar long-term depression (LTD) at the parallel fiber (PF) to Purkinje cell (PC) synapses (PF-PC LTD) has long been implicated in cerebellum-dependent motor learning. For decades, other type of plasticity in the cerebellar PCs has emerged as the other side of the engram for memory storage. Since the cerebellar PCs are sole output neurons which form inhibitory synapses onto the neurons in deep cerebellar nuclei and vestibular nuclei, the activity-dependent modulation of the cerebellar output may play a pivotal role in information processing in the cerebellar circuitry. Most recently, the synaptic depression was found to be accompanied by plasticity of intrinsic excitability (intrinsic plasticity; in this case: LTD of intrinsic excitability, LTD-IE), however, detailed mechanisms remain unclear. Using patch-clamp recordings in the floccular PCs, we present here that the synergistic modulation of the synaptic plasticity with the intrinsic plasticity determines the cerebellar output. As the synaptic LTD, the PC intrinsic plasticity was precisely tuned for an interval of 120 ms between PF and climbing fiber (CF) activation. Interestingly, spike probability elicited by PF stimulation at 20 Hz was significantly decreased after LTD induction with the 120 ms-interval stimuli. When an interval of PF and CF activation was 0 ms, the synaptic and intrinsic plasticity were not shown. In addition, the PC intrinsic plasticity was prevented by inhibition of mGluR1 and CaMKII, suggesting that the intrinsic plasticity requires activation of signaling for PF-PC LTD. The LTD-IE of the cerebellar PCs was insensitive to suppression of AMPAR internalization by application of PICK1 inhibitor. Although the PF-PC LTD was abolished by application of PICK1 inhibitor, the spike probability was decreased, indicating that the cerebellar output may more sensitively reflect the excitability change. Furthermore, the spike probability showed robust reduction when electrical stimuli were applied to the conditioned PF. Therefore, the intrinsic plasticity of PCs was confined to specific-branch of the PC dendrite. In conclusion, the activity-dependent modulation of the cerebellar output would be derived from synergies between synaptic and intrinsic plasticity of the cerebellar PCs.

Key Words: Cerebellar motor learning, Synaptic plasticity, Intrinsic excitability, Metabotropic glutamate receptor, Cerebellar Purkinje cells

P2-07

Noradrenergic modulation of cerebellar glial activity during nociception

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The cerebellum has been implicated in pain processing. Several studies revealed that the metabolic activity of the cerebellum is enhanced, implying that cerebellum might play active and/or passive role in the pain processing. However, how the cerebellum takes a part in the pain processing is still elusive. Here, using two-photon calcium imaging, we showed that the cerebellar Bergmann glia (BG) is activated by a noxious input. Interestingly, we found that BG calcium response was completely blocked when the alpha 1 adrenergic receptor (α1-AR) was genetically suppressed in the BG-specific manner, suggesting that the noxious stimuli-induced BG calcium activation was dependent on α1-AR. Furthermore, the pain-related behavior test was performed in order to investigate whether BG-specific α1-AR signaling would be involved in pain processing. Interestingly, Capsaicin-induced paw licking behavior was reduced in the BG-specific α1-AR knockdown mice, indicating the BG calcium activity is related to pain behavior. Taken together, we suggest that the pain processing in the cerebellum may be mediated by BG activity through glial adrenergic signaling

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

P2-08

Effects of intranasal oxytocin on impairments in hippocampal plasticity and recognition memory following uncontrollable stress

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Background: Nasal pretreatment with the neuropeptide oxytocin has been reported to prevent stress-induced impairments in hippocampal synaptic plasticity and spatial memory in rats. However, no study has asked if oxytocin application following a stress experience is effective in rescuing

stress-induced impairments.
Methods: Synaptic plasticity was measured in hippocampal Schaffer collateral-CA1 synapses of rats subjected to uncontrollable stress; their cognitive function was examined using an object recognition task.
Results: Impaired induction of long-lasting, long-term potentiation by uncontrollable stress was rescued, as demonstrated both in rats and hippocampal slices. Intranasal oxytocin after experiencing uncontrollable stress blocked cognitive impairments in stressed rats and in stressed hippocampal slices treated with a perfused bath solution containing oxytocin.
Conclusions: These results indicated that posttreatment with oxytocin after experiencing a stressful event can keep synaptic plasticity and cognition function intact, indicating the therapeutic potential of oxytocin for stress-related disorders, including posttraumatic stress disorder.

Key Words: Oxytocin, Synaptic plasticity, Hippocampus, Posttraumatic stress disorder

P3-01

Regulation of PKD2L1 channel by direct interaction with calmodulin

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Polycystic kidney disease 2-like-1 (PKD2L1), or polycystin-L or TRPP2, formerly TRPP3, is a transient receptor potential (TRP) superfamily member. It is a calcium-permeable non-selective cation channel that regulates intracellular calcium concentration and thereby calcium signaling. Calmidazolium (CMZ), which is well-known as a calmodulin (CaM) inhibitor, is an activator of PKD2L1 channel, but the activating mechanism remains unclear. The purpose of this study is to clarify the activating mechanism of CMZ on PKD2L1 channel. To clarify the interaction between PKD2L1 channel and CaM, we co-expressed the proteins in HEK293 cells and observed the difference in PKD2L1 currents. When co-expressed with CaM(WT) or CaM(DN), the double-negative mutant, PKD2L1 showed significantly reduced currents. To further examine whether the binding of CaM (either Ca^{2+}-CaM or apo-CaM) affects the channel currents, we identified the predicted CaM binding site, and generated deletion and truncation mutants. The mutants 588Stop, del590-600, L592/597E, and RRRK/AAAA showed significant reduction in currents losing PKD2L1 I-V curve. The results suggest that the C-terminal region from 590 to 600 is likely to be CaM-binding site and crucial for maintaining functionality of PKD2L1 channel.

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Key Words: PKD2L1, TRPP3, Ion channel, Calcium, Calmodulin, Calmidazolium

P3-02

Tricyclic antidepressant clomipramine blocks voltage-gated K^+ current in rabbit coronary arterial smooth muscle cells

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We investigated the effect of the tricyclic antidepressant clomipramine on voltage-dependent K^+ (Kv) channels in native rabbit coronary arterial smooth muscle cells. Our results showed that clomipramine inhibited vascular Kv channels in a concentration-dependent manner, with an IC50 value of 8.61 ± 4.86 μM and a Hill coefficient (*n*) of 0.58 ± 0.07. The application of 10 μM clomipramine did not affect the activation curves of the Kv channels; however, the inactivation curves of the Kv channels were shifted toward a more negative potential. The clomipramine-induced inhibition of Kv currents was not changed by the application of train pulses (1 or 2 Hz), which demonstrated that clomipramine inhibited Kv current in a state (use)-independent manner. Pretreatment with the Kv1.5 and Kv2.1 inhibitors, DPO-1 and guangxitoxin, respectively, did not affect clomipramine-induced inhibition of Kv currents. Therefore, we concluded that clomipramine inhibited vascular Kv channels in a concentration-dependent, but state (use)-independent manner, regardless of its own function.

Key Words: Clomipramine, Voltage-gated K^+ channel, Coronary artery

P3-03

Inhibition of voltage-gated K^+ current by tricyclic antidepressant desipramine on rabbit coronary arterial smooth muscle cells

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We describe the effect of a tricyclic antidepressant drug, desipramine on voltage-dependent K^+ (Kv) currents in freshly isolated rabbit coronary arterial smooth muscle cells using a conventional whole-cell patch clamp technique. Application of desipramine rapidly decreased the Kv current amplitude in a concentration-dependent manner, with an IC_{50} value of 5.91 ± 0.18 μM and a Hill coefficient of 0.61 ± 0.09. The steady-state inactivation curves of the Kv channels were not affected by desipramine. However, desipramine shifted the steady-state inactivation curves toward a more negative potential. Application of train pulses (1 or 2 Hz) slightly reduced the Kv current amplitude. Such reduction of the Kv current amplitude by train pulses increased in the presence of desipramine. Furthermore, the inactivation recovery time constant was also increased in the presence of desipramine, suggesting that desipramine-induced inhibition of the Kv current was use-dependent. Application of a Kv1.5 inhibitor (DPO-1) and/or a Kv2.1 inhibitor (guangxitoxin) did not change the inhibitory effect of desipramine on Kv currents. Based on these results, we concluded that desipramine directly inhibited the Kv channels in a dose- and state-dependent manner, but the effect was independent of norepinephrine/serotonin reuptake inhibition.

Key Words: Desipramine, Voltage-gated K^+ current, Coronary arterial smooth muscle cell

P3-04

Changes of ATP-sensitive K^+ channel expression in human umbilical smooth muscle during gestational diabetes mellitus

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We investigated the alterations of ATP-sensitive K^+ (K_{ATP}) channels in human umbilical arterial smooth muscle cells during gestational diabetes mellitus (GDM). The amplitude of the K_{ATP} current induced by application of the K_{ATP} channel opener pinacidil (10 μM) was reduced in the GDM group than in the control group. Pinacidil-induced vasorelaxation was also predominant in the normal group compared with the GDM group. Reverse transcription polymerase chain reaction and Western blot analysis suggested that the expression of K_{ATP} channel subunits such as Kir6.1, Kir6.2, and SUR2B, were decreased in the GDM group relative to the normal group. The application of forskolin and adenosine, which activate protein kinase A (PKA) and there-

by K_{ATP} channels, elicited K_{ATP} current in both the normal and GDM groups. However, the current amplitudes were not different between the normal and GDM groups. In addition, the expression levels of PKA subunits were not altered between the two groups. These results suggest that the reduction of K_{ATP} current and K_{ATP} channel-induced vasorelaxation are due to the decreased expression of K_{ATP} channels, not to the impairment of K_{ATP}-related signaling pathways.

Key Words: Gestational diabetes mellitus, ATP-sensitive K^+ channel, Umbilical smooth muscle

P3-05

DPP-4 inhibitor, vildagliptin induces vasorelaxation via activation of Kv channel and SERCA pump in aortic smooth muscle

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This study investigated vildagliptin-induced vasodilation and its related mechanisms using phenylephrine induced precontracted rabbit aortic rings. Vildagliptin induced vasodilation in a concentration-dependent manner. Pretreatment with the large-conductance Ca^{2+}-activated K^+ channel blocker paxilline, ATP-sensitive K^+ channel blocker glibenclamide and inwardly rectifying K^+ channel blocker Ba^{2+} did not affect the vasodilatory effects of vildagliptin. However, application of the voltage-dependent K^+ (Kv) channel inhibitor 4-aminopyridine significantly reduced the vasodilatory effects of vildagliptin. In addition, application of either of two sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) inhibitors, thapsigargin or cyclopiazonic acid, effectively inhibited the vasodilatory effects of vildagliptin. These vasodilatory effects were not affected by pretreatment with adenylyl cyclase, protein kinase A (PKA), guanylyl cyclase, or protein kinase G (PKG) inhibitors, or by removal of the endothelium. From these results, we concluded that vildagliptin induced vasodilation via activation of Kv channels and the SERCA pump. However, other K^+ channels, PKA/PKG-related signaling cascades associated with vascular dilation, and the endothelium were not involved in vildagliptin-induced vasodilation.

Key Words: Vildagliptin, Vasorelaxation, Kv channel, SERCA pump

P3-06

Characterization of voltage-dependent Ca^{2+} channels of human cardiac myofibroblasts and the effect of nitric oxide through cGMP-dependent mechanism

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We investigated the expression of voltage-dependent Ca^{2+} channels (VDCC) in human cardiac myofibroblasts (HCMFs) and the effect of nitric oxide (NO) on the currents and underlying mechanisms. In western blot analysis and immunofluorescence staining, the protein expression of *a*-smooth muscle actin (α-SMA), a myofibroblast marker, was strong positive in passage 13 (P13) cells of human cardiac fibroblasts (HCFs) but not P4 cells. On the other hand, calponin (a fibroblast marker) was expressed only in P4 cells but not P13 cells. These positive expression of α-SMA was observed in P12~15 cells but not P4 and P7 cells. In RT-PCR, strong mRNA expression was observed for $Ca_V1.2$ (L-type Ca^{2+} channels) and $Ca_V3.3$ (T-type Ca^{2+} channels), while weak expression was exhibited with $Ca_V2.1$ (N-type Ca^{2+} channels) and $Ca_V2.3$ (R-type Ca^{2+} channels) in the P12~15 cells of HCFs. However, only weak expression of $Ca_V2.3$ was shown in the P4 cells. The protein expression of $Ca_V1.2$ for L-type Ca^{2+} channels was also identified by western blotting. In whole cell mode patch-clamp recordings, nifedipine sensitive L-type Ca^{2+} currents ($I_{Ca,L}$) and mibefradil sensitive T-type Ca^{2+} currents ($I_{Ca,T}$) were recorded in P12~15 cells. $I_{Ca,L}$ was detected in 97.6%, but $I_{Ca,T}$ was only detected in 2.4% of the cells. When S-nitroso-N-acetylpenicillamine (SNAP; 100 μM), an nitric oxide (NO) donor, was added to the bath solution, the amplitude of $I_{Ca,L}$ was decreased. However, $I_{Ca,L}$ was not inhibited at physiological low concentration of NO (0.1~10 nM). In dose-response curve of SNAP for $I_{Ca,L}$, the half maximal inhibitory concentration (IC_{50}) was 113.2 μM and Hill coefficient was 1.258. In the pretreatment state of 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, a soluble guanylate cyclase blocker) or KT5823 (a PKG blocker) before SNAP addition, SNAP could not inhibit $I_{Ca,L}$. 8-bromo-cGMP (a membrane-permeable cGMP analogue) also decreased the currents. However, pretreatment of N-ethylmaleimide (NEM; a thiol-alkylating reagent) could not block the SNAP-inhibition effect on $I_{Ca,L}$. In addition, DL-dithiothreitol (DTT; a reducing agent) could not reverse the SNAP inhibition on $I_{Ca,L}$. These data suggest that NO inhibited the L-type Ca^{2+} channel in HCMFs through cGMP-dependent PKG pathway but not *S*-nitrosylation.

Key Words: Human cardiac myofibroblasts, L-type Ca^{2+} channels, Nitric oxide, Protein kinase G, *S*-nitrosylation

P3-07

Effect of carbon monoxide on Ca^{2+}-activated K^+ currents of human cardiac fibroblasts through the protein kinase pathways

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Cardiac fibroblasts (CFs) are the major cell type that involved in regulating the extracellular matrix in the heart and play an important role in cardiac remodeling after myocardial infarction or in response to hemodynamic overload. Ca^{2+}-activated K^+ (K_{Ca}) channels play a role in the proliferation of the fibroblasts which may play a role in cardiac remodeling. Carbon Monoxide (CO) was shown to exert a variety of pharmacological effects including cardioprotective properties. However, its mechanisms of action are not completely understood. We investigated the effect of CO on K_{Ca} channels and the mechanism in human cardiac fibroblasts (HCFs). In whole-cell mode patch clamping, application CO delivery by carbon monoxide-releasing molecule 2 (CORM-2) increased the amplitude of K_{Ca} currents. Carbon monoxide-releasing molecule 3 (CORM-3) also stimulated the KCa currents. The CORM-2 or CORM-3 stimulating effect on KCa currents were blocked by pretreatment with KT5823 (a protein kinase G inhibitor) or 1 H-[1,-2, -4] oxadiazolo-[4,-3-a] quinoxalin-1-one (ODQ; a soluble guanylate cyclase inhibitor). Additionally, pretreatment with KT5720 (a protein kinase A inhibitor) and SQ22536 (an adenylyl cyclase inhibitor) blocked the CORM-2 or CORM-3 stimulating effect on the currents. These data suggest that CO enhances KCa currents in HCFs through the PKG and PKA pathways.

Key Words: Ca^{2+}-activated K^+ currents, Carbon monoxide, Human cardiac fibroblast, Protein kinase A, Protein kinase G

P3-08

Regulation of transient receptor potential canonical (TRPC)4 by the phospholipase C pathway

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Transient Receptor Potential Canonical 4 (TRPC4) is known to be activated by G-protein coupled receptor (GPCR) stimulation. Gαq-coupled receptor stimulation was implied in the activation process of TRPC4 channel with not only activation but also evident inactivation time-course. Among Gαq-protein downstream pathway, TRPC4 and TRPC5 channels are maintained

by the directly bound membrane phosphatidylinositol 4,5-biphosphate [PI(4,5)P_2]. Here we investigated the Ca^{2+} sensitive phospholipase C (PLC) δ which has been implied in TRPC4 activity, since membrane lipid inhibits channel activity when PI(4,5)P_2 is hydrolyzed. In FRET and Co-IP experiments only PLCδ1 had direct interaction with TRPC4 but TRPC5 did not show the direct interaction with any PLCδ isoforms. By changing the extracellular free calcium concentration, we identified the sensitivity of PLCδ subtypes to Ca^{2+} is different in the sequence PLCδ1 < PLCδ3. In voltage-clamp experiments TRPC4 activity with PLCδ1 or its mutants had no difference with the only channel currents. But with the calcium sensitive PLCδ3, TRPC4 channel did not show or reduced channel activities and fast current inactivation. TRPC4 channel was intimately regulated by PI(4,5)P_2, Ca^{2+} and PLCδ1. Our data support the involvement of PLCδ1 in the regulation of TRPC4 channel hyperactivity and PLCδ3 with TRPC4 results in inactivity.

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Key Words: TRPC, Phospholipase C, Calcium, FRET

P3-09

Hydrogen peroxide constricts rat arteries by Na^+-permeable non-selective cation channels

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Oxidative stress is associated with many cardiovascular diseases, such as hypertension and arteriosclerosis. Oxidative stress reportedly activates the L-type voltage-gated calcium channel (VDCCL) and elevates $[Ca^{2+}]_i$ in many cells. However, how oxidative stress activates VDCCL and the consequence for arteries are unclear. Here, we examined the hypothesis that hydrogen peroxide (H_2O_2) regulates membrane potential (Em) by altering Na^+ influx through non-selective cation channels, which consequently activates VDCCL to induce vasoconstriction in rat mesenteric arteries. To measure the tone of the endothelium-denuded arteries, a conventional isometric organ chamber was used. VDCCL currents and Em were recorded by the patch-clamp technique. $[Ca^{2+}]_i$ and $[Na^+]_i$ were measured with microfluorometry using Fura2-AM and SBFI-AM, respectively. We found that 10 μM or 100 μM H_2O_2 increased arterial contraction, and nifedipine blocked the effects of H_2O_2 on isometric contraction. Additionally, H_2O_2 increased VDCCL currents, $[Ca^{2+}]_i$, and $[Na^+]_i$. H_2O_2 depolarized Em, and both Gd^{3+} and nifedipine blocked the depolarization and prevented the increase in $[Ca^{2+}]_i$ and $[Na^+]$i. Na^+-free medium blocked the H_2O_2-induced vasoconstriction. Taken together, the results suggested that H_2O_2 constricts rat arteries by activating Gd^{3+}- and nifedipine-sensitive, Na^+-permeable, non-selective cation channels and subsequently VDCCL channels. We suggest that unidentified Gd^{3+}- and nifedipine-sensitive, Na^+-permeable, non-selective cation channels may function as an important mediator for oxidative stress-induced vascular dysfunction.

Key Words: Hydrogen peroxide, Non-selective cation channels, Smooth muscle cells, Membrane potential

P3-10

Cariprazine inhibits hERG 1A and heteromeric hERG 1A/3.1 potassium channels

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Cariprazine is a novel atypical antipsychotic drug that is widely used for the treatment of schizophrenia and bipolar mania/mixed disorder. We used the whole-cell patch-clamp technique to investigate the effects of cariprazine on hERG channels that are stably expressed in HEK cells. Cariprazine inhibited the hERG1A and hERG1A/3.1 tail currents at -50 mV in a concentration-dependent manner with IC50 values of 4.1 and 12.2 μM, respectively. The block of hERG 1A currents by cariprazine was voltage-dependent, and increased over a range of voltage for channel activation. Cariprazine shifted the steady-state inactivation curve of the hERG1A currents in a hyperpolarizing direction and produced a use-dependent block. A fast application of cariprazine inhibited the hERG1A current elicited by a 5 s depolarizing pulse to +60 mV to fully inactivate the hERG1A currents. During a repolarizing pulse wherein the hERG1A current was deactivated slowly, cariprazine rapidly and reversibly inhibited the open state of the hERG1A current. However, cariprazine did not affect hERG1A and hERG1A/3.1 channel trafficking to the cell membrane. Our results indicated that cariprazine concentration-dependently inhibited hERG1A and hERG1A/3.1 currents by preferentially interacting with the open states of the hERG1A channel, but not by the disruption of hERG1A and hERG1A/3.1 channel protein trafficking.

Key Words: Cariprazine, hERG 1A/3.1 heterotetramer, Open channel block

P3-11

Altered GABAergic tone in STING KO mice

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Cytosolic DNA sensing activates Stimulator of Interferon Genes (STING) adaptor to induce type I interferons (IFNs) and subsequent proinflammatory cytokines (PICs) response. IFNs/PICs responses are key components of the innate immune response against pathogenic infections. In the brain, IFNs response has also been observed in the absence of infection such as in autoimmune diseases or during aging. Thus, IFNs involve in sterile brain injury and PICs enhances GABAergic tone in sterile neuroinflammation with impaired cognitive and motor function. However, despite the wealth of information that IFNs/PICs regulate the synaptic transmission, it is not clear if STING activity entailing IFNs/PICs response involve the synaptic transmission. Here, we showed that the deficiency of GABA transporter (GAT) activity resulted in enhanced extrasynaptic tonic $GABA_A$ current (I_{tonic}) in STING knockout (KO) mice. Firstly, we compared phasic and tonic $GABA_A$ current of the hippocampal dentate gyrus granule cells in STING KO and age-matched wild type (WT) mice. Major characteristic of sIPSC including frequency, amplitude and decay time kinetics were compared in the two groups. While sIPSC frequency were not different in STING KO and WT mice, tonic GABAA current (I_{tonic}) were significantly larger in STING KO mice than in WT mice. Consistently, nonselective GATs blockade (NPA, 100 μM) induced larger tonic inhibition WT than in STING KO mice. Secondly, we compared I_{tonic} with δ-$GABA_AR_s$ specific modulator THIP (1μM), induced larger tonic current in STING KO mice then WT mice. Finally, we compared effect of THIP (1μM) over GABA (5μM) shows no difference between the two groups, suggesting there is no change in α-$GABA_AR_s$ for enhanced tonic inhibition in STING KO mice. Taken together, our results suggested that diminished GAT activity/expression may enhance the tonic $GABA_A$ inhibition in STING KO mice.

Key Words: STING, $GABA_A$ receptors, GABA transporter, Tonic $GABA_A$ inhi-

bition, sIPSC

P3-12

Structure-function relationship of TRPC3 carboxyl coiled coil domain

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Canonical transient receptor potential 3 (TRPC3) amino acid sequence follows a coiled coil pattern (heptad repeat sequence) at both amino- and carboxy terminus (NT and CT, respectively). CT coiled coil domains of TRPC3 located right below the ion permeation path and next to the C-terminal CaM/inositol-1,4,5-trisphosphate receptor binding domain (CIRB). Therefore, the objective of this project was to investigate the structure and function relationship of coiled coil domains (CCD) of TRPC3 channel. Following the identification of key hydrophobic residues of the heptad repeat, coiled coil breaking mutations were introduced to the coiled coil sequences of both NT and CT-CCDs. CT-CCD breaking mutants but not the NT-CCD breaking mutants resulted a gain of function phenotype in TRPC3. The putative CT-CCD commences from M779 and continues up to end of CT. The calcium dependent inactivation is lost in the CT-CCD mutants and they show a calcium dependent gain of function. Furthermore, this gain of function phenotype of each amino acid mutation corresponds to the predicted CCD probability of each amino acid and the hydrophobicity of the amino acids. The peak of this phenotype is shown at I807 site. The coiled coil mutants show an increased permeability to calcium while permeability of magnesium, monovalent permeability and pore size remains intact. This gain of function is independent from STIM1 regulation. Disruption of the CCD seems to be affecting the sensitivity of TRPC3 for calmodulin dependent regulation. Together, these findings points towards the key role of TRPC3 CT-CCD in calcium/calmodulin-dependent regulation of TRPC3.

Key Words: TRPC3, CT, NT, Coild coild domain, Calcium dependent regulation

P3-13

pH- and calcium-dependent inhibition of hSlo3 by quercetin

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Slo3 is one of the most potent contributors to KSper. Dietary chemical compounds that can regulate the function of hSlo3 pose possible modulatory effects on human fertility. Quercetin is a common dietary flavonoid known to modulate ion channels mainly by inhibition of protein kinase, inhibition of phospholipase and allosteric binding. The aim of this study is to find the effect of quercetin on KSper ion channels and mechanism by which quercetin affect the channel function. We used HEK293 co-transfected with hSlo3 and hLRRC52 in the patch clamp experiments. The results indicate that quercetin and its analogues dose-dependently inhibited hSlo3 current. Furthermore, external and internal acidic environment enhanced the quercetin effect on hSlo3. Increasing the internal free-calcium concentration diminished the effect of quercetin on hSlo3. PI3Kinase inhibitors and inhibitors of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) downstream signaling had strong inhibitory effects on hSlo3. Pre-treatment of phosphatidylinositol-4,5-bisphosphate 3 kinaseinhibitor LY 294002 rapidly inhibited the hSlo3 current and diminished the effect of quercetin. Inhibition of PIP3 downstream pathways, mTOR and guanylyl cyclase by rapamycin and ODQ respectively, could rapidly inhibit the hSlo3 current, however those treatments failed to hinder the effect of quercetin. Therefore the results indicate that the calcium and pH defendant inhibitory effect of quercetin on Slo3 is mainly though the inhibition of phosphatidylinositol kinases.

Key Words: Slo3, LRRC52, PI(4,5)P_2, Phosphatidylinositol-4,5-bisphosphate 3 kinase, Ksper, Quercetin, Voltage-gated potassium channels

P3-14

Ketamine contracts rat arteries by facilitating the activation of serotonin 5-HT_{2A} receptors – clinical implications for the PCP derivatives-induced diseases

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Ketamine is an anesthetic with hypertensive effects, which makes it useful for patients at risk for shock. However, previous ex vivo studies reported vasodilatory actions of ketamine in isolated arteries. In this study, we re-examined the effects of ketamine on arterial tones in the presence and absence of physiological concentrations of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) by measuring the isometric tension of endothelium-denuded rat mesenteric arterial rings. Ketamine didn't affect the resting tone of control mesenteric arterial rings. However, in the presence of 5-HT (100-200 nM), ketamine (10-100 μ M) markedly contracted arterial rings. Ketamine didn't contract arterial rings in the presence of NE (10 nM), indicating that the vasoconstrictive action of ketamine is selectively 5-HT-dependent. The concentration-response curves (CRCs) of 5-HT were clearly shifted to the left in the presence of ketamine (30 μ M), whereas the CRCs of NE were not affected by ketamine. The 5-HT CRC's shift to the left by ketamine was reversed with ketanserin, a competitive 5-HT_{2A} inhibitor, indicating that ketamine facilitated the activation of 5-HT_{2A} receptor. Anpirtoline and BW723C86, selective agonists of 5-HT_{1B} and 5-HT_{2B} receptors, respectively, didn't contract arterial rings in the absence or presence of ketamine. These results indicate that ketamine specifically enhances 5-HT_{2A} receptor-mediated vasoconstriction, and that it is vasoconstrictive in a clinical setting. The facilitative action of ketamine on the 5-HT_{2A} receptor should be considered in the ketamine-induced hypertension as well as in the pathogenesis of diseases such as schizophrenia, wherein experimental animal models are frequently generated using ketamine.

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Key Words: Ketamine, 5-HT_{2A} receptor, Blood pressure, Mesenteric artery, Schizophrenia

P3-15

Measurement of ion concentration in the unstirred boundary layer with open patch-clamp pipette – implications for control of ion channels by fluid flow

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Fluid flow is an important environmental stimulus that controls many physiological and pathological processes such as fluid flow-induced vasodilation. Although the molecular mechanisms for the biological responses to the fluid flow/shear force are not fully understood, fluid flow-mediated regulation of ion channel gating might contribute critically. Therefore, fluid flow/shear force-sensitivity of ion channels has been studied using the patch-clamp technique. However, depending on the experimental protocol, the outcome and interpretation of data could be erroneous. Here, we present experimental and theoretical evidence for fluid flow-related errors and provide methods for measuring the errors and preventing them. Changes in junction potential between the Ag/AgCl reference electrode and bathing fluid was measured with an open pipette filled with 3M-KCl. Applying fluid flow from the static condition could shift the liquid/metal junction potential up to ~7 mV. Conversely, using the voltage shift induced by the fluid flow, we could estimate the real ion concentration in the unstirred boundary layer: in the static condition, the real ion concentrations adjacent to the Ag/AgCl reference electrode or ion channel inlet at cell membrane surface could be quite lower up to ~30% than in the flow condition. Placing an agar 3M KCl-bridge between the bathing fluid and reference electrode prevented the problem of junction potential shift. However, the unstirred layer effect adjacent to the cell membrane surface cannot be fixed like this; this has been rigorously discussed in this paper. In conclusion, we provided a method for measuring the real ion concentrations in the boundary unstirred layer with open patch-clamp pipette emphasizing the importance of using agar salt-bridge when studying fluid flow-induced regulations of ion currents. The novel interpretations for the fluid flow-induced modulations of ion channel currents would provide insights for designing experiments and interpreting data on the fluid shear force-regulations of ion channels.

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Key Words: Fluid flow, Shear force, Unstirred layer, Patch clamp, Ag/AgCl reference electrode, Liquid/metal junction potential, Convection, Ion channel

P3-16

Plasma membrane trafficking of PC2 regulated by TRPC4, TRPC5, Gαo, Gαi3 and Gαs

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Autosomal dominant polycystic kidney disease (ADPKD) occurs when the gene (PKD1 or PKD2) encoding polycystin-1 (PC1) or polycystin-2 (PC2) has mutation. ADPKD affects not only the kidney but also other organs and has a non-renal manifestation that forms cysts in other organs, such as the liver, seminal vesicles, pancreas, and arachnoid membrane. It also has an adverse effect on the lungs, leading to bronchiectasis and dyspnea. PC2 has many physiological functions, and shows higher expression level in the lung. The function of PC2 is related to its trafficking from the ER to the plasma membrane, but its factor has not been clearly identified. We investigated on the factors that are responsible for the regulation of membrane trafficking from the ER. PC1, TRPC1 and GSK3β increased PC2 expression as previously reported. TRPC4 and TRPC5, classified to the same subfamily with TRPC1, increased surface expression of PC2 as well as total expression. TRPC5 and PC2, in particular, were found to help trafficking each other. PC2 decreased Cs-induced TRPC5 current, while PC2 alone did not show any current. We investigated whether TRPC4 and TRPC5 directly interacts with PKD2. TRPC4 and TRPC5 did not co-immunoprecipitate with PKD2. FRET experiments FRET experiments also showed that PC2 did not interact with TRPC4 and TRPC5. Next, we investigated whether heterotrimeric G proteins control the expression of PKD2. Among constitutively active G proteins (QL mutants) tested, Gαs increased PC2 expression whereas Gαo and Gαi3 decreased it. These results suggest that TRPC4, TRPC5, Gαo, Gαi3 and Gαs regulate trafficking to the plasma membrane of PC2, which may affect the physiological function of PC2. These findings may help understand the pathological mechanisms of ADPKD, especially when accompanied with lung disease.

Key Words: Polycystin-2, Plasma membrane trafficking, TRPC, Gαo, Gαi3, Gαs

P3-17

Characterization of Piezo2 ion channel, a mechanical stimulus receptor in MCC-13 cells

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Recent parallel studies clearly indicated that Merkel cells and the mechanosensitive piezo2 ion channel play critical roles in the light-touch somatosensation. Moreover, piezo2 was suggested to be a light-touch sensing ion channel without a role in pain sensing in mammals. However, biophysical characteristics of piezo2, such as single channel conductance and sensitivities to various mechanical stimuli, are unclear, hampering a precise understanding of its role in touch sensation. Here, we describe the biophysical properties of piezo2 in human Merkel cell carcinoma (MCC)-13 cells; piezo2 is a low-threshold, positive pressure-specific mechanically activated (MA) cation channel with a single channel conductance of ~30.7pS. Application of step indentations under the whole-cell mode of the patch-clamp technique, and positive pressures ≥5 mmHg under the cell-attached mode, activated piezo2 currents in MCC-13 and human embryonic kidney 293T cells where piezo2 was overexpressed. By contrast, application of a negative pressure failed to activate piezo1 in a similar manner in Neuro2A cells. Our results are the first to demonstrate single channel recordings of piezo2. We anticipate that our findings will be a starting point for a more sophisticated understanding of piezo2 roles in light-touch sensation.

Key Words: Merkel cell, Piezo2 channel, Mechanotransduction

P3-18 (PO-A-5)

Translocatable voltage-gated Ca^{2+} channel β subunits in α1-β complexes reveal competitive replacement yet no spontaneous dissociation

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β subunits of high voltage-activated Ca^{2+} (Ca_V) channels promote cell-surface expression of pore-forming α1 subunits and regulate channel gating through binding to the α-interaction domain(AID) in the first intracellular loop. We addressed thestability of Ca_V α1B-β interactions by novel rapamycin-translocatable Ca_V β-subunits that allow drug-induced sequestra-

tion and uncoupling of the β subunit from $Ca_V2.2$ channel complexes in intact cells. Without Ca_V α1B/α2δ1, all modified β subunits, except membrane-tethered β2a and β2e, are in the cytosol and rapidly translocate upon rapamycin addition to anchors on target organelles: plasma membrane, mitochondria, or endoplasmic reticulum. In cells co-expressing Ca_V α1B/α2δ1 subunits, the translocatable β subunits co-localize at the plasma membrane with α1B and stay there after rapamycin application, indicating that interactions between α1B and bound β subunits are very stable. However, the interaction becomes dynamic when other competing β isoforms are co-expressed. Addition of rapamycin, then switches channel gating and regulation by $PI(4,5)P_2$ lipid. Thus, expression of free β isoforms around the channel reveals a dynamic aspect to the α1B-β interaction. On the other hand, translocatable β subunits with AID-binding-site mutations are easily dissociated from Ca_V α1B on the addition of rapamycin, decreasing current amplitude and $PI(4,5)P_2$ sensitivity. Furthermore, the mutations slow $Ca_V2.2$ current inactivation and shift the voltage dependence of activation to more positive potentials. Mutated translocatable β subunits work similarly in $Ca_V2.3$ channels. In sum, the strong interaction of Ca_V α1B-β subunits can be overcome by other free β isoforms, permitting dynamic changes in channel properties in intact cells.

Key Words: Voltage-gated Ca^{2+} (Ca_V) channel, Ca_V β subunits, Chemically inducible dimerization, Rapamycin, $PI(4,5)P_2$

P3-19

Suppression of hERG K^+ current and cardiac action potential prolongation by 4-hydroxynonenal via dual mechanisms

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Oxidative stress under pathological conditions, such as ischemia/reperfusion and inflammation, results in the production of various reactive chemicals. Of these chemicals, 4-hydroxynonenal (4-HNE), a peroxidation product of ω6-polyunsaturated fatty acid, has garnered significant attention. However, the effect of 4-HNE on cardiac electrophysiology has not yet been reported. In the present study, we investigated the effects of 4-HNE on several cardiac ion channels, including human ether-a-go-go-related (hERG) channels, using the whole-cell patch clamp technique. Short-term exposure to 100 μM 4-HNE ($4\text{-}HNE_{100S}$), which mimics local levels under oxidative stress, decreased the amplitudes of rapidly activating delayed rectifier K^+ current (I_{Kr}) in guinea pig ventricular myocytes (GPVMs) and HEK293T cells overexpressing hERG (I_{hERG}). MS analysis revealed the formation of 4-HNE-hERG adducts on specific amino acid residues, including C276, K595, H70, and H687. Long-term treatment (1-3 h) with 10 μM 4-HNE ($4\text{-}HNE_{10L}$), suppressed I_{Kr} and I_{hERG}, but not I_{KS} and I_{CaVL}. Action potential duration (APD) of GPVMs was prolonged by 37 % and 64 % by $4\text{-}HNE_{100S}$ and $4\text{-}HNE_{10L}$, respectively. Western blot analysis using surface biotinylation revealed a reduction in mature membrane hERG protein after treatment with $4\text{-}HNE_{10L}$. Proteasomal degradation inhibitors, such as bortezomib, prevented the $4\text{-}HNE_{10L}$-induced decrease in mature hERG, suggesting a retrograde degradation of membrane hERG due to 4-HNE. Taken together, $4\text{-}HNE_{100S}$ and $4\text{-}HNE_{10L}$ suppressed I_{hERG} via functional inhibition and downregulation of membrane expression of hERG, respectively. The exposure of 4-HNE under pathological oxidative stress may increase the risk of proarrhythmic events via APD prolongation.

Key Words: Lipid peroxidant, 4-hydroxynonenal, hERG channel, Cardiac action potential prolongation

P3-20

The Englerin A-sensing three charged residues for TRPC5 channel activation

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The classical transient receptor potential channel 5 (TRPC5), known as a nonselective cation channel (NSCC), has a crucial role in calcium influx. TRPC5 has been reported to be activated by muscarinic receptor activation and extracellular pH change, and inhibited by protein kinase C (PKC) pathway. Recent studies have also suggested that TRPC5 is extracellularly activated by englerin A (EA), but the mechanism remains unclear. The purpose of this study is to identify the EA-interaction sites in TRPC5 and thereby clarify the mechanism of TRPC5 activation. TRPC5 channels are over-expressed in human embryonic kidney (HEK293) cells. TRPC5 mutants were generatedby site-directed mutagenesis. The whole-cell patch-clamp configuration was used to record TRPC5 currents. Western analysis was also performed to see the expression of TRPC5 mutants. To identify the EA-interaction site in TRPC5, we first generated pore mutants. When screening the mutants with EA, we observed the EA-induced current increases of TRPC5 abolished in K554N, H594N, and E598Q mutants. The current increases of other mutants were reduced in different levels. We also examined the functional intactness of the mutants that had no effect by EA with TRPC5 agonists such as carbachol (CCh) or GTPγS. Our results suggest that the three residues, Lys-554, His-594, and Glu-598, in TRPC5 are responsible for direct interaction with EA inducing the channel activation. We also suggest that although other pore residues are not critical, they could partly contribute to the EA-induced channel activation.

Key Words: TRPC5, Englerin A, Pore mutant, Ion channel

P3-21

TMEM16F/ANO6, a Ca^{2+}-activated anion channel, is negatively regulated by the actin cytoskeleton and intracellular MgATP

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Anoctamin 6 (ANO6/TMEM16F) is a recently identified membrane protein that has both phospholipid scramblase activity and anion channel function activated by relatively high $[Ca^{2+}]_i$. In addition to the low sensitivity to Ca^{2+}, the activation of ANO6 Cl^- conductance is very slow (>3–5 min to reach peak level at 10 μM $[Ca^{2+}]_i$), with subsequent inactivation. In a whole-cell patch clamp recording of ANO6 current ($I_{ANO6,w\text{-}c}$), disruption of the actin cytoskeleton with cytochalasin-D (cytoD) significantly accelerated the activation kinetics, while actin filament-stabilizing agents (phalloidin and jasplakinolide) commonly inhibited $I_{ANO6,w\text{-}c}$. Inside-out patch clamp recording of ANO6 ($I_{ANO6,i\text{-}o}$) showed immediate activation by raising $[Ca^{2+}]_i$. We also found that intracellular ATP (3 mM MgATP in pipette solution) decelerated the activation of $I_{ANO6,w\text{-}c}$, and also prevented the inactivation of $I_{ANO6,w\text{-}c}$. However, the addition of cytoD still accelerated both activation and inactivation of $I_{ANO6,w\text{-}c}$. We conclude that the actin cytoskeleton and intracellular ATP play major roles in the Ca^{2+}-dependent activation and inactivation of $I_{ANO6,w\text{-}c}$, respectively.

Key Words: Anoctamin 6, TMEM16F, Calcium activated Cl^- current, Actin cytoskeleton, Magnesium ATP

P3-22

Temperature-dependent increase in the calcium sensitivity and acceleration of activation of ANO6 chloride channel variants

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Anoctamin-6 (ANO6) belongs to a family of calcium (Ca^{2+})-activated chloride channels (CaCCs), with three types of splicing variants (V1, V2, and V5) showing plasma membrane expression. Unlike other CaCCs, ANO6 requires a non-physiological intracellular free calcium concentration ($[Ca^{2+}]_i$ > 1 μM) and several minutes for full activation under a whole-cell patch clamp. Therefore, its physiological role as an ion channel is uncertain and is more commonly considered as a Ca^{2+}-dependent phospholipid scramblase. Here, we demonstrated that physiological temperature (37°C) increased the Ca^{2+} sensitivity of ANO6 under a whole-cell patch clamp; V1 was activated by 1 μM $[Ca^{2+}]_i$, whereas V2 and V5 were activated by 300 nM $[Ca^{2+}]_i$. Increasing the temperature to 42°C led to the activation of all ANO6 variants by 100 nM $[Ca^{2+}]_i$. The delay time for full activation of the three variants was significantly shortened at 37°C. Notably, the temperature-dependent Ca^{2+}-sensitisation of ANO6 became insignificant under inside-out patch clamp, suggesting a critical role of unknown cytosolic factors. Unlike the channel activity, physiological temperature did not induce the scramblase function of ANO6 with submicromolar $[Ca^{2+}]_i$ (300 nM), irrespective of variant type. Our results reveal the physiologically meaningful anion conducting property of ANO6, which might precede the scramblase activity.

Key Words: Anoctamin 6, TMEM16F, Calcium activated Cl^- current, Temperature

P3-23

PTPN6 regulates the cell-surface expression of TRPM4 channels in HEK293 cells

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Transient receptor-potential, cation channel, subfamily M, member 4 (TRPM4) channels regulate a variety of physiological and pathological processes; however, their roles as functional channels under diverse conditions remain unclear. In this study, cytosolic protein tyrosine phosphatase non-receptor type 6 (PTPN6) interacted with TRPM4 channels. We confirmed their interaction by performing co-immunoprecipitation (Co-IP) assays following heterologous PTPN6 and TRPM4 channel expression in HEK293 cells. Furthermore, biomolecular fluorescence complementation (BiFC) image analysis confirmed TRPM4-PTPN6 binding. In addition, immunoblotting and Co-IP analyses revealed that TRPM4 expression significantly decreased in the membrane fraction of cells after PTPN6 was silenced with a specific short-hairpin RNA (shRNA-PTPN6). In agreement, TRPM4-induced changes in whole-cell currents were not detected in PTPN6-silenced HEK cells, in contrast to cells transfected with a scrambled RNA (scRNA) or in naïve HEK cells. These data suggest that PTPN6 inhibits TRPM4 channel activity by disrupting TRPM4 expression. Furthermore, TRPM4 channels were expressed in the membrane of naïve cells and scRNA transfectants, but not in those of PTPN6-silenced cells. These results indicated that PTPN6 is critically associated with TRPM4 trafficking. This role of PTPN6 in TRPM4 membrane localization was also demonstrated in HeLa cells. TRPM4 overexpression significantly enhanced cell proliferation in untreated HeLa cells, but not in HeLa cells with silenced PTPN6 expression. These findings indicate that PTPN6-dependent TRPM4 expression and trafficking to the plasma membrane is critical for cell proliferation in both HEK293 and HeLa cells. Therefore, PTPN6 is a novel therapeutic target for treating pathologic diseases involving TRPM4.

Key Words: Membrane trafficking, PTPN6, TRPM4, Tyrosine phosphorylation

P3-24

Echinochrome A inhibits cardiac SERCA2A by regulating phosphorylation of phospholamban Ser16 and Thr17

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Echinochrome A (Ech A), a marine bio-product isolated from sea urchin eggs, is known to have cardioprotective effects through its strong antioxidant and ATP-sparing capabilities. However, the effects of Ech A on cardiac excitation–contraction (E-C) are not known. In this study, we investigated the effects of Ech A on cardiac contractility and Ca^{2+} handling in the rat heart. In ex vivo Langendorff hearts, Ech A (3 μM) decreased left ventricular developing pressure to 77.7±6.5% of basal level. In isolated ventricular myocytes, Ech A reduced the fractional cell shortening from 3.4% at baseline to 2.1%. Ech A increased both diastolic and peak systolic intracellular Ca^{2+} ($[Ca^{2+}]_i$). However, the ratio of peak $[Ca]_i$ to resting $[Ca]_i$ was significantly decreased. Ech A did not affect the L-type Ca^{2+} current. Inhibiting the Na^+/Ca^{2+} exchanger with either $NiCl_2$ or SEA400 did not affect the Ech A-dependent changes in Ca^{2+} handling. Our data demonstrate that treatment with Ech A results in a significant reduction in the phosphorylation of phospholamban at both serine 16 and threonine 17 leading to a significant inhibition of SR Ca^{2+}- ATPase 2A (SERCA2A) and subsequent reduced Ca^{2+} uptake into the intracellular Ca^{2+} store. Taken together, our data show that Ech A negatively regulates cardiac contractility by inhibiting SERCA2A activity, which leads to a reduction in internal Ca^{2+} stores.

Key Words: Echinochrome A, Negative inotropic effect, SERCA2A inhibition, Phospholamban phosphorylation

P3-25

Differential changes of flow-induced vasodilation mechanisms in coronary arteries from spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY)

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Increase in blood flow and shear stress are important physiological stimuli for vasodilation, which is mediated by endothelium-dependent hyperpolarization (EDH) as well as nitric oxide (NO) release. Pathological changes in EDH of coronary artery (CoA) and the relevant ion channels in coronary smooth muscle cells (CoASMCs) are poorly investigated yet. Here, using myography system, we examined the endothelium dependent relaxation (EDR) responses CoA to NS309, an activator of Ca^{2+}-activated K^+ channels (IK_{Ca} and SK_{Ca}) in endothelial cells. Also, we compared the current density of inwardly rectifying K+ channels (Kir) in CoASMCs obtained from spontaneously hypertensive rats (SHR) and their control WKY. Concentration-dependent relaxation of CoA by NS309 showed lower sensitivity in SHR than WKY, which was not altered by pretreatment with apamin (SK_{Ca} channel inhibitor). The pharmacological sensitivity implied that IK_{Ca}, rather than

SK_{Ca}, in the CoA endothelium of SHR are functionally downregulated. The release of K^+ from endothelium via IK_{Ca} would relax CoA via activation of Kir in CoASMCs. Interestingly, the amplitude of Kir current of CoASMCs was higher in SHR than WKY, suggesting a compensatory change for the EDH. Consistently, the contraction of CoA by Kir inhibitor (Ba^{2+}, 100 μM) was higher in SHR than WKY. In contrast to CoASMCs, Kir current densities in skeletal arterial myocytes and in cerebral arterial myocytes were lower in SHR than WKY. In the myography study, although the NS309-induced relaxation of CoA became commonly attenuated by pretreatment with Ba^{2+}, the dose-dependent relaxation responses of SHR was still less sensitive than WKY. Taken together, we suggest that the IK_{Ca} might be downregulated in the CoA endothelium of SHR, which might be partly compensated by the increased Kir activity in CoASMCs. The opposite changes of Kir current between CoASMs and other systemic arteries require further investigation.

Key Words: Coronary artery, Endothelium, Smooth muscle, Ca^{2+}-activated K^+ channel, Inwardly rectifying K^+ channel, Hypertension

P3-26

Connexin-43-hemichannel-mediated ATP efflux triggers arrhythmogenic Ca^{2+} waves via P2X purinoceptor current in atrial myocytes

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Myocardium is subjected to mechanical stresses and adapts to them. Atrial myocytes are exposed to high shear stress during hemodynamic overload and blood regurgitation. We have previously shown evidence that shear stress (~16 dyn/cm^2)-induced atrial global Ca^{2+} waves are abolished by the blockades of ATP release, gap junction hemichannel or P2 purinergic signaling. In this study, we assessed activation of gap junction hemichannels by shear stress, and its role in induction of arrhythmogenic currents and in proarrhythmic Ca^{2+} waves in rat atrial myocytes. Calcein dye efflux, but not oregon flux, was accelerated by shear application. The shear-induced calcein efflux was enhanced by zero external Ca^{2+}, and was suppressed by La^{3+}, but not by probenecid, suggesting activation of connexins by shear stress. Shear stress produced inward cation (Cs^+) currents at resting potentials, and this current was completely suppressed by La^{3+} or carbenoxolone, and was enhanced by quinine. The current was also partly suppressed by P2X receptor inhibition (by ~50%) or by blockade of $P2Y_1$ receptor/transient receptor potential melastatin subfamily 4 (by ~30%). Shear-induced NMDG+ current was one-fifth of the Cs+ current, showed linear voltage-dependence with a reversal at 0 mV and eliminated by introduction of anti-connexin-43 antibodies. Shear-induced ATP release from a monolayer of atrial cells, assessed by chemiluninescence, was abolished by either connexin-43 hemichannel inhibitor Gap 19 or connexin-43 knock-down. Simultaneous measurement of ATP release and Ca^{2+} images using a sniffer patch clamp and two-dimensional confocal microscopy, respectively, further revealed that ATP releases occurred at 200-300-ms prior to the onset of the Ca2+ waves under shear stress and were sustained under prolonged shear stimulus. Our data suggest that shear stress induces connexin-43-hemichannel-mediated ATP release, thereby initiating P2X purinoceptor-dependent triggered Ca^{2+} waves in atrial myocytes.

Key Words: Shear stress, Gap junction hemichannel, Connexin-43, P2X purinocptor, Ca^{2+} wave, Atrial myocytes

P3-27

Calcium/calmodulin dependent inhibition of TRPC6 is governed by the NT and CT interaction of TRPC6 channels

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Mutations in transient receptor potential cation channel, subfamily C, member 6 (TRPC6) have been identified to cause focal and segmental glomerulosclerosis (FSGS) in human patients. A total of 18 substitution mutants, one frameshift mutation and one deletion mutant have been recorded in TRPC6 isolated from the kidneys of human patients with FSGS. Previous studies have reported that some of these mutants display a gain of function. Our objective is the find how these FSGS causing mutants affect the function of TRPC6 channel. To study this, we generated all TRPC6 FSGS mutations in rat TRPC6 and tested their functionality with whole cell patch clamp technique. The Patch clamp experiments revealed that some of the CT and NT mutants display increased current amplitudes, increased calcium permeability, lacked calcium dependent inhibition (CDI) and were active at lower internal free calcium concentrations compared to wild type channel. These gain of function mutants are randomly scattered in the NT Ankyrin repeat (AKR) and CT coiled coil domain (CCD) region of TRPC6 channel when sorted according to the amino acid sequence of primary structure. However, tertiary and quaternary structure analysis revealed that all of the gain of function mutations except for a one FSGS mutation occur at the NT and CT interface of TRPC6. Furthermore, the NT Gain of function FSGS mutation sites have complementary sites at CT and vice versa. Gain of function phenotype of a FSGS mutant can be reversed when the corresponding site at either NT or CT was mutated to restore the interaction. For an example Q888K gain of function FSGS mutation could be reversed when the Y107 which is the amino acid with closest proximity to Q888 was mutated to Y107D to result in a salt bridge interaction between Q888K and Y107D. The C-terminal CaM/inositol-1,4,5-trisphosphate receptor binding domain (CIRB) is located at the CT of TRPC6 right next to the CCD. This strongly indicates the CT-NT interaction can result in a conformational change that allow the CIRB domain to interact with CaM and inositol-1,4,5-trisphosphate. FSG mutants did not display changes in response to inositol-1,4,5-trisphosphate. However, R864Q mutation which lacks the CaM binding ability but not the affinity to bind inositol-1,4,5-trisphosphate displayed a gain of function phenotype similar to the FSG gain of function phenotype.
Therefore, the current data indicate that TRPC6 CT and NT interaction is crucial for the Calcium/CaM dependent inactivation of TRPC6. The gain of function FSGS mutations interrupts this CT-NT interaction which results uncontrolled calcium influx to the podocytes which will lead to FSGS.

Key Words: TRPC6, Calmodulin, Coiled coil domain, Focal and segmental glomerulosclerosis

P3-28

Poly-phenol enriched green tea extract rescues cognitive impairment by restoring hippocampal synaptic plasticity in post-menopausal depression

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Post-menopausal major depression (PMD) is a common but serious disorder that causes severe symptoms that affect feel, think, and handle daily activities. The PMD is more abundant in post-menopausal woman because it is commonly caused by estrogen decline. Many researchs showed that synaptic strength changes by stress in cortex, hippocampus, and amygdala are implicated in depressive symptoms, which lead to abnormality of cognition and memory impairment. Despite growing evidences of green tea

as a potential therapeutic for PMD, its clinical use is limited because of adverse effects and ambiguity of its action mechanism. In this study, we newly developed poly-phenol enriched green tea extract (PeGTE) has lower neuronal toxicity than normal green tea extract (GTE), and demonstrated that PeGTE administration prevents development of learned helplessness (LH) more potently than GTE. In addition, PeGTE improved LH-induced cognitive impairment by restoration of BDNF/GluN2B expression. Taken together, our finding suggests the possibility that PeGTE may be new therapeutic strategy to increase resilience and reduce the cognitive deficits in women who are susceptible to stress and hormonal fluctuations by aging.

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Key Words: Post-menopausal major depression, Poly-phenol enriched green tea extract, Cognitive impairment, Synaptic plasticity, Hippocampus

P3-29 (PO-A-6)

Contribution of transient receptor potential channels to store-operated calcium entry in autonomic neuron-satellite glia unit

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Satellite glial cells (SGCs) ensheath the cell bodies of neurons within the autonomic ganglia. The unique anatomical arrangement suggests some signal exchanges between the autonomic neurons and SGCs. To date, however, little is known about the functional roles of the autonomic neuron-SGC units. In this study, we investigated the molecular mechanisms underlying calcium homeostasis in SGCs in responding to the various extracellular stimuli. In this regard, we isolated sympathetic superior cervical ganglion (SCG) neurons which are attached with SGCs by a partial enzymatic digestion, and then performed calcium imaging with Fura-2/AM. Both neurons and SGCs exhibited a store-operated Ca^{2+} entry (SOCE) when the internal storage of Ca^{2+} was depleted by cyclopiazonic acid (CPA) along with the removal of extracellular calcium. This depletion was further recovered rapidly on restoration of extracellular calcium via SOCE. The magnitude of SOCE was much larger in the SGCs than in the neurons. Unlike the neurons, interestingly, the SOCE in the SGCs was accompanied with a large Ca^{2+} oscillation. Using quantitative RT-PCR, we detected the expression of Orai 1/2/3 channels, stromal interaction molecules 1/2 (STIMs - endoplasmic reticulum calcium sensors), and the transient receptor potential channel canonical types 1/3/6 (TRPCs) which are responsible for SOCE. The blockade of TRPCs with lanthanides and pyrazoles significantly decreased the SOCE and the Ca^{2+} oscillations in SGCs, indicating the engagement of TRPC channels in SOCE. Taken together, the SOCE is mediated by both Orai and TRPC channels and may contribute to the Ca^{2+} signaling in the autonomic neuron-SGC units.

Key Words: Autonomic ganglia, Satellite glial cell, Calcium oscillation, Store-operated Ca^{2+} entry, TRPC

P3-30

The N-terminus of voltage-gated Ca^{2+} (Ca_V) channel β3 subunit sensitizes PIP_2 dependence of $Ca_V2.2$ channel gating

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The gating properties of voltage-gated Ca^{2+} channel (Ca_V) can be regulated by diverse cellular signaling molecules including plasma membrane phospholipids. For example, depletion of phosphatidylinositol 4,5-bisphosphate (PIP_2) by a voltage-sensing 5-phsphatase from zebra fish (Dr-VSP) dramatically attenuates the current amplitude in high-voltage activate Ca_V channels. Recently our group found that the PIP_2 sensitivities of Ca_V channels are also dependent upon Ca_V β subunits complexed with the channels. The results showed that when the $Ca_V2.2$ channels were co-expressed with cytosolic β-subunits, the current inhibition by PIP_2 depletion using activation of Dr-VSP was generally very strong, approximately up to 40%. Especially, in $Ca_V2.2$ channels with β3 subunits the current inhibition by PIP_2 depletion was more significant approximately ~60%. Here, we identified that the N-terminus of β3 subunit is the key regulator of PIP_2 sensitivities in $Ca_V2.2$/β3 channels. If the N-terminus of β3 subunits are deleted, the inhibition of Ca_V currents by PIP_2 depletion is reduced. When we made chimera β3 subunits where the N-terminus of β3 was replaced with that of β2b, PIP_2 sensitivity was decreased to that of β2b. However, there are no differences in PIP_2 sensitivities when the N-terminus of β3 subunits is substituted to neutrally charged amino acids or non-phosphorylation sites. Together, our results suggest that the N-terminus of β3 subunit plays an important role in PIP_2 regulation of $Ca_V2.2$/β3 complex.

Key Words: PIP_2, Ca_V, β3, Dr-VSP, N-terminus

P3-31

Guanabenz inhibits HCN current in MTN neurons via a2A adrenergic receptor-independent pathway

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Mesencephalic trigeminal nucleus (MTN) neurons, which are eccentrically located in the central nervous system, relay trigeminal proprioceptive inputs from the masticatory system. MTN neurons robustly express hyperpolarization activated-cyclic nucleotide modulated-cation channels (HCN), which regulate neuronal excitability. Alpha 2A noradrenergic receptor ($a2_A$ AR), a Gi protein coupled receptor well known for inhibiting HCN channels, has been suggested to be expressed in MTN neurons. In this study, we aimed to investigate whether $a2_A$ AR is functionally expressed in MTN neurons to negatively modulate HCN channels. HCN current (I_h) was recorded with MTN neurons within brainstem slice obtained from SD rats (p14-20) by using whole-cell patch-clamp recordings. Steady state I_h and tail currents were used to analyse the effect of $a2_A$ AR agonist, guanabenz (GBZ). The involvement of $a2_A$ AR on I_h inhibition was examined by a2 AR antagonist, atipamezole, and cell-permeable cAMP, 8-Br-cAMP. GBZ reduced Ih in MTN neurons, its effect being dose-dependent (IC_{50} = 8.0 μM). GBZ-sensitive Ih current was similar to those sensitive to ZD7288 (HCN blocker) but not Ba^{2+} (K_{ir} blocker), indicating GBZ mostly affected Ih. The activation curve of Ih shifted toward hyperpolarized membrane potential by GBZ treatment (control vs. GBZ; -80.7 mV vs. -91.4 mV). Surprisingly, GBZ-mediated I_h inhibition (normalized value, control vs. 1, 3, 10 uM atipamezole; 0.48 vs. 0.51, 0.58, 0.41) and negative shift of I_h activation (control vs. GBZ; -89.6 mV vs. -98.2 mV) were not reversed by atipamezole. Furthermore, intracellular cAMP perfusion by 8-Br-cAMP also failed to reverse Ih inhibition, indicating GBZ effect was not due to $a2_A$ AR activation. Our results indicate that GBZ inhibits Ih currents via $a2_A$ AR independent pathway. Further investigation remains to identify the endogenous modulator of Ih in MTN neurons.

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Key Words: Mesencephalic trigeminal nucleus neurons, Hyperpolarization-activated cyclic nucleotide-modulated cation channel (HCN), Alpha 2A noradrenergic receptor ($a2_A$ AR), Electrophysiology

P3-32

Evaluating physiological interaction between the electrogenic Na/HCO_3 transporter NBCe1-B and its cytosolic binding partner IRBIT

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The electrogenic Na/HCO_3 transporter NBCe1 is expressed in numerous organs, where it plays many important roles, including trans-epithelial HCO_3^- transport (e.g., pancreatic ducts) and maintenance of intracellular pH (e.g., astrocytes). Presently, five NBCe1 variants are known: the A variant, mainly in kidney; B ubiquitous; C, CNS; and D and E, murine reproductive organs. The extreme NH_2-terminus (Nt) of each variant is a strong determinant of transport activity. A and D have a unique Nt of 41 amino acids (aa) that, at least in A, confers high transport activity. B, C, and E have a unique Nt of 85 aa that, at least in B and C, confers low transport activity. However, B interacts with IRBIT [inositol trisphosphate (IP_3)-receptor binding protein released with IP_3], which binds to the unique Nt of NBCe1-B, and markedly enhances NBCe1-B's transport activity. Previously, others examined the interaction by fusing a fragment of the unique Nt of NBCe1-B/C/E to maltose-binding protein (MBP) for use in a MBP pull-down assay, and found that NBCe1-B (e1B) residues 1–62 are necessary for the interaction. In a recent study, we showed that the cationic cluster (residues 40–48 inclusive) is an essential element of the autoinhibitory domain that confers low activity on e1B. The purpose of the present study is to refine the structural requirements of the IRBIT-e1B interaction under physiological conditions. Here, we co-express 7 constructs of e1B together with super-IRBIT (sIRBIT, which lacks the PP1 binding site) in *Xenopus* oocytes, followed by two-electrode voltage-clamp, extracellular-surface-biotinylation, and NeutrAvidin pull-down of plasma-membrane proteins. We find that sIRBIT copurifies with biotinylated e1B protein, but only for low-activity e1B constructs for which sIRBIT enhances transport activity. sIRBIT does not copurify with the $\Delta 9_{40\text{-}48}$ e1B construct, which lacks the cationic cluster. Thus, the cationic cluster is not only essential for autoinhibition, but also for sIRBIT binding. Our approach will be useful for elucidating other motifs that may be necessary for the interaction of IRBIT with NBCe1-B, -C, or -E.

Key Words: Acid-base transporters, NBCe1, Autoinhibition, IRBIT

P3-33

Neurotensin modulates spontaneous firing of nigral dopamine neurons through region-dependent two distinct pathways

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Endogenous activity of dopamine neurons in substantia nigra pars compacta (SNc) generates regular spontaneous firing. Many neurotransmitters regulate and modulate this regular rhythm, resulting in production of diverse types of their own specific firing patterns via various ion channels and Ca^{2+} signals. However, it is not well elucidated how neurotransmitters including neurotensin (NT) induce specific firing patterns in dopamine neurons at the cellular level. NT, a peptide neurotransmitter, has been known to activate not only NALCN channels but also intracellular Ca^{2+} ($[Ca^{2+}]_i$) signals in dopamine neurons. Therefore, we have investigated how NT generates specific firing patterns in dopamine neurons at the subcellular level. Using patch clamp and Ca^{2+} measurement techniques, we observed NACLN currents, Ca^{2+} changes, and firing patterns in acutely dissociated mice SNc dopamine neurons. We found that NT has dual distinct actions on spontaneous firing: while stimulation of proximal dendritic region with local NT puffing increases tonic firing rates, stimulation of the soma decreases tonic firing, often pause of spontaneous firing. In the proximal dendritic region, NT strongly increased NALCN currents, but in the soma NT potently evoked $[Ca^{2+}]_i$ by the ER release. These results suggest that NT activates Ca^{2+} signaling and NALCN region-dependently, and NT-mediated downstream pathways are distributed in different subcellular compartments, thereby differently regulating excitability and generating specific firing patterns in nigral dopamine neurons.

Key Words: Neurotensin, Dopamine neuron, Spontaneous firing, Excitability

P3-34

Calpain inhibition mediates NMDAR responsiveness through KCNQ channels in midbrain dopamine neurons

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Midbrain dopamine neurons exhibit spontaneous firings whose activities and patterns are tightly regulated by calcium signals. The NMDA receptor (NMDAR) is a cation channel highly permeable to calcium and plays essential roles in modulation of firing patterns and dopamine release in dopamine neurons. Cytosolic Ca^{2+} influx through NMDAR can lead to the activation of the Ca^{2+}-dependent protease, calpain. Calpain activation regulates numerous downstream targets such as ROS and is highly involved in the pathogenesis of several diseases such as brain injury, Alzheimer disease and Parkinson's disease. However it remains largely unclear how calpain inhibition affect firing activities in the dopamine neurons. Therefore in the acutely dissociated midbrain dopamine neurons, we studied how calpain inhibition regulates NMDA-induced calcium signals and firing patterns. In the dopamine neurons, when we increased spontaneous firing rate with doses of NMDA, the rise in global $[Ca^{2+}]_c$ levels was correlated with the spontaneous firing rate. However, when we blocked calpain activation with membrane permeable calpain inhibitor, MDL 28170 (30 μM), the NMDA (50~100 μM)-induced Ca^{2+} rises were significantly exaggerated by contrast with those of control condition. In patch clamp recording, NMDAR-mediated regular firing patterns were significantly changed burst-phase patterns in pretreatment with MDL28170. Another membrane permeable calpain inhibitor, calpeptin (10 μM), also turned regular firing patterns into burst-pause firing patterns. In the resting condition, calpain inhibitors, MDL28170 and calpeptin do not affect in membrane potential and calcium signaling, however, they lead to spike broadening and Ca^{2+} increase due to inhibition of voltage-gated potassium channels in NMDA responses of dopamine neurons.
These consequently indicate that calpain inhibition regulates NMDA responsiveness by properties of ion channels, which mechanisms of NMDA responses to firing patterns changes in dopamine neuron may be important to understand calpain-mediated pathophysiological process such as Parkinson's disease.

Key Words: Dopamine neuron, NMDA receptor, Calpain inhibition, MDL28170, Calpeptin, Calcium, Firing patterns, Voltage-gated potassium channels

P3-35

Endogenous ROS induced by menadione accumulates intracellular calcium in mouse pancreatic acinar cells

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Reactive oxygen species (ROS) are formed as a result of partial reduction of oxygen during aerobic respiration. Under physiological conditions, ROS are controlled by intracellular free radical scavengers and antioxidant enzymes to protect cells from injuries. However, imbalance between ROS generating and scavenging systems can lead to oxidative stress which can

morphologically and functionally damage cells. Intracellular calcium (Ca^{2+}) oscillation is an initial event in digestive enzyme secretion of pancreatic acinar cells. Reactive oxygen species are known to be associated with a variety of oxidative stress-induced cellular disorders including pancreatitis. We previously reported that exogenous hydrogen peroxide accumulated intracellular Ca^{2+} in mouse pancreatic acinar cells. In this study, we investigated the effect of endogenous ROS induced by menadione on intracellular Ca^{2+} accumulation in mouse pancreatic acinar cells. Perfusion of menadione resulted in additional elevation of intracellular Ca^{2+} levels and termination of oscillatory Ca^{2+} signals induced by carbamylcholine (CCh) in the presence of normal extracellular Ca^{2+}. Antioxidant (NAC) completely prevented menadione-induced Ca^{2+} accumulation. Menadione significantly accelerated ROS generation and that was effectively protected by pretreatment of antioxidant, NAC. In Ca^{2+}-free medium, SERCA activity was completely reduced by menadione similar with thapsigargin and that was also protected by pretreatment of NAC. These results provide evidence that endogenous ROS induced by menadione could accumulate intracellular Ca^{2+} by attenuating refilling of internal Ca^{2+} stores in mouse pancreatic acinar cells.

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Key Words: Reactive oxygen species, Menadione, Intracellular calcium, Sarco/endoplasmic reticulum Ca^{2+} ATPase, Pancreatic acinar cells.

P3-36

Proximal C-terminal controls the sensitivity to fluoxetine in TREK-2 channel

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Earlier studies have demonstrated that TREK-2 two-pore domain K^+ (K_{2P}) channel is inhibited by fluoxetine and norfluoxetine, which bind to TREK-2 when the channel is in the down conformation. Some interacting residues between TREK-2 and fluoxetine have been proposed based on TREK-2 crystal structure in complex with fluoxetine, but the proposed binding region does not include the cytoplasmic carboxyl-terminus (Ct). This study was performed to investigate a potential role of Ct for the fluoxetine sensitivity in TREK-2 channel. In HEK-293A cells transfected with rat TREK-2 or mutants. Fluoxetine significantly inhibited TREK-2 channel activity in a dose-dependent manner ($p < 0.05$). The IC_{50} concentration for TREK-2 channel was 28.7±7.6 μM. Fluoxetine significantly inhibited TREK-2 channel activity in inside-out and outside-out patches, but not in cell-attached patches (inside-out; control, 0.04±0.01/ fluoxetine, 0.004±0.001, outside-out; control, 0.041±0.011/ fluoxetine, 0.009±0.006), indicating that fluoxetine may modulate TREK-2 channel through direct interaction rather than through signaling transduction pathways. To identify the binding sites of fluoxetine in the TREK-2, mutants with amino (N)-terminal (Nt) or Ct deletion were constructed (TREK-2ΔN and TREK-2ΔC). The channel activity of wild-type TREK-2 was inhibited by approximately 50% in the treatment with 30 μM fluoxetine. Fluoxetine decreased the channel activity of TREK-2ΔN like it did in TREK-2 wild-type, whereas no inhibition was observed in the channel activity of TREK-2ΔC. To further determine the essential region in the Ct, TREK-2 Ct truncation mutants (1-323, 1-338, and 1-347) were constructed. Compared to wild-type, TREK-$2_{1\text{-}323}$ and TREK-$2_{1\text{-}338}$ showed low inhibition in response to fluoxetine. TREK-$2_{1\text{-}347}$ showed similar effect to wild-type in response to fluoxetine. In addition, absences of charged six amino acids ($_{3237}$KKTKEE$_{332}$) in this region showed no significant inhibition by fluoxetine. These results indicate that proximal Ct region is critical for fluoxetine sensitivity.

Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF-2015R1A-5A2-008833, NRF-2018R1A2B6001446) grant funded by the Korea government.

Key Words: Fluoxetine, Tandem-pore domain K^+ channel, TREK-2

P3-37

Decrease of inward rectifier and voltage-dependent K^+ currents of the right coronary artery smooth muscle cells in pulmonary arterial hypertensive rats

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Voltage-gated K^+ channels (Kv) counterbalance the depolarization and L-type Ca^{2+} channel activation in vascular smooth muscle. Inwardly rectifying K^+ channels (Kir) mediate the endothelium dependent hyperpolarization and the relaxation by moderate increase of $[K^+]_{ext}$. Pulmonary arterial hypertension (PAH) induces right ventricle hypertrophy (RVH) and right heart failure (RHF). Although the previous studies demonstrated dysfunctions of coronary endothelial cells in PAH, the changes of K+ channel activities in the coronary artery smooth muscle cells are rarely investigated. Here we compared Kv and Kir current densities (I_{Kv} and I_{Kir}) in right and left coronary arterial myocytes (RCoSCs and LCoSCs) from control and monocrotaline-induced PAH rats. Monocrotaline was injected at 8 weeks, and significant increase in the pulmonary arterial pressure with the hypertrophy of right ventricle was confirmed at 11 weeks (MCT-3w) in the pilot studies. Through the whole-cell patch clamp study, we found that the I_{Kir} in the control RCoSCs was smaller than that of LCoSCs. Although I_{Kv} amplitudes were similar, the half-inactivation voltage ($V_{1/2,inact}$) was more negative in RCoSCs than LCoSCs. In MCT-3w, the amplitudes of both I_{Kv} and I_{Kir} were decreased in RCoSCs while not in LCoSCs. Also, the voltage-dependence of inactivation was partly right-shifted, and the $V_{1/2,inact}$ was not different between RCoSCs and LCoSCs. The RCoSCs-specific decreases of the I_{Kv} and I_{Kir} might partly underlie the functional impairment of coronary blood flow regulation in the PAH-induced pathological RVH and RHF.

Key Words: Coronary artery, Smooth muscle, Pulmonary artery hypertension, Kv channel, Kir channel

P3-38

Alterations of shear-activated cation channels during chronic mechanical stress-induced atrial remodeling

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Our previous findings have suggested that high shear stress activates P2-receptor-linked cation channels including transient receptor potential melastatin 4 (TRPM4), through connexin hemichannel-mediated ATP release in atrial myocytes. To know possible involvement of these shear-activated channels in atrial adaptation under chronic mechanical stress, we examined whether they are altered by chronic pressure overload (CPO), induced by transverse aortic constriction (≥35 weeks). The heart weight to body weight ratio increased significantly by CPO. The size of left atria as well as the capacitance of left atrial myocytes were larger in the CPO group (108±4.3 pF, n=22) than in the sham-operated control group (54.4±6.5 pF, n=17, $P<0.001$). When whole-cell currents triggered by brief shear stimulus (~16 dyne/cm^2; 2 s) were assessed using symmetrical CsCl-rich solutions in isolated atrial myocytes, they were found to be about two-fold larger in CPO atrial cells than sham atrial cells (CPO: 2.55±0.32 pA/pF, n=10; sham: 1.49±0.19 pA/pF, n=8, $P<0.05$). Shear-triggered $NMDG^+$ current, representing gap junction channel current, was larger in atrial myocytes from CPO rats (1.20±0.05 pA/pF, n=6) than in the myocytes from sham rats (0.63±0.12 pA/pF, n=4, $P<0.01$). Prolonged shear-induced Cs^+ currents that are mainly carried by TRPM4 channels, were significantly smaller in CPO atrial cells (at +60 mV) (3.12±0.39 pA/pF, n=6) than in sham cells (5.26±0.62 pA/pF, n=5, $P<0.05$). The sensitivity of this current to 9-phenanthrol, the TRPM4 blocker, was not changed by CPO. These results suggest that CPO induces left atrial hypertrophy with significant alterations in shear-connexin/TRPM4 signaling, and

further suggest contribution of these shear response to atrial remodeling under chronic increase in afterload, such as hypertension.

Key Words: Shear stress, Atrial myocytes, Chronic pressure overload, Connexin, TRPM4

P3-39

Effects of PCB 77 on Kv1.3 channel and Kv1.5 channel

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Polychlorinated biphenyls (PCBs) are a family of bicyclic chlorinated aromatic hydrocarbons. PCBs' unique chemical properties and the low cost of producing PCBs have contributed to their extensive industrial use. PCBs are detected in air, water, sediments, fish, and wild life and human adipose tissue, milk, and serum. Acute and long-term exposure to the compounds has been known to causes diseases such as developmental delays and motor dysfunction. The presence of PCBs in the environment presents various toxic effects in vivo and in vitro. But the mechanism is not yet fully understood. One immune-modulating mechanism is achieved by the Kv1.3 voltage-dependent potassium channel, which is expressed highly in lymphocytes including effector memory T lymphocytes. Kv1.5 channel contributes to action potential repolarization of myocytes in heart. Here we studied the effect of 3,3',4,4'-tetrachlorobiphenyl (PCB77) on human Kv1.3 channels and Kv1.5 channels expressed in *Xenopus* oocytes. We exposed the oocytes with PCB77 for 8 min and 15 min. The peak current of Kv1.3 decreased by PCB77 (0.03~5 μM) , however the peak current of Kv1.5 did not change by PCB77 (0.03~5 μM). PCB77 inhibited the amplitude of the peak Kv1.3 channel current in a concentration-dependent manner. These results suggest the possibility that PCB77 could inhibit the immune mechanism by suppressing the current of the Kv1.3 channel.

Key Words: Polychlorinated biphenyls, PCBs, PCB77, Kv1.3 channel, Kv1.5 channel

P3-40

Effects of polychlorinated biphenyl 77 on PKC activation and Kv1.5 channel current

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Polychlorinated biphenyls (PCBs) have been known as serious persistent organic pollutants (POPs), causing developmental delays and motor dysfunction. PCBs have 208 possible congeners and we investigated the effects of 3,3',4,4'-tetrachlorobiphenyl (PCB77). We observed the phosphorylation of protein kinase C (PKC) with signal transduction, cell growth, gene expression, and tumor promoter function by western blotting. We treated ventricular myocytes from guinea pig with 0.1~100 μM of PCB77 for 15~60 min. The phosphorylation of PKCII decreased significantly by 40% at 0.1 μM of PCB77. As for PKC, PCB77 increased the phosphorylation of the PKC subtype by 20% at 1~100 μM. Phosphorylation of PKC was decreased by 15% at 0.1 μM PCB77 and by 40% at 10 μM PCB77. Also, we conducted further studies on the changes of Kv1.5 channel current by PCB77. We expressed the Kv1.5 channels, which contributes to action potential repolarization of myocytes in the heart, in *Xenopus* oocytes and treated PCB77 (10~100 μM) for 8 minutes and 15 minutes. We observed that the peak current of Kv1.5 channel decreased by PCB77. The present data indicate that PCB77 could affect cardiac function possibly by activation of PKC and Kv1.5 channel function, in view of the possible accumulation of the PCBs in human body.

Key Words: Polychlorinated biphenyls, PCBs, PCB77, Protein kinase C, Kv1.5 channel, Heart

P3-41

Effects of paroxetine on kinetics of Kv1.3

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Paroxetine is the most potent inhibitor of the reuptake of serotonin of all selective serotonin reuptake inhibitors. One immune-modulating mechanism is achieved by the Kv1.3 voltage-dependent potassium channel, which is expressed highly in lymphocytes including effector memory T lymphocytes. Here we studied the rapid effects of paroxetine on the human Kv1.3 channel expressed in Xenopus oocytes. Paroxetine reduced the amplitude of the Kv1.3 channel current in a concentration-dependent manner. Paroxetine inhibited Kv1.3 steady state channel currents more than peak currents at membrane potentials from +10 to +60 mV. Paroxetine did not affect the slope degree of either activation or inactivation curves. The peak currents and steady state currents of Kv1.3 channel decreased as soon as 100 μM paroxetine containing ND96 solution. The current inhibition by 100 μM paroxetine was reversible by washing for 40 min. These results suggest that paroxetine inhibit Kv1.3 currents via a non-genomic mechanism, providing a mechanism for the immunosuppressive effects of paroxetine.

Key Words: Paroxetine, Kv1.3 channel, Xenopus oocytes

P3-42

Concentration-dependent effects of alprenolol on human Kv1.3 channel

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Alprenolol is one of the adrenergic beta-antagonists used as an antihypertensive, anti-anginal, and anti-arrhythmic agent. Alprenolol non-selectively blocks beta-1 adrenergic receptors mainly in the heart, inhibiting the effects of epinephrine and norepinephrine resulting in a decrease in heart rate and blood pressure. The Kv1.3 voltage-dependent K^+ channel is highly expressed in lymphocytes and is involved in the immune response. However, it has not been known whether alprenolol has a rapid effect on the function of Kv1.3 channel. We examined the effect of alprenolol on the human Kv1.3 channel using a *Xenopus* oocyte expression system and a two-microelectrode voltage clamp technique. Alprenolol reduced or increased the amplitude of the Kv1.3 channel current according to the concentration of the drug. At 1~100 μM of alprenolol, relatively lower concentration, the drug increased the amplitude of the Kv1.3 channel current. The current was decreased by higher concentrations of alprenolol (300~1000 μM). This inhibitory effect of alprenolol was more prominent to the steady-state currents than the peak currents. At 1~100 μM of alprenolol, the increasing effect of the drug on the steady state current was stronger than the effect on the peak currents. These results suggested that alprenolol modulates Kv1.3 currents via a non-genomic mechanism. These effects might provide an electrophysiological mechanism of alprenolol's immunosuppressive effects, probably by altering the properties of Kv1.3 channels.

Key Words: Alprenolol, Kv1.3 channel, Non-genomic

P3-43

A novel high-frequency variant of TRPV3 p.A628T in East Asians showing faster activation and higher Ca^{2+} influx by repetitive applications of chemical agonists

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TRPV3, a member of thermosensitive TRPV channel family expressed in skin and sensory nerves, is also activated by various chemical agonists. Repetitive stimulation of TRPV3 characteristically induces sensitization; cumulative increase of current amplitudes with a loss of voltage-dependence (i.e. changes in the current-voltage relation from outward rectification to linear shape). A recent genomic analysis revealed non-rare TRPV3 mutation (p.A628T) in the East Asians including the Korean population (variant allele frequency: 0.249 in East Asians, 0.007 in Europeans). To elucidate the functional significance, whole-cell patch clamp was conducted in HEK293T cells overexpressing wildtype TRPV3 (WT) and the variant (p.A628T). Repetitive warming from 23 (RT) to 37°C for 30 s with 30 s RT interval cumulatively activated outward-rectifying current in both WT and p.A628T, without I/V curve linearization. The repetitive temperature pulses to 43°C showed tendency of fast sensitization with I/V curve linearization in p.A628T. Repetitive applications of 10 μM 2-aminoethyl diphenylborate (2-APB) or 100 μM carvacrol (30 s with 30 s washout interval) induced faster activation and earlier linearization of I/V curve in p.A628T. However, farnesyl pyrophosphate (1 μM), an intrinsic lipid metabolite agonist of TRPV3, induced only partial activations of outward rectifying currents in both WT and p.A628T. In fura-2 microspectrofluorimetry, the 2-APB pulses induced faster increase of $[Ca^{2+}]_i$ in p.A628T than WT. The relative Ca^{2+} permeability ($P_{Ca/Na}$) was not different between WT and p.A628T. The physiological and pharmacological implications, e.g. differential skin sensitivity to chemical agents and high temperature, of the newly found TRPV3 variant require further investigation.

Key Words: TRPV3 variant, Thermosensitive, 2-APB, Carvacrol, Ca^{2+} permeability

P3-44

Combination of transcranial alternating current stimulation and fermented Scutellaria baicalensis ameliorates motor recovery and cortical neural excitability following focal stroke

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Transcranial alternating current stimulation (tACS) is known as non-invasive neuromodulation that alters neural excitability in the cortex. Some composites of fermented *Scutellaria baicalenis* (*FSB*) can activate intracellular signaling pathway of brain-derived neurotrophic factor. This study was aimed at evaluation of combinatory treatment of tACS and FSB on behavior recovery and cortical neural excitability in rodent focal stroke model. Focal ischemic stroke was induced by photothrombotic injury to the motor cortex of adult rats. Application of tACS with 5 Hz and 200 μA in combination with daily oral treatment of FBS was given to stroke animals for 3 weeks. Motor recovery was evaluated by rotating bean test and ladder working test. Firing activity of cortical pyramidal neurons of stroke model was monitored using multi-channel extracellular recording technique. Compared with control stroke group who did not receive any treatment, combination of tACS and FSB treatment resulted in more rapid recovery of forelimb movement following focal stroke. This combination treatment also elicited increase in spontaneous firing rate of putative pyramidal neurons. Furthermore, expression of metabolic marker for neural excitability was upregulated in peri-infract area under thallim autometallography. These results suggest that combination treatment of tACS and FSB can be a possible remedy for motor recovery in focal stroke

Key Words: Focal stroke, *Scutellaria baicalensis*, Transcranial alternating current stimulation, Thallim autometallography

P3-45

Polyamine-mediated inward rectification of TRPC4 channel

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Transient Receptor Potential Canonical 4 (TRPC4) channel is a Ca^{2+}-permeable, non-selective cation channel. TRPC4 is expressed in various tissues such as brain, thyroid, ventricular myocyte, kidney, uterus, gonads, lung and lower GI tracts. Although exact physiological role of the channel at those tissues are still remaining unknown, it has been reported that TRPC4 is essential for ileal smooth muscle contraction stimulated by parasympathetic nervous system. With its inward-rectifying I-V relationship and high Ca^{2+} permeability, TRPC4 channels permit Ca^{2+} influx once the channel is opened by muscarinic receptor stimulation by acetylcholine or by carbachol, a potent acetylcholine homologue. Molecular mechanistic study for the nature of inward rectification has been conducted in inward-rectifying potassium channels ($Kir_{2.1}$) with Mg^{2+} and polyamines being putative rectification mediators. Meanwhile, we reported that intracellular spermine blocks TRPC4 channel through electrostatic interaction with two glutamate residues. In HEK293 cell over-expressing mouse TRPC4 channel, we evaluated blocking/unblocking kinetics of spermine with electrophysiological analysis and structural modeling. We identified that there are two-different spermine binding sites (shallow site and deep site) in TRPC4 channels. These sites were different from each other in view of voltage-dependency and time-dependency. While time constant for spermine-mediated blocking showed saturation at highly depolarized voltage (over +40 mV) in $Kir_{2.1}$, blocking time constant for TRPC4 showed no saturation. The difference may be correlated with critical difference in I-V relationship of two channels at highly depolarized voltage; Kir2.1 does not show outward current burst but TRPC4 does. Based on recent cryo-EM structure of TRPC4 channels, the proposed two different binding sites in for spermine are well-correlated.

Key Words: Transient receptor potential canonical 4, Inward-rectification, Polyamine

P4-01

STIM2 regulates both intracellular Ca^{2+} distribution and Ca^{2+} movement in skeletal myotubes

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Stromal interaction molecule 1 (STIM1) along with Orai1 mediates extracellular Ca^{2+} entry into the cytosol through a store-operated Ca^{2+} entry (SOCE) mechanism in various tissues including skeletal muscle. However, the role(s) of STIM2, a homolog of STIM1, in skeletal muscle has not been well addressed. The present study, first, was focused on searching for STIM2-binding proteins from among proteins mediating skeletal muscle functions. This study used a binding assay, quadrupole time-of-flight mass spectrometry, and co-immunoprecipitation assay with bona-fide STIM2- and SERCA1a-ex-

pressing rabbit skeletal muscle. The region for amino acids from 453 to 729 of STIM2 binds to sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA1a). Next, oxalate-supported $45Ca^{2+}$-uptake experiments and various single-myotube Ca^{2+} imaging experiments using STIM2-knockdown mouse primary skeletal myotubes have suggested that STIM2 attenuates SERCA1a activity during skeletal muscle contraction, which contributes to the intracellular Ca^{2+} distribution between the cytosol and the SR at rest. In addition, STIM2 regulates Ca^{2+} movement through RyR1 during skeletal muscle contraction as well as SOCE. Therefore, via regulation of SERCA1a activity, STIM2 regulates both intracellular Ca^{2+} distribution and Ca^{2+} movement in skeletal muscle, which makes it both similar to, yet different from, STIM1.

Key Words: Stromal interaction molecule 1, Store-operated Ca^{2+} entry, Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase 1a

P4-02

Transient receptor potential C4 like channel is involved in stretch-induced spontaneous uterine contraction of pregnant rat

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Spontaneous myometrial contraction (SMC) in pregnant uterus is greatly related with gestational age and growing in frequency and amplitude toward the end of gestation to initiate labor. But, an accurate mechanism has not been elucidated. In human and rat uterus, all TRPCs except TRPC2 are expressed in pregnant myometrium and among them, TRPC4 are predominant throughout gestation, suggesting a possible role in regulation of SMC. In addition, mechanical stretch induced by hypoosmotic cell swelling activates TRPC4/5 like channel in pregnant rat myometrium, which strongly suggests involvement of TRPC4/5 to SMC in pregnant rat. But, decisive discrimination between TRPC4 and 5 for the contributions to SMC has not been elucidated yet. Therefore, to determine a major contribution between TRPC4 and 5 to the mechanical stretch-induced uterine contraction, we here investigated in detail electrophysiological and pharmacological characteristics of the mechanical stretch-induced ion channel to elucidate a molecular identity between TRPC4 and 5 using single-channel patch clamp technique. In the present results, hypoosmotic cell swelling activated a potent outward rectifying current in G protein-dependent manner in rat pregnant myocyte. Single-channel activities of the SACnp were significantly attenuated by ML-204 (3 μM), a selective blocker for TRPC4 over TRPC5 but, not affected by application of clemizole (1 μM), a selective TRPC5 inhibitor over TRPC4 in both cells. Similarly, hypoosmotic swelling-evoked currents were significantly suppressed by application of 3 μM ML-204, but clemizole (1 μM) had little effect on the Ihypo in both cells. In addition, 4) the frequencies of the stretch-induced SMCs were significantly attenuated by 3 μM ML-204 without effect on SMC amplitudes, but clemzole (1 μM) failed to affect the mechanical stretch-evoked SMC in pregnant rat myometrium. These results, therefore, strongly suggest that TRPC4 channel rather than TRPC5 significantly contributes to SAC activation in pregnant rat uterine myocytes, which play a dominant role as regulator of SMC during late gestational age.

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Key Words: Osmotic stress, Spontaneous uterine contraction, Stretch, Transient receptor potential C4

P4-03

Role of inositol 1,4,5-trisphosphate receptor type 1 in ATP-induced nuclear Ca^{2+} signal and hypertrophy in atrial myocytes

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Inositol 1,4,5-trisphosphate receptor type 1 (IP_3R1) is expressed in atrial muscle, but not in ventricle, and they are abundant in the perinucleus. We investigated the role of IP3R1 in the regulations of local Ca^{2+} signal and cell size in HL-1 atrial myocytes under stimulation by IP_3-generating chemical messenger, ATP. Assessment of nuclear and cytosolic Ca^{2+} signal using confocal Ca^{2+} imaging revealed that IP_3 generation by ATP (1 mM) induced monophasic nuclear Ca^{2+} increase, followed by cytosolic Ca^{2+} oscillation. Genetic knock-down (KD) of IP_3R1 eliminated the monophasic nuclear Ca^{2+} signal and slowed the cytosolic Ca^{2+} oscillation upon ATP exposure. Prolonged application of ATP as well as other known hypertrophic agonists (endothelin-1 and phenylephrine) increased cell size in wild-type cells, but not in IP3R1 KD cells. Our data indicate that IP3R1 mediates sustained elevation in nuclear Ca^{2+} level and facilitates cytosolic Ca^{2+} oscillation upon external ATP increase, and further suggests possible role of nuclear IP3R1 in atrial hypertrophy.

Key Words: Inositol 1,4,5-trisphosphate receptor type 1 (IP3R1), Ca^{2+} signal, ATP, Hypertrophy, HL-1 atrial myocytes

P4-04

Mitochondrial dysfunction reduces the activity of KIR2.1 K^+ channel in myoblasts via impaired oxidative phosphorylation

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Myoblast fusion depends on mitochondrial integrity and intracellular Ca^{2+} signaling regulated by various ion-channels. In this study, we investigated the ionic currents associated with $[Ca^{2+}]_i$ regulation in normal and mitochondrial DNA-depleted (ρ0) L6 myoblasts. The ρ0 myoblasts showed impaired myotube formation. The inwardly rectifying K^+ current (I_{Kir}) was largely decreased with reduced expression of KIR2.1, whereas the voltage-operated Ca^{2+} channel and Ca^{2+}-activated K^+ channel currents were intact. Sustained inhibition of mitochondrial electron transport by antimycin A treatment (24 h) also decreased the I_{Kir}. The ρ0 myoblasts showed depolarized resting membrane potential and higher basal $[Ca^{2+}]_i$. Our results demonstrated the specific downregulation of I_{Kir} by dysfunctional mitochondria. The resultant depolarization and altered Ca^{2+} signaling might be associated with impaired myoblast fusion in ρ0 myoblasts.

Key Words: Myoblast, mtDNA-depleted myoblasts, Inward-rectifying K^+ channel, Oxidative phosphorylation, Myogenesis

P4-05

Difference in calcium sensitivity between right ventricle and left ventricle due to the low expression level of calcium binding protein in right ventricle

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Left ventricle (LV) and right ventricle (LV) have distinctive structural and functional characteristics as well as heterogeneous physiological properties. The RV is exposed to a relatively low pulmonary vasculature, which results in less mechanical afterload. Consistent with these physiological differences, the RV has a thinner free wall than the LV, and the movement of its contraction is geometrically different. Despite these definite differences, the studies of basic excitation-contraction coupling and calcium homeostasis of RV has been less studied than in LV. To establish the interventricular difference, we evaluated the basic electrophysiological and calcium-contractile properties of myocyte with or without β-adrenergic stimulation. Analyses of contraction/Ca2+ signaling and action potential duration (APD) in isolated RV myocytes showed prolonged APD with less significant changes in sarcomere shortening and calcium transient, implying less efficient E-C coupling in RVF. Contraction and calcium transient of cardiomycyte were measured using IonOptix, cell geometry measurement system. Comparing with LV, RV myocytes showed round peak, slower early relaxation, and faster late relaxation, suggesting the difference of calcium sensitivity between two ventricles. To investigate the difference, we examined the expression level of calcium-binding proteins that regulate myofilament activities. Using immunoblotting from enriched protein of myofilament fraction, we observed the calcium buffering proteins was decreased in RV. Taken together, our results suggest that calcium binding proteins of RV was differ from that of LV, which induce the modified calcium sensitivity. The calcium sensitivity of RV is a clue to explain the different physiological properties of the RV.

Key Words: Right ventricle, Calcium sensitivity, Excitation-contraction coupling, Calcium binding protein

P4-06

Abnormal calcium signaling and contractions in right ventricular myocytes from pulmonary hypertension model rats

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Left ventricular failure (LVF) shows dysregulated Ca^{2+} signaling with increased risk of arrhythmia, especially in β-adrenergic stimulation (β-AS). One of the arrhythmia mechanisms is sarcoplasmic reticulum (SR) Ca^{2+}-leak through RyR, a target of cardiac NO synthase (NOS) signaling. Compared with LVF, the mechanism of arrhythmia and altered excitation-contraction coupling (E-C coupling) in right ventricular failure (RVF) and hypertrophy (RVH) under pulmonary arterial hypertension (PAH) is largely unknown. In monocrotaline-injection induced PAH rats, both functional (echocardiography) and structural (histology) changes of RVH and RVF were confirmed at around 20th and 30th day, respectively. Analyses of contraction/Ca^{2+} signaling and action potential duration (APD) in isolated RV myocytes showed prolonged APD with less significant changes in sarcomere shortening and calcium transient, implying less efficient E-C coupling in RVF. The β-AS (Isoproterenol, 10 nM)-induced prolongation of APD, Ca^{2+} transient, and sarcormere shortening in normal RV myocytes were more significant than those in LV myocytes. In RVH myocytes, these responses to β-AS became less significant, and RVF myocytes showed no significant change, implying desensitization to β-AS. Interestingly, inhibition of NOS (L-NAME, 1 mM, 30 min) combined with β-AS induced arrhythmic contractions of RVH and RVF myocytes, e.g. spontaneous contractions, delayed afterdepolarization (DAD). Confocal microscopic imaging of Ca^{2+} signals revealed abnormal Ca2+ leaks from SR in RVH and RVF myocytes when treated by both ISO and L-NAME. In conclusion, the β-AS responses cardiomyocytes became progressively insignificant along with the increased APD and Ca^{2+} transient duration in the progress of RVF. The pro-arrhythmic effects of L-NAME in RVH under β-AS suggest a critical role of NOS in RV myocytes for the E-C coupling regulation, especially SR Ca^{2+} release/uptake processes.

Key Words: Right ventricle, Pulmonary arterial hypertension, Spontaneous SR calcium leak, Arrythmia, β-Adrenergic stimulation

P4-07

Shear stress induces ATP release from ventricular myocytes via connexin hemichannel

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Mechanical stresses in cardiac muscle such as stretch and shear stress increase as intra-chamber pressure increases under hypertension, valve diseases, and heart failure. Cardiac arrhythmias and cellular remodeling are associated with these pathological conditions. Shear stress-induced longitudinal Ca^{2+} wave is thought to be mediated by autocrine activation of P2Y1 purinergic signaling in atrial myocytes (J Physiol 2016;593:5091). In this study, we directly assessed shear-induced ATP release and underlying mechanism in ventricular myocytes from murine hearts, and explored if this response is altered under pressure overload (PO), induced by transverse aortic constriction. Extracellular ATP level was increased transiently by shear stress (~16 dyn/cm^2) in rat ventricular myocytes, reaching peak value of ~3x10^{-18} moles per unit membrane area (mm^2) at 2 s, suggesting lower ATP release in ventricular cells than atrial cells under the same shear force. Removal of external Ca2+ to enhance connexins increased shear-induced ATP release in ventricular myocytes. Inhibition of gap junction hemichannels using either carbenoxolone or La^{3+} almost completely suppressed ATP release from these myocytes during shear stimulation. Shear-induced ATP releases were significantly higher in ventricular myocytes from PO mice compared to those measured in sham-operated control mice. These data indicate that shear stress induces ATP release through connexin hemichannels under shear stress and further suggest contribution of this shear response to ventricular remodeling by increased afterload.

Key Words: Shear stress, Ventricular myocytes, ATP, Connexin

P4-08

Sabinene inhibits tumor necrosis factor-α-induced atrophy in mouse C2C12 skeletal myotubes

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Sabinene, one kind of monoterpene, is present in the essential oils of many plants including *Chrysanthemum boreale* Makino and black pepper. It is reported to exhibit anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal properties. However, its activities on skeletal muscle remain unclear. In the present study, we explored the effect of sabinene on C2C12 skeletal myotube atrophy. For this study, the atrophy of differentiated myotubes from C2C12 cell line (mouse skeletal muscle cell line) was induced by treatment with tumor necrosis factor (TNF)-α for 48h. Size, atrophy marker protein expression and atrophy-linked signal molecule expression were examined in TNF-α-treated C2C12 myotubes in response to sabinene which is known to be nontoxic to human. Sabinene recovered the TNF-α-reduced

size of myotubes. Sabinene decreased the enhanced expression level of an E3 ubiquitin ligase MuRF-1and increased the reduced AKT-mTOR phosphorylation level in TNF-α-treated C2C12 myotubes. In addition, the level of reactive oxygen species in TNF-α-atrophied myotubes was decreased by treatment with sabinene. These results demonstrated that sabinene may promote recovery of TNF-α-induced atrophy in C2C12 myotubes. Therefore, sabinene may be a promise candidate agent for alleviation of the TNF-α-induced skeletal muscle atrophy.

Key Words: Sabinene, Skeletal muscle, Atrophy, C2C12 cell, Myotube

P4-09

Fenofibric acid suppresses migration and proliferation via mitogen-activated protein kinase signaling pathway in vascular smooth muscle cells in response to platelet-derived growth factor-BB

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Vascular smooth muscle cell (VSMC) migration and proliferation are important events for neointimal formation of atherosclerosis or vascular restenosis. Fenofibric acid has anti-hepatic steatosis and antioxidative activities. However, it has not been investigated whether fenofibric acid affects the functional events of vascular smooth muscle cells (VSMCs). In this study, we investigated the effect of fenofibric acid on VSMC migration and proliferation. Fenofibric acid inhibited platelet-derived growth factor (PDGF)-BB-induced migration and proliferation in VSMCs. Fenofibric acid dose-dependently suppressed migration and proliferation. In addition, fenofibric acid attenuated increased phosphorylations of mitogen-activated protein kinases (MAPKs), p38MAPK and extracellular signal-regulated kinase (ERK)1/2, in VSMCs in response PDGF-BB. These findings demonstrate that fenofibric acid inhibits migration and proliferation by suppressing the phosphorylations of p38MAPK and ERK1/2 in VSMCs. Therefore, fenofibric acid may be potential candidate for prevention or treatment of vascular disorders such as vascular restenosis or atherosclerosis

Key Words: Fenofibric acid, Vascular smooth muscle cell, Migration, Proliferation, Mitogen-activated protein kinase

P4-10

Fetuin-B activates TGF-β receptor II-mediated signaling pathway in vascular smooth muscle cells: A possible mechanism of vascular plaque rupture

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The rupture of an atherosclerotic plaque is one of the main causes of coronary artery thrombotic occlusion, leading to myocardial infarction. Our previous study showed that fetuin-B may be associated with modulation in the development of myocardial infarction that can be caused by the rupture of an atherosclerotic plaque. However, the mechanism of plaque rupture by fetuin-B remain unclear. In this study, we investigated how fetuin-B can influence vascular smooth muscle cells (VSMCs) for plaque rupture. Immunoprecipitation assay using plasma membrane proteins from VSMCs revealed fetuin-B tightly bound to transforming growth factor-β receptor II (TGF-β RII). In addition, fetuin-B significantly elevated the phosphorylation of Smad2 and Smad3 in VSMCs. Fetuin-B also stimulated nuclear translocation of phosphorylated Smad. The Fetuin-B-induced phosphorylation of Smad and its nuclear translocation were inhibited in VSMCs treated with SB431542, a selective inhibitor of TGF-β R. Fetuin-B elevated the expression of plasminogen activator inhibitor-1 (PAI-1), a risk factor for thrombosis and atherosclerosis, in VSMCs through its epigenetic modification including recruitments of both histone deacetylase (HDAC)1 and RNA polymerase (RNAP) II. Similar results were observed in matrix metalloproteinase-2 (MMP-2) expression. These epigenetic alterations were also inhibited in VSMCs treated with SB43154.These results demonstrate that fetuin-B may induce activation of the PAI-1 and MMP-2 expression in VSMCs via TGF- β receptor and Smad pathway. Therefore, these events in VSMCs by fetuin-B may contribute to vascular plaque rupture.

Key Words: Vascular smooth muscle cells, Fetuin-B, Transforming growth factor-β receptor II

P5-01

Stimulation of autophagy improves vascular function in the mesenteric arteries of type 2 diabetic mice

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Vascular dysfunction is a major complication in type 2 diabetes. It has been suggested that dysregulation of autophagy is associated with various cardiovascular diseases. However, the relationship between autophagy and type 2 diabetic vascular dysfunction remains unclear. Thus, in this study, we examined whether reduced autophagy is involved in vascular dysfunction of type 2 diabetic mice and stimulation of autophagy could improve vascular function in type 2 diabetes. Ten to 12-week old male type 2 diabetic (*db*$^-$/*db*$^-$) mice and their control (*db*$^-$/*db*$^+$) mice were treated with two different autophagy stimulators (rapamycin, 4 mg/kg/every other day, *i.p.* injection and trehalose, 3% in drinking water *ad libitum*) for 2 weeks. Isolated mesenteric arteries were mounted in the arteriograph. Pressure-induced myogenic response was significantly increased, whereas endothelium-dependent relaxation was significantly attenuated in the mesenteric arteries from type 2 diabetic mice. These results were associated with increased expressions of LC3II, p62, and beclin-1 (markers of autophagy) in mesenteric artery from type 2 diabetic mice compared to arteries from control mice. Interestingly, treatment of autophagy stimulators significantly reduced the potentiation of myogenic response and improved endothelium-dependent relaxation in the type 2 diabetic mice. Furthermore, autophagy stimulation using rapamycin and trehalose normalized expression levels of LC3II, p62 and beclin-1 in the type 2 diabetic mice. We provide evidence that type 2 diabetes impairs vascular function by dysregulated autophagy-dependent mechanism. Therefore, autophagy could be a potential target for overcoming diabetic microvascular complications.

Key Words: Autophagy, Mesenteric artery, Type 2 diabetes, Myogenic tone, Endothelium-dependent relaxation

P5-02

αKlotho protects diabetic nephropathy via stabilizing podocyte Ca^{2+} signaling

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An aging suppressor protein αKlotho is predominantly produced in the kidney and has a renoprotective role. Type 2 diabetes is a primary risk factor for chronic kidney disease. Podocytes, the gatekeeper of kidney filter, is primary target for diabetic nephropathy (DN) and Ca^{2+} signaling whose perturbation leads to disruption of glomerular filter integrity causing pro-

teinuria. It is currently unknown how αKlotho protects podocytes in DN and whether it directly regulates podocyte Ca^{2+}-permeable channels to stabilize podocyte slit diaphragm and proteinuria. Here, we find that Orai1 is a novel Ca^{2+}-permeable channels in podocytes and a culprit for proteinuria as well as TRPC5 and TRPC6. Orai1 activation disturbs the Ca^{2+}-calcineurin pathway and subsequent actin remodeling leading to transepithelial albumin leakage. While TRPC5 expression is not altered in hyperinsulinemic DN animal model, *db/db* mice, Orai1 and TRPC6 is upregulated in early and end-stage of DN, respectively. Transgenic overexpression of *Orai1* in mice and *db/db* mice cause foot process fusion and albuminuria. Administration of purified αKlotho attenuates albuminuria and downregulates Orai1 in early phase of DN. Administration of αKlotho or inhibition of Orai1-calcineurin pathway ameliorates proteinuria and restores glomerular basement membrane thickness and foot process fusion in *db/db* mice. Moreover, αKlotho suppresses both TRPC6 and TRPC5 as well as Orai1 suggesting that αKlotho regulates multiple Ca^{2+}-permeable channels in podocytes. Together, our data demonstrates that tightly balanced podocyte Ca^{2+} signaling by αKlotho is vital for maintaining glomerular filter integrity in DN. This provides novel perspectives on the pathogenesis of podocytopathy in DN and a potential new therapeutic strategy for treatment of proteinuric diseases. [NRF-2015R1D1A1A01060454 & 2017R1D1A3B0303176].

Key Words: αKlotho, Podocyte, Diabetic nephropathy, Orai1, TRPC5, TRPC6, Proteinuria

P5-03

Oxidative stress triggers hepatic stellate cell activation and fibrosis through TRPC6/MRTF-A signaling pathway

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Oxidative stress plays a crucial role in hepatic fibrosis leading to cirrhosis. Oxidative stress activates hepatic stellate cells (HSCs) initiating the fibrosis. Various HSCs activation mediators such as TGFβ and Angiotensin II are not only linked to Ca^{2+} signaling but also the potent oxidative stress inducers. However, the molecular mechanism linking oxidative stress to Ca^{2+} signaling in hepatic fibrogenesis remains unclear. Here, the molecular components and their underlying mechanisms mediated by oxidative stress to cause fibrosis were examined in hepatic fibrosis animal models; thioacetamide (TAA) administration and bile duct ligations (BDL) and *in vitro* primary HSCs activation models. We find that TRPC6 channel is predominant Ca^{2+}-permeable channel mediating HSCs activation by oxidative stress. H_2O_2 directly upregulates TRPC6 currents. Reactive oxygen species (ROS) and TRPC6 expression are significantly increased in both BDL and TAA-induced liver fibrosis animal models. Functionally, fibrosis was ameliorated by suppressing TRPC6 and its downstream effector MRTF-A in in vitro primary and cultured HSCs, respectively. Notably, deletion of TRPC6 (*TRPC6*$^{-/-}$) in mice ameliorated TAA-induced hepatic fibrosis, while transgenic overexpression of TRPC6 accelerates *de novo* expression of fibrosis markers in the liver. Moreover, production of ROS and MRTF-A pathway is decreased in TAA-induced fibrosis of *TRPC6*$^{-/-}$ mice compared to that of wild type mice. These results indicate that ROS induces TRPC6 activation mediating activation of HSCs leading to hepatic fibrosis with ROS/TRPC6/MRTF-A positive feedback loops. These results suggest new perspective on the pathogenesis of hepatic fibrosis and provide clues for novel therapeutic approach for the cirrhosis. [NRF-2015R1D1A1A01060454 & 2017R1D1A3B0303176]

Key Words: Oxidative stress, TRPC6, Hepatic stellate cell, Liver fibrosis, Calcium signaling

P5-04

Ethylenethiourea induces nephrotoxicity in male mice

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Ethylenethiourea (ETU) was mainly used as a vulcanizing catalyst for polychlorophrene and polyacrylate rubbers and used in dyes, synthetic resins, pharmaceuticals, and intermediate in antioxidant production. ETU is also one of the main metabolite of ethylenebisdithiocarbamate (EDBC) fungicides including maneb, zineb, and mancozeb. Therefore, potential occupational exposure to ETU is greatest for workers involved in machine and metal manufacturing, rubber production, and manufacture of EDBC fungicides. The pathways of potential human exposure to ETU are dermal contact, ingestion, and inhalation. The toxicological studies of ETU were extensively investigated to the endocrine disruption, teratogenesis, goiter, and carcinogenicity. Extensive study on the carcinogenicity demonstrates that ETU increases adenocarcinomas and thyroid follicular adenomas in rodents. ETU has also demonstrated developmental toxicity including central nervous system and skeletal structure. Recently, it has been reported that high dose of ETU (300 mg/L) resulted in ultrastructure alteration in proximal tubular epithelial cells. In the present study, we evaluated that the changes of visceral organ weight, cholesterol levels in serum, renal and liver function index, and epigenetic miRNA expression levels in C57BL/6 mouse with chronic exposure of ETU for 58 weeks. Chronic exposure of low dose ETU(250 ppm) induced toxicological effects which as followed; 1) lowered body weight, 2) increased triglyceride and cholesterol in serum, 3) increased blood urea nitrogen(BUN) and creatine levels, 4) induced extreme malfunction of kidney including decreased number and size of glomerulus, 5) and induced severe hydronephrosis or poly-cystogenesis compared to the control. Also, ETU diet increased expression levels of miR-1971, miR-155, miR-135, miR-125, and miR-21, as known to biomarker for renal injury and fibrosis, in kidney. In the cause of polycystic kidney disease, ETU diet increased expression levels of miR-17~92 cluster, known as an oncogenic miRNA cluster and renal cyst growth, and miR-182, an novel regulator of actin cytoskeleton and cyst progression. Taken together, these data suggest that chronic exposure to ETU, at low concentration without causing acute toxicity, evoked renal dysfunction such as glomerular dysfunction and renal cyst development.

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Key Words: Ethylenethiourea, Polycystic kidney disease, miR-17~92 cluster, Nephrotoxicity

P5-05

Inhibition of ERK1/2-mTORC1 axis ameliorates proteinuria and fibrogenic action of TGF-β in adriamycin-induced glomerulosclerosis

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Transforming growth factor-β (TGF-β) plays crucial roles in the development of focal segmental glomerulosclerosis (FSGS), however, key molecular pathway remains unknown. Here we described the pathogenic mechanism that links mammalian target of rapamycin complex1 (mTORC1) activation by TGF-β in murine model of FSGS induced by adriamycin (ADR) injection. We noted that ADR clearly augmented p-Smad3 and podocyte specific p-S6RP expression after day 1 and these changes persisted till day 14. ADR-induced mTOR activation was completely prevented by SB431542, a pharmacological inhibitor of TGF-β receptor-I (TGF-RI) activation. Treatment of SB431542

or rapamycin significantly suppressed glomerular fibrosis, Col4α3 and PAI-1 expression and restored nephrin, synaptopodin, WT-1, podocin and podocalyxin caused by ADR. FDA-approved MEK inhibitor trametinib/GSK1120212 blunted TGF-β1-induced mTORC1 activation in podocytes. Trametinib, even at lower doses, effectively ameliorated ADR-mediated TGF-β, PAI-1, fibronectin, α-SMA upregulation and prevented proteinuria with elevated serum albumin level. Notably, rapamycin suppressed upstream Smad3 and ERK1/2 phosphorylation ex vivo (isolated glomeruli) and *in vivo* studies. ERK inhibition also down-regulated p-Smad3, indicating that there are pathologic paracrine signalings among glomerular cells to amplify TGF-β-ERK1/2-mTORC1 axis by forming a positive feedback loop. Taken together, accentuated TGF-β-ERK1/2-mTORC1 pathway is suggested as an important therapeutic target for glomerulosclerosis. Blocking this vicious loop by trametinib might offer a new strategy in ameliorating albuminuria and FSGS progression. [NRF-2017R1D1A3B0303176 & Myung Sun Kim Memorial Foundation]

Key Words: TGF-β, Focal segmental glomerulosclerosis, Cell signaling, Fibrosis, Proteinuria

P5-06

Understanding of the role of SREBP-1c neddylation in hepatic lipogenesis and validation of a neddylation inhibitor as a therapeutic for hepatic steatosis

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Neural precursor cell expressed, developmentally down-regulated 8 (NEDD8) is known to regulate protein stabilization and activity by binding to the substrate protein, a process known as neddylation. Neddylation has been recently shown to play a role in regulating lipid metabolism. In the liver, deregulated lipid metabolism caused non-alcoholic fatty liver disease (NAFLD). NAFLD represents a spectrum of liver disease that, can lead to simple steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis and ultimately hepatocellular carcinoma. However, the effects of neddylation on NAFLD have not previously been studied. Sterol regulatory element-binding protein-1c (SREBP-1c) has crucial roles in NAFLD. Chronic activation of SREBP-1c with increased lipogenic activity contributes to the development and progression of several pathological conditions, such as fatty liver. Here, we demonstrate that the NEDD8 based post-translation modification (neddylation) of SREBP-1c is essential for lipogenesis in HepG2 cells. NEDD8 is robustly conjugates with SREBP-1c, leading to SREBP-1c stabilization and its transcriptional activities. Based on these result, we tested whether a NEDD8-activting enzyme inhibitor, NAE1 i, had an anti-fatty liver formation. The result showed that NAE i decreased lipid accumulation in HepG2 cells and reduced both SREBP-1c expression and its transcriptional activity. In addition, treatment of NAE1 i was effective in attenuating lipogenic genes expression. In conclusion, our results suggest that neddylation of SREBP-1c contributes to hepatic steatosis formation and its inhibitor may be a novel therapeutic target for the treatment of hepatic steatosis.

Keywords: Neddylation, Obesity, NAFLD, Transcription factor, SREBP 1c

P6-01

Role of JHDM in the regulation of hepatic steatosis

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Non-alcoholic fatty liver disease (NAFLD) is caused by excessive fat accumulation in the hepatocytes. Mild steatosis may develop into aggressive forms of hepatic fibrosis, cirrhosis and carcinoma. Sterol regulatory element binding protein (SREBP)-1c is the major transcription factor that regulates hepatic lipid accumulation and it has been demonstrated that patients with steatosis and NASH, which is characterized by excessive hepatic *de novo* lipogenesis, contain SREBP-1c stimulation. However, the molecular mechanisms involved in the regulation of SREBP-1c remain incompletely understood. Jumonji C histone demethylase (JHDM) is known as the demethylase of the histone H3K9 through its Jmj C domain while it binds to H3K4me3 with the PHD domain. JHDM is also known to have associated with metabolism-related transcription factors. In this study, we identified the role of JHDM in the hepatic steatosis through regulation of hepatic transcription factor. In hepato-carcinoma cells, ectopic expression of JHDM decreases SREBP-1c protein and mRNA levels of target genes. In addition, JHDM down-regulates SREBP-1c protein stability, and JHDM directly interacts with SREBP-1c through protein-protein interaction. In accordance with these findings, Oil Red O staining showed that elevated lipid accumulation by oleic acid in a dose-dependent manner was suppressed by ectopic expression of JHDM. These results suggest that JHDM plays a role as a repressor in the progression of hepatic steatosis through the association with SREBP-1.

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Key Words: JHDM, SREBP-1c, NAFLD

P6-02

Hydrogen sulfide augmented hypoxia-induced ANP secretion via HIP1a and PPAR-γ pathway

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Hypoxia is a common disorder which is induced by deficient oxygen supply or insufficient blood distribution. In hypoxic condition, ATP-sensitive potassium (K_{ATP}) channel is overexpressed as a compensatory mechanism. Recently, we have reported that sodium hydrosulfide (NaHS) stimulated high stretch induced-atrial natriuretic peptide (ANP) secretion partially via K_{ATP} channel. However, whether NaSH affects ANP secretion during hypoxia remains obscure. The purpose of the present study is to discover the impact of NaHS on ANP secretion during hypoxia and to unravel its signaling pathway. Isolated beating rat atria were perfused with buffer exposed to different O_2 tension (from 100% O_2 to 100% O_2, normoxia; to 50% O_2, hypoxia; to 10% O_2, anoxia). The ANP secretion increased negatively correlated with O_2 tension. NaHS (50 μM) did not show any significant effect on ANP secretion in normoxic condition but augmented ANP secretion in hypoxic condition. Hypoxia increased the expression of peroxisome proliferator-activated receptor (PPAR)-γ protein, but not the expression of HIF1α protein and eNOS phosphorylation. The augmentation of NaHS-induced ANP secretion during hypoxia was blocked by the pretreatment with K_{ATP} channel blocker (glibenclamide), HIF1α inhibitor (2-methoxyestradiol), and PPAR-γ inhibitor (GW9662) but enhanced by the pretreatment with K_{ATP} channel activator (pinacidil). These results suggest that NaHS augmented hypoxia-induced ANP secretion partly through K_{ATP} channel and PPAR-γ.

Acknowledgement: Supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NO 2017-R1A2B-4002214).

Key Words: H2S, Atrial natriuretic peptide, NaHS, Hypoxia, K_{ATP} channel, PPAR-γ.

P6-03

Effect of intensive and repetitive by thermal exposed on the central nervous system sudomotor activity

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The sweating response is modulated in two different ways depending on adaptation conditions. In this work, we examined sudomotor activities before and after intensive and repetitive heat exposure. Nine male volunteers were exposed to 30-min half-body immersion in hot water (42 ± 0.5℃) at the same time of day on alternate days for 3 weeks. All experiments were performed in an automated climate chamber. Tympanic temperature and skin temperatures were measured. Mean body temperature was calculated. Sudomotor activities, including sweat onset time, sweat rate and volume, activated sweat gland density (ASGD) and output (ASGO), were tested in four regions of the skin chest, abdomen, upper back and thigh. Basal local tympanic temperature and mean body temperature were found to decrease by 0.15 ℃ and 0.16 ℃, respectively. As a typical data (upper back), sweat onset time increased by 33.6% after heat acclimation. After heat acclimation, sweat rate decreased by 14.7%, sweat volume decreased by 15.5% and ASGO also decreased by 11.1%. ASGD decreased by 4.1% after heat acclimation without statistical significance. The data suggest that intensive and repetitive heat exposure induces suppression of sudomotor activities within 3 weeks.

Key Words: Heat exposure, Heat acclimation, Sweat rate, Sweat gland density, Sweat gland output

P6-04

Study of active sweat gland density and sweat gland output per single gland activated by thermal exposed sudomotor activity in the humans

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Modification of sweating could be due to changes in activated sweat gland density (ASGD) and/or activated sweat gland output (ASGO). However, how the two factors are changed in tropical natives remains unknown. In addition, the distributions of ASGD and ASGO over the body in tropical natives are unclear. The present study determined regional and inter-ethnic differences in ASGD and ASGO during passive heating between tropical natives (Africans) and temperate natives (Republic of Korean). Heat load was carried out by immersing the half body into a hot water bath (42 ± 0.5℃) for 30 min. All experiments were performed in an automated climate chamber. Tympanic temperature and skin temperature were measured. Mean body temperature was calculated. Sudomotor activities including sweat onset time, sweat rate, ASGD, and ASGO were examined in eight regions of the skin. Africans had smaller increase in mean body temperature during passive heating than Koreans. The onset time of sweating was much more delayed in Africans compared to Koreans. In response to thermal load, ASGD and ASGO differed between body regions in Africans and Koreans. In most skin regions, ASGD and ASGO were decreased in tropical Africans compared to those in temperate Koreans. Trunk portion including chest, upper back, lower back, abdomen had greater swear rate, ASGD, and ASGO compared to peripheral segments including upper arm, forearm, leg, and thigh in both ethnic groups. Distribution patterns of ASGD over the body appeared to be similar in both Africans and Koreans at the peak of thermal loading. In conclusion, the present study demonstrates that sudomotor activity in tropical Africans is suppressed with decreased ASGD and ASGO over the body surface compared to temperate Koreans.

Key Words: Thermal sweating, Heat acclimatization, Sweat glands density, Sweat gland output, Tropical, Temperate

P6-05

Insulin regulates adrenal steroidogenesis by stabilizing SF-1 activity

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Development of metabolic syndrome is associated with hyperactivity of the HPA axis characterized by elevated levels of circulating adrenal hormones including cortisol and aldosterone. However, the molecular mechanism leading to the dysregulation of the HPA axis is not well elucidated. In this study, we found that insulin regulates adrenal steroidogenesis by increasing the expression and activity of steroidogenic factor 1 (SF-1) both *in vitro* and *in vivo* and this insulin effect was partly through inhibition of FoxO1. Specifically, insulin increased the protein and RNA levels of SF-1 and steroidogenic target genes. Further, adrenal SF-1 expression was significantly increased by hyperactivation of insulin signaling in mice. Together with the elevated SF-1 expression in adrenal glands, hyperactivation of insulin signaling led to increased aldosterone and corticosterone levels. On the other hand, suppressing the insulin signaling using streptozotocin markedly reduced the expression of adrenal SF-1 in mice. In addition, overexpression of FoxO1 significantly suppressed SF-1 and its steroidogenic target genes implying that the positive effect of insulin on SF-1 activity might be through suppression of FoxO1 in the adrenal gland. Taken together, these results indicate that insulin regulates adrenal steroidogenesis through coordinated control of SF-1 and FoxO1.

Key Words: SF-1, Insulin, Adrenal gland, FoxO1

P6-06

P110β in the ventromedial hypothalamus regulates glucose and energy metabolism

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Phosphoinositide 3-kinase (PI3K) signaling in hypothalamic neurons integrates peripheral metabolic cues including leptin and insulin to coordinate systemic glucose and energy homeostasis. PI3K is composed of several subunits, each of which has several unique isoforms. However, the role of the PI3K subunits and isoforms in the ventromedial hypothalamus (VMH), a prominent site for the regulation of glucose and energy homeostasis, is unclear. Here, we investigate the role of subunit p110β in steroidogenic factor-1 (SF-1) neurons of the VMH in the regulation of metabolism. Our data demonstrates that deletion of p110β in SF-1 neurons disrupts glucose metabolism, including insulin sensitivity. In addition, deletion of p110β in SF-1 neurons leads to whitening of brown adipose tissues and diet-induced obesity due to blunted energy expenditure. These results highlight a critical role for p110β in the VMH in the regulation of glucose and energy homeostasis.

Key Words: Phosphoinositide 3-kinase, Ventromedial hypothalamus, Ener-

gy metabolism

P6-07

Investigating the cell-Nonautonomous roles of the nuclear hormone receptor NHR-49 in the nervous system of *Caenorhabditis elegans*

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The central nervous system plays a key role in regulating whole-body metabolism. Examples of neuronal regulation of fat metabolism and lifespan have also been reported for *C. elegans*. NHR-49 is one of the 284 nuclear hormone receptors expressed in *C. elegans* and is an important regulator of fat metabolism and lifespan. Recently, it was found that NHR-49 expression in the neurons is sufficient to restore many of the nhr-49-dependent functions in the worm. To glean insight into the neuronal regulation of whole-body metabolism, we plan to investigate the tissue-specific role of the ubiquitously expressed nuclear hormone receptor, and elucidate the neuronal circuitry and neurotransmitters involved in signaling to the periphery. Preliminary results show that unc-31 deletion only partially obstructs the lifespan extension and gene expression phenotypes in the NHR-49 neuronal rescue strain, suggesting that a more complex signaling process underlies its effects.

Key Words: Metabolism, Cell-nonautonomous, Caenorhabditis elegans, Nuclear hormone receptor, Neuron

P6-08

Equipotent deteriorating effect of angiotensin A to angiotensin II on cardiac I/R injury

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Angiotensin (Ang) A was first identified in human plasma and it differs from Ang II in Ala (1) instead of Asp (1). The aim of this study is to investigate whether Angiotensin A (Ang A) has an effect on ischemia-reperfusion (I/R) injury in Langendorff hearts. After sacrificing Sprague-Dawley rats, the hearts were perfused with Krebs-Henseleit buffer for a 20 min pre-ischemic period with and without Ang A followed by 20 min global ischemia and 50 min reperfusion. Pretreatment with Ang A (0.1, 1, 10, and 100ug/kg) for 2 hrs before ischemia deteriorated effects on post-ischemic left ventricular end-diastolic pressure and post-ischemic left ventricular developed pressure induced by reperfusion compared to untreated hearts. Ang A also increased infarct size and lactate dehydrogenase levels in effluent, and decreased coronary flow during reperfusion. Similarly, Ang II also showed deteriorating effects on cardiac function after I/R injury. Interestingly, Ang A but not Ang II stimulated high stretch-induced ANP secretion from isolated perfused beating atria. The inhibitory effect of ANP secretion by Ang II was completely blocked by the pretreatment with AT1R antagonist. The stimulatory effect of Ang A was partially blocked by the pretreatment with AT1R and AT2R antagonists. Further research will elucidate its interactions in cardiovascular pathophysiology and its possible therapeutic implications.

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Key Words: Angiotensin A, Angiotensin II, Ischemia, Reperfusion, Atrial natriuretic peptide, Receptor

P7-01

CACC and ENaC mediate LPS-induced disruption of epithelial barrier

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Calcium-activated chloride channels (CACC) and epithelial sodium channels (ENaC) are major regulators of fluid homeostasis, membrane potential and epithelial secretion. Especially, salivary secretion is known to be largely dependent on these ion channels. In this manner, the alteration of ion channels is associated with diverse diseases including cancer and autoimmune diseases. Besides, gram-negative bacteria lipopolysaccharide (LPS) increases the susceptibility of cells to pathogenic disorders including septic shock, gastroenteritis, inflammation, and autoimmune diseases. Recently, several studies have reported that the rapid effect of LPS on intracellular cell signaling relate to derangement of calcium channels and epithelial barrier in cells. However, the pathogenic role of CACC and ENaC in LPS treated epithelial cells has not been fully examined. Therefore, the aim of this present study is to investigate whether LPS evokes the activities of ion channels. We further investigated the specific involvement of CACC and ENaC in LPS induced disruption of epithelial barrier function. To determine the effect of LPS on epithelia, the immortalized rat submandibular epithelial cell lines, SMGC6, were used. To measure the activities of CACC and ENaC, short circuit current (Isc) was continuously monitored by Ussing chamber under voltage clamping condition. Transepithelial potential difference (Vm) was monitored under current clamping condition. Epithelial membrane integrity was estimated by Transepithelial electrical resistance (TER) and macromolecular permeability assay with FITC-dextran. Zo-1 expression was represented by immunocytochemistry staining and real time PCR. LAL endotoxin quantitation kit was used to measure second bacterial infection.
Apical application of LPS increased a short circuit current, demonstrating transient inward current. The effect of LPS on Isc was nearly abolished by treatment of ENaC and CACC inhibitors, whereas basolateral administration of other inhibitors of NKCC and N+K+ATPase had no effect on the Isc. These results validated LPS alternatively increases activation of CACC and ENaC. LPS also impaired cellular barrier function; increased permeability; and downregulated Zo-1 due to depolarization induced tyrosine phosphorylation. Inhibitors of ENaC and CACC attenuated the effect of LPS on TER, permeability and Zo-1 expression suggesting that activation of ENaC and CACC is responsible for the epithelial barrier disruption. Lastly, LAL endotoxin assay showed that the impairment of epithelial barriers by LPS increased susceptibility to secondary bacterial infections but was rescued in the presence of CACC and ENaC inhibitors.
We conclude that LPS evokes an activation of CACC and ENaC resulting in the disruption of epithelial barrier integrity.

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Key Words: CACC, LPS, ENaC

P7-02

Crif1 deficiency inhibits the invasive growth of keloid fibroblasts via TGF/SMAD signaling pathway

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A keloid is an abnormal proliferation tissue in excessive response to damaged skin tissue, which appears in the wound healing process. Keloid fibro-

blasts(KF) are the main cells that induce keloid disease. The migration of KF may play an important role in infiltrating abnormal tissue and forming keloid tissue, but the mechanism has not been elucidated. In this study, we investigated whether CR6-interacting factor 1(CRIF1) deficiency-induced mitochondrial dysfunction had an effect on cell migration, proliferation and extra cellular matrix synthesis in human primary KF. Our results revealed that downregulation of CRIF1 reduced cell migration, cell proliferation and extracellular matrix synthesis in KF compared with SiControl cells. TGF/SMAD pathway-related proteins play the main role in synthesizing extracellular matrix. Western blot data showed that CRIF1 deficiency downregulated SMAD2 and SMAD3 by increasing the inhibitory SMAD protein(SMAD7 andSMURF2) in KF compared with SiControl cells. Our results proved that crif1 knockdown-induced mitochondrial dysfunction decreased cell migration, cell proliferation, extracellular matrix synthesis, which that CRIF1 may be used as a therapeutic target protein in the treatment of keloid diseased.

Key Words: Keloid, Keloid fibroblast, CRIF1, SMAD

P7-03

Comparison for molecular mechanisms of different pruritus state in mice

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Pruritus leads to scratching behavior and severe scratching behavior results in skin barrier dysfunction. An interruption of itching-scratching circuit helps to prevent and/or treat these dysfunctions. we investigated how gene expressions change pattern at different pruritus states in mice. The candidate genes were selected by literature review: reference genes, immune response genes, ion channel genes. All mice were anesthetized and clipped. They were divided into 4 groups: group 1 non-stimuli control; group 2 vehicle control; group 3 DNCB-treated group for sustained state; group 4 skin-scratching stimuli group for transient state. After exposure to stimuli, scratching behavior was more observed at stimuli groups compare with control groups. The next day after stimuli, all mice were sacrificed, and Skin tissues were taken for total RNA isolation. Gene expression levels were determined by RT-PCR and Real-time qPCR. In a sustained state, 4 genes of which were linked Tnf signaling pathway were markedly expressed. In a transient state, 4 genes of which were linked NK/NK-1R pathway were markedly expressed. The genes identified are expected to provide itch relief.

Key Words: Skin pruritus, Ion channels, TNF-α, NK/NK-1R

P8-01

NecroX-5 shows an anti-inflammation and mitochondrial biogenesis modulation roles to protect hypoxia-reoxygenation injury in rat hearts

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NecroX compounds have been shown to protect the liver and heart from ischemia-reperfusion injury. In this study, we verified whether the Necrox-5 modulates 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. Level of mitochondrial biogenesis related proteins has dramatically decreased and level of pro-inflammatory proteins was increased in HR treatment heart. However, treated with NecroX-5 significantly attenuated those HR-induced proteomic alterations, practically which are involved in oxidative phosphorylation and metabolic function. NecroX-5 treatment improved mitochondrial complex activities, markedly higher peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1α) expression levels were observed in the NecroX-5-treated group. In addition, HR- or LPS-induced TNF-α and TGF-β1 and phosphorylation of Smad2 productions were reduced with NecroX-5 supplement. The findings suggested the cardio-protective effect of NecroX-5 against cardiac HR injuries by modulating mitochondrial biogenesis and exerting anti-inflammation actions.

Key Words: NecroX-5, Hypoxia/reoxygenation, Inflammation, Mitochondria

P8-02

Endothelial nitric oxide synthase uncoupling in CR6 interacting factor-1 deficiency endothelial cells is related to tetrahydrobiopterin.

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Tetrahydrobiopterin (BH4) has responsibilities as a cofactor for the reaction of many enzymes. One is the biosynthesis of the neurotransmitters in brain. Tetrahydrobiopterin also related to catalyst for the production of nitric oxide. BH4 is synthesized by two pathways, de novo and recycling pathway. In vascular endothelium, de novo pathway is the primary pathway for BH4 synthesis. Endothelial nitric oxide synthase (eNOS) generates NO in blood vessels, which plays a major role in regulating vascular function. eNOS is composed of two kinds of domains, a reductase domain and an oxidase domain. Oxidative domain displays binding sites for heme group, zinc, BH4, and the substrate L-arginine. Reductase domain and Oxidase domain are linked by calmodulin-binding sequence. In the vascular endothelium, NO is synthesized by eNOS from L-arginine and molecular oxygen, which binds to the heme group of eNOS, is reduced and finally incorporated into L-arginine to from NO and L-citrulline. Without BH4, the structure of the eNOS becomes unstable and the substrate L-arginine cannot bind. Then the oxygen in the heme group receives the electrons instead of the substrate and canges to reactive oxygen species. eNOs that forms ROS instead of NO is called uncoupling eNOs. CR6 interacting factor 1 (CRIF-1) is essential for the translation and integration of mitochondrial oxidative phosphorylation complex, CRIF-1 deficiency induces mitochondrial dysfunction and mitochondrial reactive oxygen species. Our previous studies shown that NO generation and vasodilation decrease and ROS production increases in CRIF-1 knockout endothelial cell. So, we investigated eNOS and its cofactor BH4 in CRIF-1 knockout endothelium. First, eNOS inhibitor L-NAME and substrate L-arg were treated to confirm eNOS uncoupling in CRIF-1 knockout endothelial cells. It was confirmed that eNOS was uncoupled through reduction of ROS by L-NAME and lack of reactivity to L-arginine in CRIF-1 deficiency endothelial cells. Next, the amount of BH4 in endothelium was determined by high pressure liquid chromatography (HPLC). In CRIF-1 deficiency endothelial cell, total biopterin, BH4, BH4/Total ratio was decreased. To determine the effect of ROS induced by CRIF-1 deficiency on BH4, we treated N-acetylcysteine (NAC), a ROS scavenger. Reduction of ROS restored NO production and reactivity to L-arginine. In addition, total biopterin, BH4, and BH4/Total ratio were restored. In conclusion, ROS induced by CRIF-1 deficiency is major factor on BH4 declined and eNOS uncoupling.

Key Words: eNOS uncoupling, BH4, CRIF-1, ROS

P8-03

Protective effect of anthocyanin-rich extract from red Chinese cabbage on vascular inflammation in hyperlipidemic apolipoprotein E-deficient mice

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Anthocyanins have a broad spectrum of biomedical functions, including improving immune responses and reducing risk for chronic diseases. The aim of this study was to evaluate the role of anthocyanin-rich extract from red Chinese cabbage on vascular inflammatory responses. In this study, the anti-inflammatory activities of anthocyanin-rich extract from red Chinese cabbage (ArCC) (18% cyanidin) were demonstrated based on inhibitory effects on cultured endothelial cells and on hyperlipidemic apolipoprotein E-deficient (ApoE-/-) mice. ArCC treatment suppressed the expression and transcription of adhesion molecules in tumor necrosis factor-α-stimulated endothelial cells. ArCC significantly inhibited aortic inflammation in hyperlipidemic ApoE-/-mice. The aortas from these mice exhibited markedly lower leukocyte infiltration, lower concentrations of blood inflammatory cytokines, and reduced plaque formation than those from control mice did. We demonstrate for the first time that ArCC suppresses aortic vascular inflammation, using in vivo imaging, most likely by inhibiting expression of adhesion molecules and inflammatory cytokines. These data may contribute to the development of a promising natural approach in chronic inflammatory diseases management.

Key Words: Anthocyanin-rich red Chinese cabbage, Apolipoprotein E-deficient mice, In vivo imaging, Vascular cellular adhesion molecule, Vascular inflammation

P8-04

Inhibitory role of APE1/Ref-1 in phosphate-induced vascular smooth muscle cell calcification and phenotype changes

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Vascular calcification is strongly associated with pathogenesis of atherosclerosis, diabetes, and chronic kidney disease. However, the role of apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1) in inorganic phosphate (Pi)-induced vascular smooth muscle cell (VSMC) calcification remains unknown. In this study, we investigated the possible role of APE1/Ref-1 in Pi-induced VSMC calcification. VSMC calcification was induced by the exposure of cells to Pi. Alizarin red S staining or a calcium deposition assay was performed to measure calcium deposition. A phenotype change of VSMCs was determined based on the expression of alpha-smooth muscle actin (α-SMA) and smooth muscle protein 22-alpha (SM22α). Pi decreased the endogenous APE1/Ref-1 expression and its promoter activity in VSMCs. Adenoviral overexpression of APE1/Ref-1 but not that of an APE1/Ref-1 double redox mutant inhibited Pi-induced calcification in VSMCs and in an ex vivo organ culture of a rat aorta. Pi-induced intracellular and mitochondrial reactive oxygen species production were also inhibited by overexpression of APE1/Ref-1. Pi exposure induced a loss of the smooth muscle phenotype, based on the expression of α-SMA and SM22α; however, overexpression of APE1/Ref-1 prevented the Pi-induced loss of the smooth muscle phenotype. Taken together, our results reveal that Pi-induced VSMC calcification is associated with a decreased level of APE1/Ref-1 expression. APE1/Ref-1 overexpression suppressed Pi-induced VSMC calcification and prevented a loss of the smooth muscle phenotype. Our findings provide important molecular insights into the role of APE1/Ref-1 in preventing VSMC calcification.

Key Words: APE1/Ref-1, Inorganic phosphate, Vascular smooth muscle cells, Vascular calcification

P8-05

The ABCA1/STAT6 pathway is initiated by apoptotic cells and reinforced by activation of PPARγ/LXRα pathway in macrophages

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We investigated whether ABCA1/STAT6 signaling is initiated and reinforced by activation of PPARγ and liver X receptor (LXR)α in macrophages after exposure to apoptotic cells, using specific siRNAs for ABCA1, STAT6, and PPARγ or their antagonists. The interactions between mouse bone marrow-derived macrophages or RAW 264.7 cells and apoptotic Jurkat cells induced STAT6 phosphorylation as well as PPARγ expression and activation. Knockdown of ATP-binding cassette transporter A1 (ABCA1) after the transfection of macrophages with ABCA1-specific siRNAs reduced STAT6 phosphorylation as well as PPARγ mRNA and protein expression following apoptotic cell stimulation. ABCA1 knockdown also reduced apoptotic cell-induced LXRα expression at mRNA and protein level. Moreover, inhibition of STAT6 with specific siRNAs or the pharmacological inhibitor AS1517499 reversed the induction of PPARγ, LXRα, and ABCA1 by apoptotic cells. PPARγ-specific siRNAs or the PPARγ antagonist GW9662 reversed apoptotic cell-induced increases in LXRα and ABCA1 mRNA and protein levels. Thus, these results suggest that the apoptotic cells trigger ABCA1/STAT6 pathway which is reinforced through activation of the PPARγ/LXRα/ABCA1 pathway in macrophages.

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Key Words: ABCA1, STAT6, PPARγ, Macrophages

P8-06

Development of new indexes to evaluate acute atopic dermatitis-like inflammation

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The role of certain cytokines in the development of acute atopic dermatitis-like inflammation has not been clearly elucidated. This study was undertaken to develop new indexes to evaluate acute skin inflammation. For this purpose, we examined the expression pattern of inflammatory cytokines including Interleukin (IL)-33 in human dermal fibroblasts, mouse macrophages, and mouse models. We especially focused on observed changing in interleukin (IL) -33, which is produced as endocrine and epithelial cells as well as in various tissues and hematopoietic cells (especially macrophages). Human dermal fibroblasts (HDF) and macrophage RAW264.7 cells were treated with three pro-inflammatory cytokines (LPS, TNF-α and IFN-γ). The RT-PCR was performed to observe the mRNA level of cytokines expressed in cells after treatment. Acute dermatitis-like inflammation was induced by 2,4-dinitrochlorobenzene (DNCB) in Balb/c mice. After 4 weeks treatment, RT-PCR were performed to observe the mRNA level of cytokines in skin lesions of mice. Serologic analysis was also performed by enzyme-linked immunosorbent assay (ELISA) to check IgE, interleukin (IL) -4, IFN -γ level. H&E staining was performed for histological analysis. IL-1β, IL-6, and IL-8 expression was increased by LPS and TNF-α but decreased by IFN-γ when

HDF was treated with pro-inflammatory cytokine to induce the inflammatory immune responses. IL-33 was up-regulated in all conditions, especially in the treatment of TNF-α and IFN-γ. In RAW264.7, IL-6 and IL-10 expression was increased by treatment. IL-33 was up-regulated in LPS or IFN-γ, but not in TNF-α. Plasma IgE was increased in balb/c mice treated with DNCB for 4 weeks, similar to atopic dermatitis. However, there was no change in IL-4 and IFN-γ. We were also detected acute dermatitis lesions characterized by erythma, scaling, and bleeding. The expression of inflammatory cytokines was decreased of IL-1β, IL-6, IL-13 and IL-5 but IL-10 were slightly increased in DNCB-induced mice skin lesion. IL-33 showed no change. H&E staining of skin lesion confirmed thickening of the epidermis and increased lymphocyte infiltration. Taken together, the various cytokines changes in vitro and in vivo model of DNCB confirmed by this study could be used as a useful indicator to determine acute dermatitis status. Also, it can be used to confirm the effect of treatment in acute phase of dermatitis. In the future, we will verify the relationship between IL-33 and pro-inflammatory cytokines in the development of atopic dermatitis.

Key Words: Atopic dermatitis-like inflammation, Fibroblasts, Macrophages, Balb/c mice, IL-33

P8-07

Pro-inflammatory cytokine induces transient receptor potential vanilloid 1 (TRPV1) activation in dermal fibroblasts

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Transient receptor potential vanilloid 1 (TRPV1) channels reported on primary sensory neurons and non-neuronal cells in various tissues including keratinocytes, sebocytes, glandular epithelium, and mast cells belonging to skin tissue. In epithelial tissue (keratinocytes, corneal fibroblasts, foreskin fibroblasts), activation of TRPV1 due to noxious stimuli has been reported to induce pro-inflammatory cytokine release and participate in inflammation induction. However, the relationship between TRPV1 and pro-inflammatory cytokines in human dermal fibroblasts (HDF) is not yet known. In this study, we examined the expression of TRPV1 by pro-inflammatory cytokines and vice versa. Activation of TRPV1 by pro-inflammatory cytokines was detected by reverse transcription polymerase chain reaction (RT-PCR) and Western blot analysis. Expression of TRPV1 mRNA in HDF by pro-inflammatory cytokines were decreased in LPS and increased in TNF-α and IFN-γ. Western blot reactions were up-regulated with pro-inflammatory cytokines, respectively. This study confirmed the increase of TRPV1 expression by various proinflammatory cytokines in HDF by RT-PCR and Western blot. TRPV1 is susceptible to ligand-dependent activation and appears to be involved in inflammation or immune mechanism transmission, particularly when induced by IFN-γ in HDF. In future studies, we will investigate whether TRPV1 current is actually increased by IFN-γ treatment, and how TRPV1 expression leads to inflammation-related cytokines secretion and what kinds of signal transduction processes are involved.

Key Words: TRPV1, Fibroblasts, Pro-inflammatory cytokines

P9-01

The effects of actomyosin contractility on acute myeloid leukemia cells

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Myosins constitute a superfamily of ATP-dependent motor proteins that conventionally play a crucial role in muscle contraction and in a wide range of other cellular and physiological functions. In recent years, studies have increasingly demonstrated that myosins and their related up- or down-stream molecules, play irreplaceable roles during tumorigenesis and cancer-related diseases. Inhibitors of myosin activity have been described to hold therapeutic potential in diverse solid tumors. However, the role of myosin in blood cancer is largely unknown. Blebbistatin is the most commonly used and well-characterized small molecular inhibitor of class II myosins, which is a key component for the actomyosin contractility of diverse cancer cells. In this study, we reported that blebbistatin generally inhibits cell survival, growth and migration, promotes DMSO-induced differentiation of acute leukemia cell line HL-60. By using RNA sequencing method, we found that the several proto-oncogenes are significantly decreased by blebbistatin treatment, and the transcriptional change was mediated by YAP signaling pathways. These results show that actomyosin contractility may serve as a new therapeutic target for future strategies of formation and development of cancer.

Key Words: Actomyosin, Contractility, Acute myeloid leukemia

P9-02

The Relationship between RhoGDI2 and cell migration in CR6- Interacting Factor 1 deficient HUVECs

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Aims: The Rho GDP-dissociation inhibitor (RhoGDI), a downregulator of Rho family GTPases is typified by its ability to prevent nucleotide exchange and membrane association. Although RhoGDI2 has been identified as a tumor suppressor gene for cellular migration and invasion, little is known about its role in endothelial cell migration. In this study, we examined the expression of RhoGDI2 in CR6-interacting factor 1 (CRIF1) deficient human umbilical vein endothelial cells (HUVECs) and its role in cell migration. **Results:** We found that the expression of RhoGDI2 was considerably increased and therefore, cell migration was decreased in CRIF1 deficient HUVECs. Furthermore, the phosphorylation of MAP Kinases ERK and JNK were found to be increased whereas; phosphorylation of Akt was decreased in CRIF1 deficient cells. To further investigate the molecular mechanism of RhoGDI2-induced cellular migration, HUVECs were transfected with RhoGDI2 small interfering RNA (siRNA). The results showed that depletion of RhoGDI2 significantly recovered cell motility and phosphorylation of Akt. We also examined the effect of CRIF1 downregulation on other major proteins involved in endothelial cell migration. **Conclusion:** The results in our study demonstrate for the first time that RhoGDI2 plays an important role in cell migration of CRIF1 deficient endothelial cells. We also uncovered the possible proteins and pathways involved in this process.

Key Words: RhoGDI2, CRIF1, Cell migration, MAPK, Akt

P9-03

Study on pathogenesis of atherosclerosis using IDH2: relationship between IDH2 and mitophagy, mtUPR

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Aim: Mitochondrial dysfunction is a risk factor for vascular disease by over-expressing ROS caused by some stimuli in mitochondria which causes oxidative stress and enhances vascular inflammatory response. Isocitrate dehydrogenase 2 (IDH2) is $NADP^+$ dependent mitochondrial enzyme and an antioxidant enzyme that produces NADPH in the antioxidant system. Mitophagy and mitochondrial Unfolding Protein Response (mtUPR) are internal defense mechanism in mitochondria. In this study, we investigated whether IDH2 knockdown causes mitochondrial dysfunction then Mitophagy and mtUPR *in vitro* in HUVECs and *in vivo* in IDH2 knock out mice.
Results: We showed that knockdown of IDH2 expression induced depolarization of mitochondrial membrane potential (MMP). Mitochondrial dynamics is mitochondrial fusion and fission. Knockdown of IDH2 increased Drp1 (fission protein) and mfn1 (fusion protein) compared with Tom20 (control). IDH2 deficiency increase Mitophagy related protein PINK-1 and Parkin expression and mRNA level (PINK-1, Parkin, BNIP3, NIX, FUNDC-1). Moreover, knockdown of IDH2 induced mtUPR mRNA level (USP30, Clpp) *in vitro*. In addition, IDH2 deficiency increases mtUPR mRNA level and decreases PINK-1 and Parkin protein expression *in vivo*. **Conclusion:** Our data show that IDH2 deficiency induces mitochondrial dysfunction and then Mitophagy and mtUPR expression in endothelial cells. These findings provide novel strategy for the development of therapeutic agents for restoring mitochondrial and endothelial function.

Key Words: IDH2, Mitochondria, Mitophagy, mtUPR, Endothelial cells

P9-04 (PO-B-1)

Determining the deubiquitinating enzymes regulating the adipose derived mesenchymal stem cells senescence

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Clinical application of mesenchymal stem cells (MSCs) requires large quantities for injection or infusion. Evidences indicates that although stem cells remain active into old age, changes in stem cells and their microenvironments inhibit their regenerative potential. Understanding intrinsic stem cell changes as well as concomitant changes to the stem cell niche and systemic environment is crucial for development of regenerative medicine strategies. Ubiquitin proteasome mainly controlled protein degradation and key regulator of fundamental cellular processes. Deubiquitinating enzymes (DUBs) cleave ubiquitin from proteins and other molecules. A number of DUBs regulate processes associated with cell proliferation and apoptosis, and as such represent candidate targets for cancer therapeutics. The purpose of this study is to screen the DUBs relating to the mesenchymal stem cell senescence and to determine their function. Human adipose derived MSCs (hAD-MSCs) were obtained from the lipoaspirate after informed consent. hAD-MSCs were cultured until passage 14, and senescence was confirmed by beta-galactosidase assay, telomere length measurement, and senescence related genes by PCR. Using microarray and multiplex PCR, differential expressions of DUBs in Passage 1, 7, 14 cells were analyzed. Based on the data from microarray and multiplex PCR, final candidate DUBs were selected and determined by the gene and protein levels individually. Next, we synthesized siRNA for the three candidates and knock down of the three proteins were analyzed by western blot 48 hours after transfection in the hAD-MSC. Using Passage 7 cells, the loss of function for the final candidate were performed using PCR. Passage 14 cells have the characteristics of aged cells significantly compared to the Passage 1 and 7 cells in every aspect. Microarray demonstrated that USP1 showed the most prominent difference among the 95 DUBs between Passage 1 and 14 cells. However, in the multiplex PCR data showed USP12 and TNFaIP3 had the highest change during senescence. Collectively, USP1, 12, and TNFaIP3 were selected as the final candidates. PCR and western blot for the final candidates showed the consistent results in the USP1 and TNFaIP3. However, the opposite expression between gene and protein were observed in the USP12. siRNA for three DUBs were successfully constructed and determined. We transfected all 3 genes and found that the si-USP1 accelerate the hAD-MSC senescence independently of telomere. USP1 might be the therapeutic candidate for hAD-MSC therapy by controlling stem cell senescence.

Key Words: Deubiquitinating enzymes (DUBs), Stem cell senescence, Human adipose derived MSCs (hAD-MSCs), USP1

P9-05

CR6 interacting factor 1 deficiency induced anticancer effects by inducing mitochondrial dysfunction in breast cancer

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Background: Mitochondria are the main organelles for energy production and metabolism, and mitochondria in cancer cells have a unique metabolic feature, which plays an important role in the regulation of cancer cell growth and proliferation. Therefore, mitochondria can be exploited as a target for anti-cancer therapeutic intervention. Downregulation of CR6 interacting factor 1 (CRIF1), an essential mitoribosomal factor involved in the biogenesis of mitochondrial oxidative phosphorylation (OXPHOS) complexes, was previously shown to impair mitochondrial function in endothelial cells.
Aim: In this study, we investigated whether CRIF1 deficiency had an effect on suppressing breast cancer growth and tumor development. **Results:** Our results showed that downregulation of CRIF1 suppressed cell proliferation and inhibited cell migration in both MCF-7 and BT-549 breast cancer cells. Moreover, the silence of CRIF1 decreased mitochondrial OXPHOS complex I, II assembly, induced production of reactive oxygen species, and hyperpolarized the mitochondrial membrane potential. TP53-induced glycolysis and apoptosis regulator (TIGAR) is an inhibitor of glycolysis and promoter of pentose phosphate pathway (PPP), which has a high expression in human breast cancers. To define the impact of CRIF1 silence on glycolysis and the PPP, we detected the expression of TP53 and TIGAR in breast cancers. CRIF1 downregulation regulated glycolytic activity via suppression of TP53 and TIGAR with reduced NADPH synthesis. Furthermore, the inhibition of CRIF1 reduced HIF-1α stabilization under hypoxia condition in breast cancer cells. **Conclusion:** Taken together, silenced CRIF1 suppressed breast cancer cells survival via destroying mitochondrial function and inhibiting TIGAR-regulated PPP metabolic pathway.

Key Words: CR6 interacting factor 1, Mitochondrial dysfunction, Breast cancer, TIGAR

P9-06

Nitric oxide modulates lipocalin-2 expression via regulation of lipocalin-2 protein stability 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

P9-07 (PO-B-2)

EPHB6 mutation induces cell adhesion-mediated paclitaxel resistance via EPHA2 and CDH11 expression

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Mutations affect gene functions related to cancer behavior including cell growth, metastasis, and drug responses. By analyzing pharmacogenomic profiles, we predicted and demonstrated that EPHB6 mutation induce paclitaxel resistance in lung cancer, melanoma, and liver cancer cells. We also found that conformational change of EPHB6 mutant reduced recruitment of c-Cbl, diminishing EPHA2 degradation. Enhanced EPHA2 signaling induced expression of adhesion molecules resulting in acquisition of cell adhesion-mediated drug resistance (CAM-DR). We also report that the paclitaxel resistance was achieved by expression of *CDH11* and subsequent activation of RhoA/focal adhesion kinase (FAK). In conclusion, we suggest that *EPHB6* mutation induces paclitaxel resistance by activating EPHA2/CDH11/RhoA/FAK signaling axis, which can be a novel diagnostic or therapeutic target for the treatment of cancer patients with paclitaxel resistance.

Key Words: Paclitaxel, Cell adhesion-mediated drug resistance, EPHB6, EPHA2, CDH11

P9-08

CR6-interacting factor 1 deficiency increases premature senescence by impairment of anti-oxidant system in endothelial cells

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CR6-interacting factor 1 (CRIF1) is a protein that exists in mitochondria and interacts with large ribosomal subunit, the deficiency of CRIF1 induces mitochondrial dysfunction and mitochondrial reactive oxygen species (mtROS). Oxidative stress, which is defined as a disturbance between mtROS and anti-oxidant molecules, is one of the most critical factors contributing to endothelial dysfunction. The consequence of mitochondrial dysfunction is cellular apoptosis and senescence. The aorta SA-b-gal staining ratio in endothelium-specific CRIF1 knockout mice was increased compared with wild-type mice. Sirtuin 3 (SIRT3), a mitochondrial deacetylase, plays a major role in mitochondrial biogenesis and is related to oxidative stress. Furthermore, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a), which is a downstream protein of SIRT3, plays a major role in apoptosis, inflammation, and proliferation in endothelial cells. Our data confirmed that CRIF1 downregulation destroyed the anti-oxidant system in mitochondria by decreasing SIRT3 expression. SIRT3 downstream gene, mRNA and protein level of SOD2 were level dramatically decreased in CRIF1 deleted endothelial cells. SOD2 is an important antioxidant against oxidative stress. Thus, CRIF1-mediated SOD2 reduction may accumulate mtROS production in mitochondria. CRIF1 deficiency induced apoptosis and senescence progression in HUVECs by altering their morphology, β-galactosidase activity, expression of molecular senescence marker (P-p53 (S15), p21) and apoptosis marker (bcl-2, bax, caspase-3). In addition, we proved that SIRT3 overexpression could attenuate CRIF1 deficiency induced senescence in endothelial cells. We also demonstrated the same results in CRIF1 knockout mice. Our data identify a distinct senescence response and provide a mechanism by which mitochondrial dysfunction can drive aging. The final purpose of the study is to delay the development of senescence already in vascular damage.

Key Words: CRIF1, mtROS, SIRT3, Senescence

P9-09

Discovery of smoking-specific mutations using machine learning in lung cancer

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Lung cancer is the leading cause of death in Korea with about 17,000 deaths in 2014, accounting for 28 percent of all cancer deaths. Biomarkers that predict smokers' probability of developing lung cancer have a great impact on prognosis and treatment. In this study, we discovered smoking-specific mutations in lung cancer using machine learning (ML). The Cancer Genome Atlas-LUAD provided data for 490 LUAD patients with somatic non-silent mutations and clinical information. They were divided into smoking group and non-smoking group. To identify smoking-related mutations, four feature selection methods (Information Gain, Chi-squared test, MRMR, Gini index) and four classifiers (Naïve Bayes, K-NN, SVM, Decision Tree) were used. We performed 5 fold-validations to find out the efficient algorithm. A total of 75 smoking-related genes were selected by ML and 22 of them were identified as smoking-specific genes. These mutations were associated with overall survival and disease free survival. The feature selection methods and performances of classifiers were useful to find gene mutations associated with smoking in lung cancer patients. We found efficient ML algorithms to seek

smoking-related gene mutations in lung cancer. These gene mutations could be biomarker to predict occurrence of lung cancer in smoker.

Key Words: Lung Cancer, The cancer genome atlas (TCGA), Machine learning, Mutation, Smoking mutant

P9-10

Association of specific gene mutations derived from machine learning with survival in lung adenocarcinoma

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Lung cancer is the second most common cancer in the United States, following prostate cancer in men and breast cancer in women, and is the leading cause of mortality in cancer patients. Biomarkers predicting survival of patients with lung cancer have a profound effect on patient prognosis and treatment. However, until now, predictive biomarkers for survival and their relevance for lung cancer have not been well known. We performed machine learning with data from patients with lung adenocarcinoma (LUAD) to find gene mutations that could be used as survival-predicting biomarkers. LUAD data from The Cancer Genome Atlas (TCGA) were used to conduct machine learning to discover survival-specific gene mutations. To identify survival-specific mutations according to the various clinical factors, four feature selection methods (information gain, chi-squared test, minimum redundancy maximum relevance (MRMR), and correlation) were used. The extracted survival-specific mutations of LUAD were applied individually or as a group for Kaplan-Meier survival analysis. Mutations in *MXXX* and *GXXX* were significantly associated with patient mortality, and those in *ZXXX* and *SXXX* were associated with patient survive. Mutations in *DXXX* and *MXXX* were significantly negatively associated with overall survival, and the mutations in *ZXXX* were positively associated with overall survival. Mutations in *MXXX* were significantly negatively associated with disease-free survival, and the mutations in *DXXX* and *ZXXX* were positively associated with disease-free survival. The genes *DXXX*, *SXXX*, and *ZXXX* were significantly positively associated with survival in patients with LUAD; however, the opposite was true for *DXXX* and *MXXX* mutations. In this study, machine learning was conducted to obtain information necessary to discover specific gene mutations associated with the survival of patients with LUAD. The mutations in the above five genes could predict survival rate and non-disease survival rate in patients with LUAD and are important biomarker candidates.

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Key Words: Lung adenocarcinoma, The cancer genome atlas (TCGA), Machine learning, mutation, survival, biomarker

P9-11 (PO-B-3)

Cardiovascular drug, echinochrome A enhances cardiac differentiation from embryonic stem cell via PKCiota inhibition

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Echinochrome A (EchA) is a marine bioproduct extracted from sea urchins having antioxidant, antimicrobial, anti-inflammatory, and chelating effects, and is the active component of the clinical drug histochrome. We investigated the potential use of Ech A for inducing cardiomyocyte differentiation from mouse embryonic stem cells (mESCs). We also assessed the effects of Ech A on mitochondrial mass, inner membrane potential (Δψm), reactive oxygen species generation, and levels of Ca^{2+}. To identify the direct target of Ech A, we performed *in vitro* kinase activity and surface plasmon resonance binding assays. Ech A dose-dependently enhanced cardiomyocyte differentiation with higher beating rates. Ech A (50 μM) increased the mitochondrial mass and membrane potential but did not alter the mitochondrial superoxide and Ca^{2+} levels. The *in vitro* kinase activity of the atypical protein kinase C-iota (PKCι) was significantly decreased by 50 μM of Ech A with an IC_{50} for PKCι activity of 107 μM. Computational protein-ligand docking simulation results suggested the direct binding of Ech A to PKCι, and surface plasmon resonance confirmed the direct binding with a low K_D of 6.3 nM. Therefore, Ech A is a potential drug for enhancing cardiomyocyte differentiation from mESCs through direct binding to PKCι and inhibition of its activity.

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Key Words: Echinochrome A, Mitochondria, Cardiac differentiaon, Embryonic stem cell

P9-12

Role of mito-K_{ATP} channel in formation of the de-energized mitochondrial membrane potential

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Mitochondria are organelles which play a critical role in the generation of metabolic energy in cells. In previous study, we showed de-energized mitochondria in absence of mitochondrial substrates still have a membrane potential. To pursue the mechanism of that, firstly we tried the possibility of the involvement of mito-K_{ATP} channel. We tested the effects of Pi /ATP, K_{ATP} blockers/opener, and K^+ replacement on the de energized mitochondria. In this study, NADH, FAD and ΔΨm were monitored using permeabilized ventricular myocytes of the rat. The ΔΨm of de-energized mitochondria was about - 22 mV. When we add both Pi and ATP, ΔΨm was dramatically hyperpolarized to about 65 mV and the removal of ATP returned ΔΨm to the initial value. In addition, FAD signals were slightly increased which reflected FADH oxidation, which means FADH is still existed in the mitochondria in the absence of substrate. Interestingly, Pi itself could decrease FAD signal, which means the decrease of FADH consumption. NADH signal change was changed but negligible. Since ATP could inhibit K_{ATP} channel, we tested pharmacological agents to activate or to inhibit K_{ATP} channel. However, all agents blocking K_{ATP} channel did not show any significant effects. Interestingly, Diazoxide (DZX), K_{ATP} channel opener, itself could depolarize the Ψm in basal condition to about 0 mV, however, the addition of Pi could return Ψm to basal level. Interestingly, Oligomycin A (OligoA), F_1F_0-ATPase inhibitor, showed the similar effects as DZX, depolarizing Ψm and reversing by Pi. However, ATP induced hyperpolarization was not affected by DZX but fully inhibited by OligoA. K^+ replacement with NMDG did not show any change on the basal Ψm but slowed down the speed of ATP induced hyperpolarization and made it transient. However, the change NMDG to K^+ could reverse and showed the full effect of ATP on Ψm. From these results, K_{ATP} channel may not participated on the formation of the resting Ψm of the de-energized mitochondria, however, the opening of K_{ATP} channel could depolarize it and Pi might inhibit the effect of DZX or OligoA on the resting

Ψm. ATP-induced hyperpolarization may be induced by the reverse mode of F_1F_0-ATPase. The K^+ environment seemed to be important for the activity of F_1F_0-ATPase. The mechanism of formation of the resting Ψm is still not clear, however, the reverse mode of F_1F_0-ATPase by the residual ATP hydrolysis might contribute it but the present results can not answer how ATP can be formed without substrates. It still further study on them.

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Key Words: K_{ATP}, Diazoxide, Oligomycin A, ATP, Pi

P9-13 (PO-B-4)

Influence of pharmacological inhibition of AKT by novel inhibitor HS1793 in relapsed multiple myeloma

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Multiple myeloma (MM) is a neoplastic plasma cell disorder with high disease recurrence rates. Novel therapeutic approaches capable of improving outcomes in patients with MM are urgently required. The AKT signaling plays a critical regulatory role in MM pathophysiology, including survival, proliferation, metabolism, and has emerged as a key therapeutic target. Here, we identified a novel AKT inhibitor, HS1793, and defined its mechanism of action and clinical significance in MM. HS1793 disrupted the interaction between AKT and heat shock protein 90, resulting in protein phosphatase 2A-modulated phosphorylated-AKT (p-AKT) reduction. Moreover, we observed reductions in the kinase activity of the AKT downstream target, IκB kinase alpha, and the transcriptional activity of nuclear factor kappa B, which induced mitochondria-mediated cell death in MM cells exclusively. We confirmed the cytotoxicity and specificity of HS1793 via PET-CT imaging of a metastatic mouse model generated using human MM cells. We also evaluated the cytotoxic effects of HS1793 in primary and relapsed MM cells isolated from patients. Thus, HS1793 offers great promise in eliminating MM cells and improving therapeutic responses in primary and relapsed/refractory MM patients.

Key Words: AKT, Anti-cancer drug, Cancer, Cell death, HS1793, HSP90, Multiple myeloma

P9-14

A noble finding of miRNAs in neurogenic differentiation of human adipose tissue derived mesenchymal stem cells

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MicroRNAs (miRNAs) are small noncoding RNAs that emerge as regulators of stem cell lineage such as proliferation, development, differentiation, and apoptosis. We hypothesized that miRNA was involved in the neurogenic differentiation of mesenchymal stem cells. Here, the role of miRNAs in neurogenic differentiation of human mesenchymal stem cells (MSCs) is investigated. By performing a miRNA-mRNA paired microarray screening, we identified miR-4650-5p and miR-3146 among the most upregulated miRNAs during neurogenic differentiation. After selection of the miRNAs, we investigated the ability of neurogenic differentiation of miRNAs in human adipose tissue-derived MSCs (hADSCs). We found that miR-4650-5p or miR-3146 was increased the most of neuronal gene expressions by a quantitative PCR. Using bioinformatics and functional assay, we confirmed that miR-4650-5p and miR-3146 potentially targeted on JNK and GSK3β to regulate Wnt signaling pathway. Overall comparative analysis revealed that Wnt signaling was enhanced more potently and played a more important role in neurogenic differentiation of hADSCs. These findings suggest that the miR-4650-5p and miR-3146 expression contributes the neurogenic differentiation of MSCs by increasing the neuronal genes and Wnt signaling pathway. The miRNAs regulation and downstream pathway network suggested the important role of miRNAs and Wnt signaling in the neurogenic differentiation of MSCs.

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Key Words: Mesenchymal stem cell, MicroRNA, Neurogenic differentiation, Wnt signaling, JNK

P9-15

Differentiation of human bone marrow-derived stem cells into neuron-like cells by histone deacetylase inhibitors

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Mesenchymal stem cells (MSCs) have an ability to differentiate into multiple lineages, therefore, the possibility of neurogenic differentiation is important as a target for the clinical field. Wnt signaling, which is one of the remarkable regulators, plays a role in the development of the central nervous system and regulates the controlling neuronal differentiation. We hypothesized that regulating of Wnt signaling both activation and inhibition participated in the neurogenic differentiation of human adipose tissue-derived MSCs (hADSCs). In the present study, we developed the neurogenic differentiation of cells using an Anandamide, a Wnt5a activator, and Box5, a Frizzled-5-dependent Wnt5a antagonist, and studied the mechanisms for further differentiation *in vitro*. We treated Anandamide or Box5 and found that the Anandamide-treated cells have features such as neuron-like cells; exhibited distinct bipolar or multipolar morphologies with branched processes. Following PCR and quantitative PCR experiments, neuronal gene expressions were increased with Anandamide treatment; it was the same result; the protein levels of NFL and Tuj1 were highly expressed by immunofluorescence staining. We studied mechanisms of differentiation and found that Wnt signaling and downstream MAP kinase, especially GSK-3β pathway, were involved in neurogenic differentiation following Wnt5a activator. Especially, Wnt4 and Wnt11, which are a group of non-canonical Wnts, protein levels were highly increased after treatment of Anandamide; the Wnt5a activator could regulate the non-canonical Wnts signaling broadly. In addition, Anandamide activated through regulating Dvl2 and Dvl3 and resulted in expression of Axin level following highly increasing phosphorylated-JNK. Taken together, Wnt5a activator regulated the most of non-canonical Wnt signaling and the downstream pathway, especially controlling GSK-3β and JNK levels in the neurogenic differentiation of MSCs.

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Key Words: Mesenchymal stem cell, Neurogenic differentiation, Wnt signaling, Anandamide, Box5

P9-16

Secretory acetylated APE1/Ref-1 requirement for suppression of tumor growth in triple-negative breast cancer in vivo

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Background: Because triple-negative breast cancer (TNBC) represents a relatively small proportion of all BCs, but a relatively large proportion of BC-related death, more effective therapeutic strategies for the TNBC management are needed. We previously demonstrated that the stimulation of apoptosis by the binding of secretory, acetylated apurinic apyrimidinic endonuclease 1/redox factor-1 (Ac-APE1/Ref-1) to receptor for advanced glycation end products (RAGE) was essential for the TNBC death in response to hyperacetylation. The aim of present study was to assess potential therapeutic efficacy of secretory Ac-APE1/Ref-1 orthotopic TNBC xenografts in vivo. **Methods:** Secretion of Ac-APE1/Ref-1 into the blood was analyzed by enzyme linked immunoassay. Binding of secretory, Ac-APE1/Ref-1 to RAGE in tumor tissue was assessed by proximity ligation assays. The effect on tumor growth and development of TNBC xenografts using three different cell lines was analyzed by microcomputed tomography and in vivo imaging system. Cell death signaling through RAGE stimulation was immunologically assessed. The signaling in RAGE-overexpressing tumors was also compared with one of RAGE-knockout tumors. **Results:** Hyperacetylation in xenografts caused secretion of Ac-APE1/Ref-1 into the blood, where secreted Ac-APE1/Ref-1 bound directly to RAGE in hyperacetylated tumor tissues. Hyperacetylation in the TNBC xenograft induced strong inhibition of tumor growth and development. Hyperacetylation also caused cell death in tumors, accompanied by increased RAGE expression and generation of reactive oxygen species, which induce apoptosis. Additionally, tissues exhibited markedly higher counts of apoptotic bodies and a reduced proliferation index and neovascularization as compared to control tumors. Ac-APE1/Ref-1-stimulated apoptotic cell death was markedly reduced in RAGE-knockout tumors as compared with RAGE-overexpressing tumors, even in the presence of hyperacetylation. The role of secreted Ac-APE1/Ref-1 was confirmed in other hyperacetylated TNBCs xenografts using BT-459 and MDA-MB-468 cells, demonstrating its relevance as an anti-cancer molecule. **Conclusions:** Our findings suggest that the Ac-APE1/Ref-1 possesses potent chemotherapeutic efficacy against hyperacetylated TNBCs based on stimulation of cell death by RAGE-dependent triggering of Ac-APE1/Ref-1, warranting further evaluation of Ac-APE1/Ref-1 as an anti-cancer agent.

Key Words: Triple-negative breast cancer, Hyperacetylation, Secreted Ac-APE1/Ref-1, RAGE

P9-17

Discovery of common triple basic residues in the middle C-terminal of TREK K^+ channels (KCNK2 and KCNK10) responsible for the activation by low-level PIP_2

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TWIK-Related two-pore domain K+ channels (TREKs) are activated by acidic pHi, membrane stretch, temperature, and arachidonic acid (AA). In contrast, physiological PIP2 shows dual effects; (1) removing intracellular ATP activate TREKs via lowering intrinsic PIP2 while further scavenging with poly-L-Lys (pLL) abolished their activity, (2) activation of co-expressed voltage-sensitive PIP2 phosphatase (Dr-VSP) induces initial activation and subsequent inhibition of TREKs (Woo et al., 2016). We have reported that Lys (K^{330}) in the early proximal C-terminal (pCt) are responsible for the PIP2-mediated inhibition because their neutralization ($K^{330}A$) induced tonic high activity of TREK-2 irrespective of ATP (Woo et al., 2018). Here we focus on the triple successive Arg in the middle Ct (mCt), and investigate their Ala substitution ($R^{340-2}A$ and $R^{355-7}A$ in TREK-1 and -2, respectively). Both mutants showed very low basal activity, were activated neither by eliminating cytoplasmic ATP nor by stimulating the co-expressed Dr-VSP. Phosphatidic acid, an anionic phospholipid agonist with smaller head group, could not activate the mutants. However, the activations of by acidic pHi, arachidonic acid, and temperature were basically intact. However, the mechanosensitivity (i.e. membrane stretch-dependent activation) was suppressed in $R^{355-7}A$ TREK-2. Combined neutralizations of the pCt Lys (K330A/R355-7A) did not rescue the suppressed activity while acidic pHi activation was intact. Neutralization of the more distal triple cationic residues (e.g. $R^{377-9}A$ in TREK-2) did not affect the activation properties and spontaneously activated under ATP-free. Taken together, we propose a novel model of inhibitory and stimulatory regulation of TREKs by PIP_2 via electrostatic interaction with cationic amino acids in the pCt and mCt, respectively.

Key Words: TREK channel, KCNK2, KCNK10, PIP2

P9-18

RhBMP-2 diminished the growth of pancreatic cancer cells via activation of hippo pathway

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Despite that the use of recombinant human bone morphogenetic protein (rhBMP)-2 has been debated for a decade due to its oncogenic characteristics. However, the underlying molecular mechanism and safety issues of rhBMP-2 usage remain poorly understood. In this study, we investigated the effect of rhBMP-2 and its signaling pathways involved in pancreatic cancer cell using MIA Paca-2 cells. RhBMP-2 significantly inhibited proliferation of CRC cells in dose-dependent way by MTT assay. Cell cycle arrest in G1 phase was induced at 24h after rhBMP-2 treatment. RhBMP-2 also stimulated Smad4, p53 and p21 levels, and reduced cyclin D1, cyclin-dependent kinase (CDK) 4 and CDK6 activities. On the other hand, rhBMP-2 treatment resulted in reduced protein expression levels of poly (ADP-ribose) polymerase (PARP) and caspase-9 whereas those of cleaved PARP and cleaved caspase-9 were significantly increased in CRC cells. In addition, rhBMP-2 increased MST1, MST2, Mob1, p-Mob1, Sav1, and p-YAP protein levels. Therefore, our results indicate that rhBMP-2 suppresses pancreatic cell proliferation which is mediated via inactivation of hippo signaling pathway. Therefore, targeting BMP-2 may constitute a potential therapeutic strategy for human pancreatic cancer.

Key Words: Pancreatic cancer, rhBMP-2, Hippo signaling pathway, Apoptosis, Cell cycle

P9-19

HN1 contributes to migration, invasion, and tumorigenesis of colorectal cancer by regulation of autophagy

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Colorectal cancer has a high mortality rate among cancers worldwide and 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 specific functional significance of HN1 in colorectal cancer cells remains largely unknown. In this study, we investigated the underlying molecular mechanisms by which HN1 regulates proliferation, metastasis and autophagy in colorectal cancer cells. Knockdown of HN1 significantly decreased the viability of colorectal cancer cells. Knockdown of HN1 inhibited the invasion and metastasis of colorectal cancer cells. Moreover, downregulation of HN1 induced autophagy. Loss of HN1 decreased the expression of p62, whereas the LC3 II expression was increased. Therefore, our results suggest that HN1 regulates growth, metastasis, and autophagy of colorectal cancer cells and targeting HN1 may constitute a therapeutic strategy for colorectal cancer.

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

P9-20

SREBP1, targeted by HN1, modulates tumorigenesis of hepatocellular carcinoma

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Hpatocellular carcinoma (HCC) is the most common type occurring in adults, and induces greater rate of death in people with cirrhosis. Since the hematopoietic- and neurologic-expressed sequence1 (HN1) gene is detected a high expression level in various cancers, and is identified that associates with metastatic carcinoma progression, neural development, nerve and retina regeneration, its functional significance in HCC remains unclear. Thus, we investigated the biological role of HN1 in HCC using HepG2 and SNU449 cells. Silencing of HN1 significantly diminished the viability of HCC cells whereas overexpression of HN1 stimulated the viability of HCC cells. Silencing of HN1 increased apoptotic proteins and increased the number and size of colonies. In addition, silencing of HN1 inhibited the invasion and metastasis of HCC cells whereas overexpression of HN1 promoted the invasion and metastasis of HCC cells. In gene expression profiling, we identified 130 upregulated genes and 379 downregulated genes after HN1 silencing in HCC cells. Putative gene networks revealed suppressed expression of proteins associated with lipogenic signaling pathway. Silencing of HN1 significantly inhibited the expression levels of SREBP1 and SREBP2 of HCC cells whereas overexpression of HN1 increased the expression levels of SREBP1 and SREBP2 of HCC cells. In addition, silencing of SREBP1 also diminished the expression levels of HN1 and suppressed the survival and metastasis of HCC cells. In cholesterol assay and triglyceride assay, silencing of HN1 inhibited the lipid formation of HCC cells whereas overexpression of HN1 promoted the lipid formation of HCC cells. Taken together, HN1 encourages the proliferation, invasion and metastasis of HCC cells in part through the activation of SREBP signaling pathway. Therefore, our results suggest that targeting HN1 may constitute a potential therapeutic strategy for HCC.

Key Words: HN1, Hepatocellular carcinoma cells, Cell proliferation, Lipogenesis, Microarray

P9-21

Role of JAK3 in migration and differentiation of nestin-positive progenitor cells in the spinal cord

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JAK3 pathway play roles in cell growth, proliferation, differentiation and migration. During the development of the spinal cord, neural progenitor cells (NPCs) can proliferate and differentiate into neurons or glial cells depending on the developing stage. These NPCs express nestin, one of the intermediate filaments as a progenitor marker. The nestin-positive progenitor cells also can migrate into the lesion after the spinal cord injury. In our previous study, JAK3 signaling knocking down inhibits astrogliogenesis whilst induces dendrocytes including neurons and oligodendrocytes from the cortical progenitor cells. Also, nestin-positive spinal progenitor cells can Jak3-depedently differentiate into Iba1-positive microglia in growth factor-enriched conditions. In the present study, role of Jak3 in proliferation, differentiation and migration during the development of the spinal cord was investigated. The NPCs were obtained from the embryonic spinal cord in E13.5 mice, and the scratch wound model in serum-enriched condition were used. The spinal NPCs can proliferate, differentiate into neurons, and also migrate into the lesion. The nestin NPCs cells first were migrated, and migrating neurons were followed with growing cone. The leading nestin-positive cells seemed to be shuttle boats with mounting in operation. JAK3 inhibition remarkably reduced the nestin-positive cells, whilst neurogenesis from the NPCs were not affected. Also, JAK3 inhibition reduced GFAP-positive gliogenesis from the spinal NPCs. JAK3 signaling might be important in genesis of migrating nestin-positive shuttle cells, and also cytoskeletal arrangement of the migrating cells in the spinal cord. The detailed mechanism of JAK3 is needed further. Taken together, JAK3 signaling play critical roles in the migration of NPCs during the development of the spinal cord.

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Key Words: JAK3, Development, The spinal cord, Neural progenitor cell, Cell migration

P9-22

Sirtuin 6 inhibits liver cancer progression by modulating UPA and MMP9

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SIRT6 (sirtuin 6) is a member of sirtuin family of NAD+-dependent enzymes and plays a key role in DNA repair, telomere maintenance, and cellular metabolic processes. Several recent studies reported that SIRT6 functions as a tumor suppressor. However, the precise molecular mechanism of SIRT6 in human hepatocellular carcinoma (HCC) has not been clearly understood. Therefore, we have investigated the importance of SIRT6 function in HCC cell lines, HepG2 and SNU449. SIRT6 was highly expressed in human HCC cells. Overexpression of SIRT6 significantly diminished the viability of HCC cells whereas silencing of SIRT6 stimulated the viability of HCC cells. Overexpression of SIRT6 increased expression of cleaved-PARP and cleaved-caspase9 and decreased the PARP, caspase9, and caspase3.Knockdown of SIRT6 increased the number and size of colonies. In addition, overexpression of SIRT6 significantly inhibited the invasion and metastasis of HCC cells whereas silencing of SIRT6 increased the invasion and metastasis abilities of HCC cells in a time dependent manner. Moreover, overexpression of SIRT6 inhibited vimentin, UPA, and MMP9 protein levels while silencing of SIRT6 in HCC cells increased the protein levels of vimentin, UPA, and MMP9. P-β-catenin levels was increased by overexpression of SIRT6 and was decreased by silencing of SIRT6. In vitro, knockdown of SIRT6 significantly promoted the tumor growth. These data suggest that SIRT6 inhibits the proliferation, invasion and metastasis of HCC cells and may play as a tumor suppressor in HCC cells.

Key Words: SIRT6, Hepatocellular carcinoma cells, Metastasis, Cell proliferation, β-catenin

P9-23

JAK3 as a determinant in migration of GABAergic interneurons

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The proper function of cerebral cortex requires a balanced and coordinated network with the excitatory glutamatergic projection neurons and the inhibitory GABAergic interneurons. During development, these different types of neurons are originated from spatially and molecularly segregated progenitors and are moved along the different migratory pathways. Disturbed neuronal migration gives rise to neurological or neuropsychiatric disorders, such as congenital epilepsy, autism spectrum disorder, and schizophrenia. Here, we examined JAK3 as a determinant of migration and differentiation of GABAergic interneurons during developmental stage. More than 70% of interneurons are produced in medial ganglionic eminence (MGE) and move to the cortex by the mode of tangential migration in mouse embryonic day 13.5 to 15.5. In the present study, we found the JAK3 expression of the lateral migrating stream of GABAergic interneurons in the E13.5 and E15.5 embryonic brain was prominent. In *ex vivo* slice culture also, interneurons from MGE explant moved to the cortex explant, however, inhibition of JAK3 delayed the interneuronal migration toward the cortex. In in vitro neuroprecursor cell cultures from MGE in E13.5 mice, MGE-originated interneurons could cross the scratched space, and pharmacological or genetic inhibition of JAK3 significantly decreased the migration of interneurons. These results suggest the possibility that JAK3 is a determinant of proper migration of GABAergic interneurons from MGE to cortex during corticogenesis.

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Key Words: Interneuron, Migration, JAK3, Corticogenesis

P9-24

In vivo treatment with Gas6 inhibits EMT in primary murine alveolar type II cells and lung fibrosis

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Emerging evidence suggests that epithelial-mesenchymal transition (EMT) is a major event in idiopathic pulmonary fibrosis (IPF). We investigated whether growth arrest-specific protein 6 (Gas6) inhibits EMT in mouse alveolar type II (AT II) epithelial cells and lung fibrosis induced by bleomycin. In a model of bleomycin (BLM)-induced lung fibrosis, Gas6 administration prevents EMT in primary AT II cells in including changes in morphology and the mRNA expression of EMT markers, such as decreased E-cadherin and increased N-cadherin and α-SMA. BLM-induced upregulation of mRNA expression of EMT-regulating transcription factors in primary AT II cells was also reversed by Gas6. Moreover, treatment with Gas6 further enhanced BLM-induced mRNA levels of these molecules in AT II cells. Similarly, Gas6 further enhanced BLM-induced PGE_2 and PGD_2 secretion into the culture media. The reduced expression of E-cadherin and the enhanced expression of N-cadherin, α-SMA, type 1 collagen α2, and fibronectin at the protein and gene levels in lung tissue 14 and 21 d after BLM treatment were also reversed by Gas6. BLM-induced increases in active TGF-β1 levels and hydroxyproline were also inhibited by Gas6.

Key Words: Gas6, Alveolar type II epithelial cells, Epithelial-mesenchymal transition, Lung fibrosis, Bleomycin

P9-25

Incidence and management of adverse events associated with panobinostat in gastric cancer cells

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Histone deacetylase inhibition is an additional unique mechanism of action with established biological relevance in gastric cancer. Panobinostat (LBH-589) is an experimental drug developed by Novartis for the treatment of various cancers. It is a hydroxamic acid and acts as a non-selective histone deacetylase inhibitor (HDAC inhibitor). Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related deaths worldwide. Despite the significant progress made in gastric cancer chemotherapy, advanced disease remains largely incurable and novel efficacious chemotherapies are urgently needed. The purpose of this study was to identify genes in gastric cancer cells that are differentially regulated by panobinostat to provide the functional role of HDAC inhibition in gastric cancer. Panobinostat significantly inhibited the proliferation of SNU484 cells in a dose-dependent manner and resulted in a significant inhibition of colony formation in SNU484 cells. Panobinostat significantly increased apoptosis as indicated by cleaved poly (ADP-ribose) polymerase (PARP) and cleaved caspase-9 and diminished caspase-3 protein levels in SNU484 cells. Statistical analyses of gene expression data from panobinostat treated cells revealed that 2814 genes were significantly upregulated, while 1788 genes were down-regulated in SNU484 cells. Putative canonical pathways showed that ATM signaling and G2/M DNA damage checkpoint genes were significantly altered by panobinostat treatment. Therefore, our present study shows that panobinostat inhibits proliferation of gastric cancer cells by induce cell apoptosis through G2/M cell cycle DNA damage regulation.

Key Words: Panobinostat, Gastric cancer cells, Apoptosis, Gene expression profiling, Cell cycle

P9-26

Anoctamin1 does not function as ion channel in head and neck squamous cell carcinoma due to lack of surface expression

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Anoctamin1 (ANO1) gene (*TMEM16A*) encodes a calcium-activated chloride channel (CaCC) in various epithelial cells, and its role has also attracted attention in cancer research due to its changed expression level. In several tumors including head and neck squamous cell carcinoma (HNSCC), the expression of ANO1 is significantly amplified, and the ANO1 knock-down reduces cell migration and/or proliferation. However, the electrophysiological role of ANO1 in the tumor biology is still unclear. Here, we detected a highly over-expressed ANO1 in HNSCC patients and significant correlation between the expression level of ANO1 and prognosis by analyzing TCGA database. We further measure the current of ANO1 (I_{ANO1}) in five type of HNSCC, breast cancer (BCa), and prostate cancer (PCa) cell lines using whole-cell patch clamp. I_{ANO1} was detected in BCa and PCa, while not in any HNSCC cell line. Confocal imaging and immunoblot analysis of HNSCCs revealed no significant expression of ANO1 in the plasma membrane despite the high cytosol expression. In contrast, BCa and PCa cells show unequivocal membrane expression, consistent with the I_{ANO1} recordings. Moreover, in the present of ANO1-specific inhibitors, the migration and proliferation of cancer cell was reduced only in BCa and PCa cell lines with the surface expresses ANO1, but not in HNSCC. Taken together, our results suggest that ANO1 does not function as ion channel in HNSCC due to the lack of surface expression. The surface expression of ANO1 is a prerequisite for the modulation of cancer cell proliferation and migration by the ANO1 channel inhibitor.

Key Words: ANO1, TMEM16A, Head and neck squamous cell carcinoma,

Surface expression

P9-27

Inactivation of Akt pathway by ursolic acid plus paclitaxel suppressed growth of esophageal cancer cells

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Ursolic acid (UA) is a 3β-hydroxy-12-urs-12ene-28-oic acid and is one of the pentacyclic triterpenoids present in different plants, herbs, and fruits. Extensive research has been conducted over the last few decades to clarify the role of UA in various cancers. Despite the fact that paclitaxel is widely used as chemotherapy agents against several types of cancer, their combined effects with natural compound on esophageal squamous cell carcinoma (ESCC) have never been fully elucidated. Therefore, we explored the effects of UA as well as its combination treatment with paclitaxel in ESCC cells by using TE-12 and TE-8 cell lines. UA plus paclitaxel treatment inhibited the proliferation of TE-12 cells and TE-8 cells in a dose-dependent manner when compared to treatment with UA or paclitaxel alone. In colony formation assay, UA potentiated the inhibition of colony formation by paclitaxel when compared to treatment with a single agent. Combination treatment substantially induced apoptosis as indicated by increased levels of cleaved polyADP-ribose polymerase (PARP) and cleaved caspase-9 protein. UA plus paclitaxel treatment significantly inhibited the invasion and metastasis of TE-12 and TE-8 cells. In addition, combination treatment increased the protein levels of p-Akt and decreased FOXM1 in ESCC cells. These results suggest that UA effectively potentiates the efficacy of chemotherapeutic agents such as paclitaxel via inhibition of proliferation and metastasis by inactivation of FOXM1 in ESCC cells. Taken together, UA enhances the therapeutic efficacy of paclitaxel in esophageal cancer and is a potential clinical anticancer agent for the prevention and/or treatment of esophageal cancer.

Key Words: Ursolic acid, Esophageal squamous cell carcinoma, Apoptosis, FOXM1

P9-28

Expression of TonEBP/NFAT5 is associated with migration in NSCLC Cells

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TonEBP/NFAT5 (tonicity responsive enhancer binding protein/ nuclear factor of activated T cells 5) is transcription factor that play important roles in regulation of chronic inflammatory disease and maintenance of kidney. Recent studies have shown that TonEBP/NFAT5 is closely related to the malignancy of cancer cells. Therefore, the correlation between the migration of cancer cells and TonEBP/NFAT5 expression in NSCLC (non-small cell lung cancer) cells was investigated. RNAi-mediated knockdown of TonEBP/NFAT5 was decreased the migration in A549 cells and PC9 cells. Also overexpression of TonEBP/NFAT5 increased the migration of cancer cells. Previous studies have shown that macrophages promote the malignant transformation of cancer cells by increasing the migration of cancer cells. In this study we have found that the expression of TonEBP/NFAT5 was increased by macrophages that increase the migration in NSCLC cells. And increased cell migration by macrophages decreased when the TonEBP/NFAT5 was knockdown. These results suggest that TonEBP/NFAT5 may be an important regulator of malignant transformation of cancer cells.

Key Words: Migration, NSCLC, TonEBP/NFAT5

P9-29

Cytokines secreted from macrophage induce cisplatin resistance and migration in A549 cells

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Macrophages have been known to promote malignant behaviors of tumor by affecting tumor development, progression, metastasis, and invasion. In this study, we investigate whether cisplatin resistance and migration of cancer cells according to the various types of cytokines secreted from macrophage. First we analyze the differences of secreted cytokines between monocyte and macrophages and then selected CCL21, CXCL1, CXCL2, and CXCL3. To investigate the effect of CCL21, A549 cells were induced selective suppression using siRNA of CCR7 (CCL21 receptor) and then cisplatin resistance or cell migration was observed. RNAi-mediated knockdown of CCR7 was decreased the effect induced by macrophages. However, treatment of CCL21 was not induced cisplatin resistance and migration. CXCL1, CXCL2 and CXCL3 bind to the CXCR2 receptor and regulate cellular mechanisms. Therefore, we inhibited the activity of CXCR2 by antagonist or siRNA. Cisplatin resistance and migration were decreased after treatment of CXCR2 antagonist (SB265610) and RNAi-mediated knockdown of CXCR2. But single treatment of CXCL1, CXCL2 and CXCL3 did not induce cisplatin resistance and migration. The reason for not showing the effect is that the single treatment of cytokine is not enough. From these results, we can conclude that increased cisplatin resistance and migration by macrophages is likely due to CXCL1, CXCL2, CXCL3, and CCL21.

Key Words: A549, Cisplatin, Cytokine, Macrophage, Migration

P9-30

The role of TREK1 in cancer cell epithelial-mesenchymal transition

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The expression of the two-pore domain mechanosensitive potassium channel, TREK1, correlates with the grade and stage of cancer, suggesting that TREK1 is a novel therapeutic molecule target in cancer cells. Activation of epithelial mesenchymal transition (EMT) is known to be involved in invasion, recurrence, and chemotherapy drug resistance, but no association between TREK1 and EMT has been reported. Here we investigated whether TREK1 is associated with EMT development because it could disrupt cell homeostasis by altering cell membrane potential. We have shown that cell membrane potential is depolarized inTREK1 siRNA transfected cancer cells. Also we observed changes in vimentin, E-cadherin and N-cadherin as EMT markers using human TREK1 small interfering RNA (TREK1 siRNA) and rat TREK1 plasmid DNA (rTREK1p) to determine whether TREK1 contributes to EMT regulation. The level of mRNA of cells transfected with TREK1 siRNA or rTREK1p alone and cells treated with negative control siRNA (NC siRNA) was observed using real-time RT-PCR. Cells transfected twice over 5 days of TREK1 siRNA showed an increase in vimentin and N-cadherin compared to negative control siRNA, whereas vimentin, a mesechymal marker, decreased in rTREK1p overexpressing cells compared to NC siRNA cells. The epithelial marker E-cadherin was slightly reduced in cells transfected with TREK1 siRNA. Real-time RT-PCR analysis showed that TREK1 siRNA transfecting cells increased the binding homeobox transcription factor (Zeb1) and slug compared to NC siRNA cells, and this factor was restored by rTREK1p overexpressing cells. Western blot and immunocytochemistry results were also similar to real-time RT-PCR results. In the TREK1 siRNA treated cells, vimentin and N-cadherin were increased but E-cadherin was slightly decreased. Nuclear slug and ZEB1 protein were increased in TREK1 siRNA transfected cells. Taken together, these results suggest that TREK1 is involved in the EMT process of cancer cells

Key Words: Epithelial-mesenchymal transition, TREK1, Small interfering RNA, E-cadherin, Vimentin

P9-31

Molecular target of nobiletin in rotenone-induced mitochondrial dysfunction and apoptosis

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We recently reported that nobiletin, a polymethoxylated flavone from *Citrus sunki* Hort. ex Tanaka, significantly protects primary cortical neurons against glutamate toxicity by reducing neurotoxic mitochondrial Ca^{2+} overload through mitochondrial K^+ channels-mediated partial mitochondrial depolarization. In this study, we explored other possible mitochondrial molecular targets of nobiletin on the neuroprotective action. The results demonstrated that nobiletin significantly reduced a complex I inhibitor rotenone-induced mitochondrial ROS and neuronal cell death. In contrast, nobiletin did not reduce a complex III inhibitor antimycin A-induced mitochondrial ROS and neuronal cell death in both primary cortical neurons and pure isolated brain mitochondria from Sprague-Dawley rats. In addition, nobiletin significantly increased ATP production in dose-dependent manner. The results may suggest that nobiletin regulates rotenone-induced mitochondrial dysfunction through electron transport system (ETC) complex 1, although the action mechanism is not fully understood yet. Taken together, we propose that nobiletin may be a promising neuroprotective agent against neurodegenerative diseases and neuroinflammation regulating through not only mitochondrial K^+ channels but also mitochondrial ETC complex I.

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Key Words: Mitochondria, Complex I, Rotenone, ROS, Cell death, Nobiletin

P9-32 (PO-B-5)

Impairment of NHE6 recruitment to synaptic vesicle by SCAMP5 deficiency decreases quantal size at glutamatergic synapses

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The quantal size of synaptic vesicle (SV) is regulated by a chemical gradient (ΔpH) and membrane potential (Δψ) generated by the vacuolar H+-ATPase. The relative roles of ΔpH and Δψ vary with the type of neurotransmitters, and uptake of glutamate is known to more depend on the electrical component of Δψ than ΔpH. Monovalent cation/H^+ exchanger plays an important role in establishing Δψ, and thus the proper sorting of this protein to SV is of utmost importance for regulating the quantal size of glutamate release, but the underlying mechanism that mediates the sorting of this protein to SV remains poorly understood. In this study, we demonstrated that sorting of (Na^+/K^+)/H^+ exchanger 6 (NHE6) to SV is regulated by its interaction with Secretory carrier membrane protein 5 (SCAMP5) at hippocampal excitatory synapses. We showed that the 2/3 loop domain of SCAMP5 interacts with the C-terminal region of NHE6 and depletion of endogenous SCAMP5 by shRNA or overexpression of 2/3 loop mutant hinders the sorting of NHE6 to SV in cultured rat hippocampal neurons. Using optical imaging with a fluorescent glutamate sensor iGluSnFR, we demonstrated that the amount of glutamate released spontaneously or by stimulation decreased with SCAMP5 knockdown (KD). This result was further corroborated by the electrophysiological recording in which SCAMP5 KD results in a decreased miniature excitatory postsynaptic current (mEPSC), thus supporting that disturbed localization of NHE6 to SVs reduces presynaptic quantal size. Together, our results suggest that SCAMP5 has a critical role in proper sorting of NHE6 to SVs and subsequent regulation of quantal size at glutamatergic synapses.

Key Words: NHE6, SCAMP5, Synaptic vesicle, Quantal size, Glutamatergic synapse

P9-33

TASK-5 two-pore domain K^+ channel controls sensitivity to H_2O_2 in MCF-7 and MDA-MB-231 breast cancer cells

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Little is known about physiological role of TWIK-related acid-sensitive K+ (TASK)-5 channel, because it 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, among TASK channels tested, mRNA expression was the highest in breast cancer cells (MCF-7 and MDA-MB-231), and it is predominantly expressed at the mitochondrial region. TASK-5 mRNA expression level was similar between MCF-7 and MDA-MB-231, but protein level was higher in MDA-MB-231 than those in MCF-7 cells. MCF-10A, a non-tumorigenic epithelial cell line, also expressed TASK-5 protein with similar expression level to that in MDA-MB-231. Treatment with hydrogen peroxide (H_2O_2), which induces cell death, increased TASK-5 mRNA and protein expression levels in a time- and dose-dependent manner. Compared to MCF-10A, MDA-MB-231 cells were less sensitive to H_2O_2, whereas MCF-7 cells showed hypersensitivity in response to H_2O_2 treatment. In addition, treatment with H_2O_2 increased VEGF expression levels, and the VEGF expression levels increased in cells overexpressed with TASK-5. In MCF-7 cells overexpressed with TASK-5, H_2O_2 induced low cell death compared to vector transfected cells. Overexpression of TASK-5 in breast cancer cells significantly increased proliferation, migration, and invasion compared to vector-transfected cells. These results show that TASK-5 expression levels control sensitivity to H_2O_2 in breast cancer cells. In addition, high TASK-5 expression level could increase proliferation, migration, and invasion through regulation of VEGF.

Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF-2015R1A-5A2-008833, NRF-2018R1A2B6001446) grant funded by the Korea government.

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

P9-34

Inhibition of autophagy promoted Rk1 induced apoptosis of neuroblastoma through the inhibition of autophagosome-lysosome fusion

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Autophagy can result in cellular adaptation, as well as cell survival or cell death. Modulation of autophagy has been increasing regarded as a promising cancer therapeutic approach. In this study, we screened several ginsenosides extracted from *Panax ginseng* and showed that Rk1 inhibit late stage autophagy (autophagosome and lysosome), possibly through changes in autophagy regulator protein expression. Rk1 treatment dose dependently increased GFP-LC3 puncta formation and expression of SQSTM1 in neuroblastoma cells. Also, Autophagy flux inhibitor, chloroquine treatment fur-

ther enhanced the effect of Rk1 induced apoptosis. These results suggest that minor ginsenosides Rk1 is a novel autophagy inhibitor and could function as a potent anti-cancer agent, and that combination therapy with classical chemotherapeutic drugs might be promising compounds to have therapeutic effect on neuroblastoma cell lines.

Key Words: Neuroblastoma, Ginsenosides Rk1, Apoptosis, Autophagy

P9-35

Downregulation of survivin inhibits epithelial mesenchymal transition (EMT) and promotes RK1-induced apoptosis in neuroblastoma cells

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Neuroblastoma is a solid tumor that occurs mainly in children. Malignant neuroblastoma has poor prognosis because existing chemotherapeutic drugs are not effective. The epithelial-mesenchymal transition (EMT) is an important factor in neuroblastoma metastasis, which targeting EMT is a potential therapeutic strategy. Survivin was abnormally elevated in many cancers. In this study, we investigated minor ginsenoside Rk1 inhibits EMT and invasion in neuroblastoma cell lines and the relevance of survivin. We found that Rk1 treatment (0, 10, 20, and 30 μM) inhibited cell migration and invasion by wound-healing and transwell assays. Rk1 significantly altered EMT marker proteins with increased E-cadherin, but decreased Snail, N-cadherin, Slug and Vimentin expression. Rk1 also down-regulated survivin gene and protein expression in neuroblastoma cells by qPCR, Western blot and immunofluorescence. After survivin down-regulated with siRNA, Rk1 induced apoptosis was obviously promoted. RNA interference of survivin was found to be a potent inhibitor of neuroblastoma cells growth and metastasis formation. Also, Rk1 inhibits EMT and invasion of neuroblastoma by down-regulating survivin. These result suggest that combination therapy with Rk1 and knockdown of survivin may be a potentially effective agent for the treatment of neuroblastoma

Key Words: Neuroblastoma, Ginsenoside Rk1, Survivin, EMT, SiRNA

P9-36

Survivin knockdown increased anti-cancer effect of CK in human malignant neuroblastoma cells

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Neuroblastoma is a solid tumor that is found mostly in children. Malignant neuroblastoma has poor prognosis because conventional chemotherapeutic agents are hardly effective. Survivin is a member of the inhibitor of apoptosis family proteins and is involved in tumor cell survival and Invasion, and differentiation. Survivin is highly upregulated in neuroblastoma and correlated with poor prognosis. This study, we examined consequence of survivin knockdown by siRNA survivin and then treatment with ginsenoside CK in neuroblastoma cells. We found that CK treatment (0, 2, 5 and 10 μM) inhibited cell migration and invasion by wound-healing and transwell assays. CK also down-regulated survivin gene and protein expression in neuroblastoma cells by qPCR, Western blot and immunofluorescence. Malignant neuroblastoma SK-N-BE (2) and SH-SY5Y cells were highly expressed of survivin protein. We transfected neuroblastoma cell with siRNA survivin, treated with CK, and confirmed knockdown of survivin mRNA and protein levels. Survivin knockdown and CK treatment of neuroblastoma cells induced morphological features of neuronal differentiation and apoptotic cell death. Combination of survivin siRNA and CK promoted neuronal differentiation biochemically by increase in expression of NSE and E-cadherin and also decreases in expression of Notch-1 and PCNA. Also, combination therapy was highly effective inducing, respectively, morphological and biochemical feature of apoptosis. Collectively, combination therapy with Rk1 and knockdown of survivin may be a potentially effective agent for the treatment of neuroblastoma.

Key Words: Neuroblastoma, CK, Survivin, Apoptosis

P10-01

Administration of Banhasasim-tang (BHSST) ameliorates irritable bowel syndrome-like symptoms through TRPA1 or NaV 1.7 channels in a zymosan-induced mouse model

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Irritable bowel syndrome (IBS) is a functional bowel disease in which recurrent abdominal pain is associated with defecation or a change in bowel habits. To assess the effects of Banhasasim-tang (BHSST) on IBS, we used a mouse model of colonic zymosan injection presenting with diarrhea-predominant IBS-like symptoms. Oral BHSST administration restored colon length and weight change, and minimized body weight loss without affecting food intake. In BHSST-treated mice, the submucosal thickening and epithelial lining of the colon were inhibited and were similar to those of naive mice. Also, serum tumor necrosis factor-alpha levels were markedly suppressed. These effects were comparable to those of sulfasalazine (SSZ) or amitriptyline (AMT), an anti-inflammatory drug. Furthermore, BHSST inhibited transient receptor potential ankyrin 1 (TRPA1) or voltage gated Na^+ (NaV) 1.7 channels, which was involved in visceral nociception in IBS. Taken together, these data suggest that BHSST may have potential as a medicinal food for IBS by acting TRPA1 or NaV 1.7 channels.

Key Words: Irritable bowel syndrome, Banhasasim-tang, Transient receptor potential channel, voltage gated Na+ channels

P10-02

Linalyl acetate mitigates the pulmonary endothelial dysfunction in a rat model of COPD with hypertension

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Endothelial dysfunction is an early, reversible step in the pathogenesis of cardiovascular and respiratory diseases. Improving endothelial function is considered an effective, preventive strategy. Here, we investigated pulmonary endothelial dysfunction in a newly developed rat model of chronic obstructive pulmonary disease (COPD) with hypertension. Linalyl acetate (LA), a natural compound well known for its anti-inflammatory and anti-hypertensive effects, prevented supersensitivity to SNP in the pulmonary artery and decreased blood pressure. Increased levels of pro-inflammatory mediators and enlargement of alveolar airspaces were also alleviated by treatment with LA. We found that vasorelaxation induced by the nitrovasodilator, sodium nitroprusside (SNP), was abnormally increased in the pulmonary artery, but not in the aorta. Moreover, endothelial nitric oxide synthase expression was significantly downregulated resulting in a serum nitrite deficiency. Together, our results demonstrate that the pulmonary endothelium is vulnerable in the condition of COPD with hypertension, and suggest the potential of LA in treating pulmonary endothelial dysfunction through its endothelium-protective effects.

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Key Words: COPD, Hypertension, Pulmonary endothelial dysfunction, Supersensitivity, Nitrovasodilator, Linalyl acetate

P10-03

Lancemaside A from *Codonopsis lanceolata* prevents hypertension by inhibiting NADPH oxidase 2-mediated oxidative stress in hypertensive rats

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Codonopsis lanceolata (CL) has been used as a traditional medicine due to its anti-obesity, anti-cancer, memory improvement, and anti-inflammatory properties. CL was recently reported to have anti-hypertensive effects. Lancemaside A (LMA) is a representative triterpenoid saponin which is main constituent of CL. LMA was found to have beneficial effects, reducing inflammation and oxidative stress, and mitigating memory impairment. To date, however, no studies have evaluated the ability of LMA to prevent the hypertension. This study investigated the anti-hypertensive properties of LMA, as well as assessing their mechanisms of action in hypertensive rats.
Hypertensive rats were orally treated with LMA (1, 20, or 40 mg/kg) or nifedipine (10 mg/kg) as a positive control daily for 3 weeks. Blood pressure and heart rate were measured by volume pressure transducer, and myograph study was conducted to measure vascular tone. Protein levels were measured by western blot.
In hypertensive rats, LMA dose-dependently reduced systolic blood pressure, but did not significantly alter vascular tone. LMA doses of 20 mg/kg and 40 mg/kg significantly reduced the expression of endothelial nitric oxide synthase, nuclear factor kappa B, and NADPH oxidase 2 (NOX2) in the aorta, and 40 mg/kg LMA significantly reduced serum total protein and malondialdehyde concentrations. These effects were similar to those in normotensive rats.
These findings indicate that LMA prevents hypertension by inhibiting NOX2-mediated oxidative stress. LMA may act as a preventive agent for hypertension. This research was supported by KFS-2016005C10-1719-AB01 and NRF-2018R1D1A1B07050048.

Key Words: Lancemaside A, Anti-hypertension, NADPH oxidase, Oxidative stress, Nuclear factor kappa B; Endothelial nitric oxide synthase

P10-04

Exercise training attenuates long-term high-fat diet-induced impairment of mitochondrial structure and function in mice skeletal muscle.

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Obesity induces various chronic diseases associated with mitochondrial impairment. Given exercise training positively affects skeletal muscle and mitochondrial structure and function, exercise training could be an important regulator of obesity-induced alterations of skeletal muscle and mitochondria. However, the effects of high-fat diet-induced obesity and exercise training on mitochondrial structure, function, and mitochondria-mediated apoptosis in skeletal muscle have not been sufficiently elucidated. Thus, we examined whether exercise training affects high-fat feeding-induced alterations of skeletal muscle and mitochondrial structure, function, and mitochondria-mediated apoptosis in skeletal muscle. Following obesity was induced by 20 weeks of 60% high-fat diet (HFD) in male C57BL/6 mice, treadmill exercise training (EX) was performed at 13~16 m/min, 40~50 min/day, 6 days/week for 12 weeks. And then, skeletal muscle and mitochondrial morphology, mitochondrial function, mitochondrial dynamics, mitophagy, and mitochondria-mediated apoptosis were analyzed in red gastrocnemius (combined type I and II fiber). Exercise training for 12 weeks attenuated obesity-induced mitochondrial dysfunction in permeabilized myofibers, indicating that reduction of mitochondrial O2 respiration and Ca2+ retention capacity was improved and elevation in mitochondrial H2O2 emission by obesity was reduced in HFD + EX compared with HFD. In addition, exercise training also ameliorated obesity-induced imbalance of mitochondrial dynamics, demonstrating that obesity-induced decrease in fusion (Mfn2, Opa1) protein levels were elevated (41% and 121%, respectively) and conversely, increase in fission (Drp1, Fis1) protein levels were reduced in HFD + EX compared with HFD (40% and 68%, respectively). Dysregulation of mitophagy by obesity was mitigated in HFD + EX with reductions in PINK1 protein level (- 250%). Pro-apoptotic markers, Bax (- 35%), mPTP opening (- 44%), cytochrome c (- 23%), and cleaved caspase-3 were reduced and apoptosis (TUNEL positive myonuclei) was ameliorated in HFD + EX compared with HFD. In conclusion, the current study strongly demonstrated that obesity-induced impairments of skeletal muscle and mitochondrial structure, function, and mitochondria-mediated apoptosis were attenuated by aerobic exercise training, suggesting that exercise training as an effective intervention plays a therapeutic role in protecting against obesity-induced impairments of skeletal muscle and mitochondria.

Key Words: Exercise, Obesity, Mitochondrial function, Mitochondrial structure, Skeletal muscle

P10-05

Seasonal effect on resting energy expenditure is age and percent body fat dependent

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The seasonal variation of resting energy expenditure (REE) is still under debate. This study aims to examine the seasonal changes in REE and its relevant factors among Korean adults.
A total of 867 healthy volunteers (385 men and 482 women) aged 20-69 years were split into four seasonal groups and subgroups of age intervals, body mass index (BMI), and percent of body fat (PBF) quartiles. REE, body composition, glucose metabolism, thyroid hormones, and catecholamines have been strictly measured.
Seasonal factor contributed to the calculation of REE independently to anthropometric indices with the additional variation reduced from 6 to 2% among younger and older persons. Adjusted REE in the winter was 5.4-13.9%, 7.8-14.3%, and 8.6-11.9% higher than that in the summer in every age, BMI, and PBF subgroups, respectively. T3 and log-transformed norepinephrine (NE_{log}) were higher whereas log-transformed epinephrine (EPI_{log}) was lower in the winter compared to that in the summer. The magnitude of winter-summer difference in REE and T3 and of summer-winter difference in EPI_{log} reduced 3 folds between the lowest and highest intervals of age and PBF, whereas the difference in NE_{log} was constant across age and PBF intervals. No typical change in the seasonal difference in REE and its relevant biomarkers across BMI intervals.
In summary, the season was an independent predictor of REE and its effect was attenuated by the increment of age and PBF, but not BMI.

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Key Words: REE, EPI, BMI, PBF, Korean

P10-06

Aerobic exercise training decreases cereblon and increases AMPK signaling in the skeletal muscle of STZ-induced diabetic rats

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Cereblon (CRBN) has been reported as a negative regulator of adenosine monophosphate-activated protein kinase (AMPK). Aerobic exercise training has been shown to increase AMPK, which resulted in glucose regulation in skeletal muscle. However, the expression level of CRBN and its association with the physiological modulation of glucose are still unclear. Male Sprague-Dawley rats (5-week-old, n = 18) were assigned to control, streptozotocin (STZ, 65 mg/kg)-induced diabetic group, and STZ + exercise (STZ + EXE) group with six rats in each group. Rats in the STZ + EXE group exercised by treadmill running (20 m/min, 60 min, 4 times/week) for 8 weeks. Compared with the STZ group, blood glucose was significantly decreased in the STZ + EXE group. The skeletal muscle of rats in the STZ + EXE group showed a significant decrease in CRBN levels and an increase in AMPK, protein kinase B, peroxisome proliferator-activated receptor gamma coactivator 1-alpha, fibronectin type III domain-containing protein 5, glucose transporter type 4, superoxide dismutase 1, and uncoupling protein 3 levels. These results suggest that CRBN is a potential regulator of glucose homeostasis in the skeletal muscle. Moreover, our results suggest that aerobic exercise training may provide an important physiological treatment for type 1 diabetes by decreasing CRBN and increasing AMPK signaling in skeletal muscle.

Acknowledgement: This work was supported by the National Research Foundation of Korea, and the funding was granted by the Ministry of Education of Korea (2010-0020224).

Key Words: Aerobic exercise, CRBN, AMPK, Type 1 diabetes, Skeletal muscle, FNDC5

P10-07

Resistance exercise improves mitochondrial function to rescue OLETF rats hearts

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Diabetic cardiomyopathy (DC) is a hallmark complication of long-standing hyperglycemia caused by various metabolic and mitochondrial disturbances. Physical activity such as exercise not only enhances the condition of healthy individuals but could also improve the status of those with disease. However, the beneficial effects of resistance exercise (RE) in the prevention of DC and cardiac mitochondrial dysfunction are uncertain. Therefore, this study investigated whether RE attenuates DC by improving mitochondrial function using an in vivo rat model of diabetes. Fourteen Otsuka Long-Evans Tokushima Fatty rats were assigned to sedentary control (SC, n=7) and RE (n=7) groups at 28 weeks of age. Long-Evans Tokushima Otsuka rats were used as the non-diabetic control. The RE rats were trained by 20 repetitions of climbing a ladder 5 days per week. The RE rats exhibited higher glucose uptake and lower lipid profiles, indicating enhanced energy metabolism. RE significantly increased the ejection fraction and fractional shortening compared with the SC rats. The RE rats had more cardiac mitochondria, which increased mitochondrial biogenesis via higher expression of PGC-1α and TFAM. RE reduced proton leakage and reactive oxygen species production, with increased membrane potential. These results were accompanied by higher SOD2 and lower UCP2 and UCP3 levels in the RE group. These data suggest that RE is effective at ameliorating DC by improving mitochondrial function, which may contribute to the maintenance of diabetic cardiac contractility.

Key Words: Diabetic cardiomyopathy, Resistance exercise, Cardiac function, Mitochondrial function

P10-08

Nano-LC-ESI-MS/MS reveals circadian modulation of the cardiac proteome underpins differential adaptation to morning or evening exercise training

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Circadian related changes in the cardiac proteome may underlie differential cardiac adaptation to morning or evening exercise training. Global proteome changes underpin adaptations to exercise training, which is a potent stimulus to improve cardiovascular health, but the impact of time-of-day on exercise-induced cardiac adaptation is unknown. The aim of the present study was to determine the effect of morning or evening exercise training on the cardiac proteome and cardiovascular adaptations. Eight weeks old Sprague Dawley rats underwent either morning (ME) or evening exercise (EE) with treadmill running, 60 min/day, 5 days/week for twelve weeks compared to non-exercise trained controls (MC and EC). Differences in body weight, organ weight and cardiac function were assessed. Nano-LC-ESI-MS/MS was used for quantification of differences in the global cardiac proteome. Exercise training decreased body weight, but cardiac mass was not significantly different between groups trained in the morning or evening. Stroke volume and cardiac output were significantly higher in the morning compared to evening exercise trained rats ($p<0.05$). Global proteomics identified 1647 proteins in the heart. Of these 194 proteins showed circadian regulation. We identified 826 proteins that were commonly or divergently modulated by exercise training regardless of time of day. However, 278 and 188 proteins were modulated only by morning exercise training or only by evening exercise training respectively. Ingenuity Pathway Analysis revealed differentially modulated proteins were involved a range of molecular pathways including mitochondrial dysfunction, oxidative phosphorylation, and calcium signaling. In conclusion, cardiac adaptations appear to be greater in response to morning rather than evening exercise training.

Key Words: Exercise, Cardiac function, LC-MS, Proteomics, Heart, Circadian

P10-09

Plasma catecholamine and physical activity levels in Korean elderly people with orthostatic hypotension

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Purpose: Orthostatic hypotension (OH) is defined as a drop of ≥20 mmHg or ≥10 mmHg in systolic or diastolic blood pressure (BP), respectively, within 3 minutes of standing. OH is apparent as a result of autonomic nervous system dysfunction. However, few studies have directly examined the association between catecholamine and physical activity levels related to OH. This study aimed to identify differences in catecholamine (Epinephrine and Norepinephrine) and physical activity levels among Korean elderly with and without OH. In a cross-sectional study, 217 elderly people were recruited

in a South Korean city. Convenience sampling methods were employed, and data were collected using (1) participant blood samples analyzed for plasma catecholamine level and (2) K-PASE questionnaire to assess physical activity level. Using SPSS for Windows version 21.0, data were analyzed by descriptive analysis, χ^2-tests, t-tests, and Pearson's correlation tests. The plasma Norepinephrine level was significantly higher in the OH group than in the non-OH group (t=-2.298, p=.023). In addition, physical activity level was significantly lower in the elderly with OH than without OH (t=2.735, p=.007). **Conclusion:** These results showed that the mechanism for OH may be associated with plasma catecholamine and physical activity levels. These findings provide further understanding of the OH phenomena and highlight the need for interventions that address elderly people with OH.

Acknowledgement: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1D1A3B03934143).

Key Words: Catecholamine, Physical activity, Orthostatic hypotension, Elderly

P10-10

Effect of exercise training on vascular reactivity in high fat diet induced hypertensive rats

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Obesity and hypertension are complex diseases that share the similar risk factors such as physical inactivity and increase the intake of high fat diet. The purpose of our study was to investigate the effects of daily exercise training on high-fat diet induced vascular reactivity in systemic arteries. Sprague Dawley rats were randomly assigned to the following groups: 1) control diet (Control), 2) control diet with exercise training (Control-E), 3) high-fat diet (HFD), and 4) high-fat diet with exercise training (HFD-E) for 16 weeks. Blood pressure and body weight were monitored weekly. At the final weeks, the isometric tension was measured using multiwire myograph technique from isolated mesenteric (MA) and deep femoral arteries (DFA). The dose-dependent concentration of phenylephrine (PhE) and acetylcholine (ACh) (0.05-10 μM) were applied. Data were analyzed with student t-test or ANOVA. After 16 weeks, there were no significant differences in body weight among the groups. The mean arterial pressure and heart rate of HFD and HFD-E rats were increased compared to control and control-E groups. The contractile response to PhE-induced vasoconstriction were enhanced in MAs and DFAs from HFD rats compared to control. PhE-induced vasocontraction remained higher in MAs and DFAs from HFD-E group compared to control. The maximal contraction of PhE were decreased in MAs but not in DFAs from control-E groups compared to control. Furthermore, there was no difference of endothelium-dependent relaxation in MAs from HFD and HFD-E compared to control groups. However, the enhancement of endothelium-dependent relaxation were observed in Control-E compared to control rats. Our study demonstrated that high-fat diet induced hypertension might associate with the enhancement of alpha-adrenergic receptors expression in systemic arteries. Daily exercise training could not prevent high-fat diet induced vascular dysfunction. We conclude that exercise training may not be effective in enhancing vascular relaxation in mesenteric and femoral arteries of rats fed high fat diets.

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Key Words: High-fat diet, Mean arterial pressure, Exercise training, Vascular reactivity, Systemic arteries

P11-01

Deep neural network-based classifiers to detect experimental seizures

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Manually reviewing electroencephalograms (EEGs) is labor-intensive and demands automated seizure detection systems. To construct an efficient and robust event detector for experimental seizures from continuous EEG monitoring, we applied many different models of deep neural networks on experimental EEGs. The pilocarpine-induced status epilepticus model was generated by i.p. injection of pilocarpine hydrochloride. EEG monitoring was conducted with stereotaxically implanted epidural recording electrodes for 2 weeks between 4 and 7 weeks after pilocarpine injection. Mice underwent continuous monitoring by a wireless video/EEG monitoring system. Convulsive seizures were defined by repetitive epileptiform spiking (≥ 3 Hz) that persisted for more than 3 seconds and was confirmed by video recordings. Seizure activity was marked at the beginning and end of each event by human inspectors to train deep neural networks. Total 4704 hours of EEGs were used as training set and 4272 hours of EEGs were used as test set. The 5-second EEG data segments were converted to periodograms, images of periodograms, or images of EEG itself for fully connected deep neural network, convolutional neural network (CNN), or CNN, respectively. Each segment was processed alone or three 2-minutes separated segments were processed together by recurrent neural network (RNN). True positive rate was 100% regardless of model. Positive predictive value was also 100% except for simple models using only periodogram. Even simple model showed only a < 0.0004% false detection time. Furthermore, scanning and classifying 8977 hours of training and test EEG datasets took only 2 to 4 hours with a personal computer. These results demonstrate that deep neural networks can classify convulsive seizures with great accuracy and low computational burden, highlighting the feasibility of our automated seizure detection algorithm.

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Key Words: Seizure, EEG, Deep learning, Neural network

P11-02

Teaching cardiac excitation-contraction coupling using a mathematical computer simulation of human ventricular myocyte

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Understanding the excitation-contraction (E-C) coupling of cardiomyocytes along with the electrophysiological mechanisms of their characteristic long action potential duration (APD) is one of the important learning goals in physiology and in the pathophysiology of heart failure. Although the students could enumerate the processes of E-C coupling, the integrative interpretation of the responses simultaneously occurring in the actual situation of contraction-relaxation cycle is generally far more difficult due to dynamic interaction of underlying factor. To achieve more efficient as well as scientifically sound understanding of cardiac cellular physiology, we adopted the mathematical computer simulation model of human ventricular myocytes (cardiac E-C_Sim). Here, we describe the mathematical model and computer program about E-C coupling and the overall process which used in physiology course. We also report on the survey results from the questionnaires answered by the students. Through the analysis we could understand how the students had approached the problem-solving and reached the teaching goals through constructive discussions on the unique dynamic cellular

phenomena of the cardiac E-C coupling.

Key Words: Medical education, Excitation-contraction coupling, Computer simulation, Mathematical model

P12-01

Development of multi-functional theragnostic magneto-ceria nanoparticles for cancer radiotherapy

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Radiotherapy is still a promising therapy to treat head and neck cancer. However radiation may severely harm normal tissues such as salivary glands adjacent to the tumor mass. Salivary glands damaged by radiation gradually lose their functions and normal structural characteristics.
In this study we developed multi-functional theragnostic hetero-nanocubes that can radio-protect normal tissues while radio-sensitize cancer tissues. This nanocube has FION magnetic core covered with mesoporous silica-ceria oxide conjugates (FION-CeNP). Ceria oxide nanoparticles are widely known as autocatalytic reactive oxygen species (ROS) scavengers. FION-CeNPs were again coated with 25kDa Polyethyleneimine (PEI) and cell-penetrating peptide (TAT) to deliver functionally restorative genes such as Aquaporin 5 to target tissues. Fully synthesized FION-CeNPs showed 50nm hydrodynamic size with +50mV zeta potential. Radio-protective ability of FION-CeNPs was tested by using ex vivo embryonic submandibular salivary glands (eSMG) 48hrs after 10Gy irradiation. FION-CeNP-pretreated eSMGs showed 1.5 fold higher bud number and significantly lowered apoptotic rate of ckit/AQP-5+ pro-acinar cells and parasympathetic ganglion than untreated group. Extra-dermally generated magnetic field enhanced uptake and gene delivery efficiency of FION-CeNP to mouse submandibular glands for 4 folds compared to conventional PEI molecules. These combined effects of gene delivery and ROS scavenging successfully rescued salivary flow and histological characteristics of adult mouse submandibular glands from damages caused by 20Gy of irradiation. In addition, highly concentrated magnetic field could induce aggregation of FION-CeNPs, which deactivated FION-CeNPs' ROS scavenging ability with increased immuno-toxicity and radio-sensitivity. By locally manipulating strength and shape of magnetic fields FION-CeNPs can act as radio-protector or radio-sensitizer. In CT26 xenograft mouse model FION-CeNP-pretreatment with magnetic agglomeration resulted 60% decrease in tumor size with significantly increased cleaved caspase 3 18days after the 5 times of 2Gy irradiation. Also FION-CeNPs showed great potentials as diagnostic materials. FION-CeNP showed high r2 relaxivity (760/mM s) in 3T MR scanner. FION-CeNPs showed high signal to noise ratio when subjected to ultrasound imaging machine.
In conclusion, multi-functional FION-CeNPs have both therapeutic and diagnostic properties with enhanced gene delivery and ROS scavenging ability.

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Key Words: Nanoparticle, Radiation-induced xerostomia, Salivary gland, Oropharyngeal cancer

P12-02

Corylifol C inhibits osteoclastogenesis by inhibition of ROS and induces downregulation of c-Src in osteoclast

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An excessive increase in osteoclast differentiation and bone resorption gives rise to various bone-resorptive diseases. Lately, the study of anti-resorptive agents from natural compounds has become a topic of interest. Corylifol C is a compound isolated from the seeds of Psoralea corylifolia that has been used as a traditional medicine in Asia. Corylifol C has previously been shown to have weak antioxidative effects; however, its effect on osteoclast differentiation and bone resorption remains unclear. In this study, we investigated the effects of Corylifol C on osteoclast differentiation and bone resorption. Corylifol C dose-dependently inhibited RANKL-induced osteoclast differentiation from 5uM. It is evaluated on bone marrow-derived monocytes (BMMs) by a tartrate-resistant acid phosphatase (TRAP) staining and TRAP activity assay. Expression of RANKL-induced osteoclastogenesis-related marker genes including acid phosphatase 5(ACP5), matrix metalloproteinase-9(MMP-9), dendritic cell-specific transmembrane protein(DC-STAMP), d2 isoform of vacuolar (H+) ATPase V0 domain(Atp6v0d2), cathepsin K(Ctsk), chloride channel 7(CLCN7) and nuclear factor of activated T-cells 1(NFATc1) was inhibited by Corylifol C treatment. The inhibitory effect of Corylifol C on the expression of NFATc1 was also confirmed by Western blot analysis. Moreover, Corylifol C inhibits RANKL-induced bone resorption demonstrated by a bone resorption assay. The activation of c-Src by ROS are key steps in enhancing OC survival and differentiation. Corylifol C inhibited expression of c-Src protein and mRNA and decreased the generation of RANKL-mediated reactive oxygen species (ROS) in BMMs at 5uM. These findings suggest that Corylifol C inhibits osteoclast differentiation by inhibition of ROS and induces downregulation of c-Src in osteoclast. Our results revealed that Corylifol C could be a potential therapeutic agent of the treatment of bone-resorptive diseases. Further investigations are required to evaluate the effects of Corylifol C on pathologic osteoclast formation and bone erosion in vivo.

Key Words: Corylifol C, Osteoclast differentiation, Bone resorption, ROS, c-Src

P12-03

Sestrin 2 regulates osteoclast differentiation through interaction with p62 and TRAF6

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Sestrin 2(Sesn2), an autophagy inducer, interacts with p62 and Keap1 and prevents oxidative stress via Keap1-Nrf2 pathway. In addition, previous reports showed that p62 interacted with TRAF6 and leads to the activation of osteoclast differentiation. However, the role of Sesn2 in osteoclast differentiation is unknown. In the present work, we investigated the effects of Sesn2 in osteoclast differentiation with Sesn2 knock-out (Sesn2-/-) mice. The bone mass of Sesn2-/- mice increased more than that of wild type mice by μCT analysis. Also, the formation of multinuclear osteoclasts was inhibited in Sesn2-/- mice. Furthermore, NFATc1 and osteoclastogenesis - related gene expression were significantly diminished in Sesn2-/- mice during osteoclast differentiation. RANKL-induced TRAF6 downstream pathways were delayed in osteoclasts of Sesn2-/- mice. The interaction of p62 and TRAF6 also decreased in Sesn2-/- mice. These results suggest that Sesn2 regulates osteoclast differentiation via the interaction with p62 and TRAF6.

Key Words: Sestrin 2, Osteoclast, p62, TRAF6, RANKL

P12-04

Repurposed drugs for angiogenesis inhibitors using database analysis system

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Drug repositioning, which is the application of known drugs and compounds for treatment, has been recognized worldwide as a new drug development method in the pharmaceutical market for its cost savings and time-consuming process. As tumor cells are known to stimulate the growth of new vessels, drug repositioning to inhibit angiogenesis could be utilized to rediscover drugs that effectively inhibit the tumor cell proliferation. Through analysis with GEO2 Enrichr tool in GEO (Gene Expression Omnibus), an open gene expression database, the upregulated and downregulated genes in tumor angiogenesis experiments were identified. The top 50 drug candidates that counter those effects were rediscovered and listed in order by their 1-cos (α) values. We listed several drugs to relocate its effect on anti-tumor activity. Qualification is following: less cytotoxicity, faster cell migration and greater tube formation. From these candidate substances, already known angiogenesis inhibitors were excluded and the remaining candidate drugs with low side effects were further investigated. We analyzed the listed chemicals on cytotoxicity and angiogenesis. Six listed candidates (C, H, V, P, L, and J) with different concentrations (1 nM ~ 10 μM) were prepared. HUVECs at passage 3 were applied with each drug of the different concentrations. Cytotoxicity of the chemicals was measured using MTT and angiogenesis using tube formation and scratch wound assay. The effect of the candidates of the cell-viable concentrations on tube formation was examined. At nanomolar concentration, three chemicals had little effect on cell viability; however, they inhibited angiogenesis. The newly rediscovered angiogenesis inhibitors could be quickly applied in the medical field and used in clinic compared *de novo* drug development. However, these repositioned drugs must be tested *in vitro* and *in vivo* beforehand to fully assess their anti-angiogenic effects and potential side effects.

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Key Words: Drug repositioning, LINCS, Connectivity map, Angiogenesis

P12-05

FAK-integrin mediated cell migration by far-infrared in rat

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Far infrared (FIR) is a region in the infrared spectrum of electromagnetic radiation. The efficacy of FIR was well been reported as wound healing effect but signaling were not definitely confirmed yet. In recent studies, wound healing and skin blood flow increase through cell migration by FIR radiation. In other study show that integrin-ECM signals were affected with FAK-mediated cell migration. Cell migration incurred the wound healing effects by Integrin-extracellular matrix (ECM) signaling. Cell -migration and -adhesion were stimulated by FAK-integrin complex which in protein of transmembrane. However, poor understanding of the mechanisms. In this study, we made cloth which FIR radiated fabric then, nine rats were dressed by FIR fabric. The animals were divided into one control group and two experimental groups. Rat skeletal muscle tissue was used for Western blot, mRNA microarray. Also, wound healing assay via primary cell culture. To observe the integrin-mediated cell migration pathway, integrin-related genes were screening by mRNA microarray data. Also, we investigated protein expression with Western blot. We prove that variation of integrin-mediated cell migration enhanced rat skeletal muscle throughout FAK-integrin signaling pathway by FIR radiation. Our results show that integrin was significantly increased in microarray analysis and Western blot. FAK-Integrin mediated cell migration related genes as integrin α5, c-jun N-terminal kinase (JNKs), Vascular cell adhesion protein 1 (VCam1) and transforming growth factor beta 1 (tgfb1) were sorted in mRNA microarray analysis. Real-time PCR was performed to re-verified mRNA expressions that mRNA microarray analysis. Moreover, FIR affects Integrin and ECM located in cell membrane through FAK-related cell migration pathway by Western blot results which increased FAK, Paxillin, and collagen. Therefore, we demonstrate that the FIR radiation affects FAK-integrin mediated cell migration

Key Words: Cell migration, FIR, FAK, Integrin, Microarry

P12-06

Stimulation of platelet-derived growth factor mediated cell migration by far-infrared radiation in rat

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Far-infrared (FIR) radiation and vibration frequency characteristics of the human body allows FIR heat to penetrate deeper (2.5 cm) under the skin to the muscles, blood vessels, lymphatic glands and nerves than warmed air. Recently, studies show that FIR effects in blood circulation, skin microcirculation, promoted new vessels, wound healing, ROS reduce, NOS up-regulation. In the present study, we investigated the roles of platelet-derived growth factor (PDGF)-mediated cell migration by FIR radiation. We made rat cloth from conventional fabric for control group and difference percentage of FIR-radiated fabric for two experimental groups (10%, 30%). Nine 12-weeks old Sprague-Dawley rats were used and divided into one control group and two experimental groups. To observe the mRNA expression for microarray analysis, total RNA was extracted from each sample and prepared for microarray analysis by Affymetrix GeneChip® Rat Gene 2.0 ST Arrays. Our results indicate that PDGF-mediated cell migration pathway was up-regulated by FIR. In microarray analysis, between the control and 30% groups, 218 genes were significantly regulated. Not a few experimental evidences suggested the PDGF-mediated cell migration pathway was affected on FIR radiation, such as EGFR, integrin, actin, and collagen. However, in Western blot results of FAK, essential protein of PDGF-mediated cell migration pathway, did not change much. Our further study will focus on other genes on PDGF-mediated cell migration by 218 genes of mRNA expressions microarray data.

Key Words: PDGF, Cell migration, FIR, Microarray

P12-07

Physiological role of the murine bitter taste receptor *Tas2r108*

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The taste is closely related to the intake of food, and it is also influenced by the type of food ingested, nutrition and health status. The bitter taste of the five basic tastes plays an important role in the survival of humans and animals to avoid toxic and harmful substances. Mammals type 2 taste receptors (T2Rs) perceive bitter taste and the murine T2Rs consist of a family

of 35. We found that *Tas2r108* was expressed at the highest level in tongue papillae and exocrine glands. However, physiological functions of *Tas2r108* remain poorly be understood. Calcium imaging was employed to identify specific ligand of *Tas2r108* expressed in Chinese hamster ovary (CHO) -K1 cells. The transfected plasmids include the coding sequences of the first 45 amino acids of rat somatostatin receptor 3, which enhances expression in the cell membrane. The chimeric expression of G protein subunit Gα-16gust44 helps the CHO cells be activated by bitter taste compound such as quinine and cycloheximide, resulting in mobilization of intracellular calcium ions. *Tas2r108* full sequence cRNA probe would rule out the less specific signals in various tissues. The physiological roles of *Tas2r108* would be clarified by knock-out mice construction.

P12-08

Flos Magnoliae and its chemical constituent linoleic acid suppress CD4+ T lymphocyte activation *via* store-operated calcium entry

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Intracellular calcium signaling is crucial for type 2 helper T cell and mast cell activation, which is important for allergic inflammation. It is initiated by antigen-mediated receptor stimulation that trigger store-operated calcium entry (SOCE) via ORAI1 calcium channel. *Flos Magnoliae* (FM) is widely used for the treatment of allergic diseases such as allergic rhinitis and asthma. Although FM has an immune cell suppression effect, whether it can modulate calcium signaling in immune cells is unclear. Anti-allergic effects of FM might result from the inhibition of SOCE in T cells. We investigated whether FM constituents inhibit SOCE. A 70% ethanolic extract of FM (FM_{EtOH}) and four other fractions were prepared [(water (FM_{H2O}), butanol (FM_{BuOH}), ethylacetate (FM_{EtOAc}), and hexane (FM_{Hex})]. To elucidate whether FM and its constituents can inhibit SOCE, a conventional whole-cell patch clamp study was performed in hSTIM1 and hORAI1-overexpressing HEK293T cells (HEK_{ORAI1}). Intracellular calcium concentration was determined by Fura-2 dye and cytokine production measurement in Jurkat T lymphocytes. FM_{EtOH} (0.03 mg/mL) and its fractions, especially FM_{Hex} (0.01 mg/mL), significantly inhibited SOCE and IL-2 cytokine production in Jurkat T lymphocytes; SOCE and inhibition of interleukin (IL)-2 production rate were similar for all fractions, except for FM_{EtOAc}. GC/MS analysis showed that the major component of FM_{Hex} was linoleic acid (LA). FM_{Hex} at 0.01 mg/mL (which was equivalent to 10 μM LA) not only inhibited SOCE but also IL-2 production in Jurkat T lymphocytes. Moreover, it inhibited calcium signaling induced by CD3 receptor stimulation in Jurkat T lymphocytes. In conclusion, FM_{EtOH} and its chemical constituent LA suppressed CD4+ T lymphocyte activation, at least in part, by inhibiting I_{SOCE}. Thus, the inhibition of I_{SOCE} may be a potential strategy to inhibit immune responses in inflammatory conditions.

Key Words: *Flos magnoliae*, Store-operated calcium entry, Interleukin-2, T lymphocytes

P12-09

Flos Magnoliae modulates chloride secretion *via* ANO1 inhibition in airway epithelial cells

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Flos Magnoliae (FM, Chinese name: Xin-yi) is an oriental medicinal herb commonly used for symptomatic relief from allergic rhinitis, sinusitis, and headache. FM has also been used in traditional Chinese and Korean medicine formulations. It has been reported to inhibit histamine release from mast cells and cytokine secretion from T cells. However, the mechanism of action of FM on anoctamin-1 (ANO1) ion channel, which is responsible for nasal hypersecretion in allergic rhinitis, has not been elucidated. Therefore, we investigated whether FM and its chemical constituents can regulate the activity of ANO1. A 30% ethanolic extract of FM (FM_{EtOH}) was prepared, and five major constituents of FM_{EtOH} were identified. By using a conventional whole-cell patch clamp, we revealed that FM_{EtOH} (30, 100, and 300 μg/mL) and its chemical constituent tiliroside inhibited ANO1 activity in ANO1-overexpressing HEK293T cells. In addition, we showed that the treatment of airway epithelial cell line Calu-3 with interleukin 4 significantly increased ANO1 current (I_{ANO1}), but not cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride current (I_{CFTR}). FM_{EtOH} and tiliroside specifically modulated IANO1. In this study, we identified a novel mechanism underlying the alleviation of allergic rhinitis by FM_{EtOH}. FM_{EtOH} and its chemical constituent tiliroside can be potent agents for the prevention and treatment of allergic rhinitis.

Key Words: *Flos magnoliae*, Tiliroside, Allergic rhinitis, Hypersecretion, ANO1, Calcium-activated chloride channel, Anti-allergic effect

P12-10

Cardioprotection in ischemic/reperfusion heart injury conferred by natural pyridine nucleoside NPS A

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Sponges belonging to the genus Neopetrosia contain diverse bioactive metabolites. In a previous study, we observed that Neopetroside A (NPS A), a natural pyridine nucleoside that contains α-glycoside bond, could upregulate mitochondrial functions without cytotoxicity. In this study, we examined the physiological effects of NPS A on mitochondrial metabolism and heart function and its role in cardioprotection against ischemia/reperfusion (I/R) injury. NPS A reduced *ex vivo* I/R-induced damage in hearts of 8-week-old male Sprague Dawley rats by preserving hemodynamics and mitochondrial respiration capacity. In an *in vivo* model, NPS A also exhibited significantly smaller infarct size of 8-week-old C57BL6 mice subjected to left coronary artery ligation myocardial infarction surgery. The effects in *in vivo* and *ex vivo* could be attributed to the increased cellular and mitochondrial functions such as increased glycolysis, oxidative phosphorylation, and metabolic processes which were observed using rat H9c2 cells. Interestingly, NPS A increased mitochondrial function and NAD^+/NADH ratio. Using *in vitro* kinase activity assays, we showed that NPS A inhibits the GSK-3β. A docking simulation study demonstrated that NPS A could interact with GSK-3β. Furthermore, NPS A increased the NAD^+/NADH ratio via the NRF2-NQO1 pathway, which is how NPS A can exert its effect on metabolic and cellular processes. NPS A, a natural marine compound, can enhance mitochondrial metabolism and protect the heart against I/R-damage via inhibition of GSK-3β without toxicity. The combined effects regulated by NPS a treatment can protect the heart against acute I/R damage and chronic myocardial infarction.

Key Words: Neopetroside A, Marine pyridine nucleoside, Mitochondria, ischemia/reperfusion injury, GSK-3 inhibition

P12-11

Modulation of *FNDC5* transcription by glucocorticoid receptor

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Irisin is a hormone secreted by skeletal muscle during exercise which can influence energy and metabolic homeostasis. It is a cleaved and secreted fragment of fibronectin type III domain-containing protein 5 (FNDC5). Elucidation of the *FNDC5* gene regulation mechanism is necessary to clarify the function of irisin as a potential therapeutic target in human metabolic diseases. Thus, we investigated the genetic and epigenetic mechanisms that regulate expression of the *FNDC5* gene. *FNDC5* mRNA was strongly expressed in major energy-dependent human tissues; including heart, brain, liver, and skeletal muscle. Promoter analysis of the *FNDC5* gene revealed that the core promoter region of the *FNDC5* gene contained one CpG island that was located just upstream of the transcriptional start site for variants 2 and 3. Treatment with the histone deacetylase inhibitor, sodium butyrate, and 5-azacytidine, a demethylating agent, increased mRNA expression of *FNDC5* in Huh7 cells. Prediction of transcription factor binding sites suggested that glucocorticoid receptor was involved in the regulation of *FNDC5* expression, and indeed, cortisol treatment increased mRNA expression of *FNDC5* in Huh7 cells. Collectively, these findings offer insight into the genetic and epigenetic regulation of *FNDC5*, providing the initial steps required for understanding the role of irisin in the metabolic homeostasis.

Key Words: Irisin, *FNDC5*, Glucocorticoid receptor

P12-12

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: Mitochondrial pyruvate dehydrogenase phosphatase 1, Differentiation, Embryonic stem cell, Cardiomyocytes

P12-13

Osteoporosis prevention effect *of Parthenocissus tricuspidata* extract

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Osteoporosis is not a symptom of the disease itself, but the bone is not found until massive bone fractures occur. Currently, osteoporosis treatments inhibit osteoclast function, which causes bone resorption, that is, the process of calcium excretion from bone. We have focused on balancing osteocyte formation and destruction by increasing osteoclast formation as well as osteoclast inhibition. We have demonstrated the efficacy of Songdam(*Parthenocissus tricuspidata*), which is effective in improving arthritis, myalgia and diabetes using hepatocyte cells that can differentiate into osteoblasts. *Parthenocissus tricuspidata* was extracted into ethanol, spray dried, or freeze dried. SD-Rat Ovariectomy was performed at 6 weeks of age to make osteoporosis model, and then *Parthenocissus tricuspidata* spray drying/freeze drying powder were administered orally. DPD, PYD, ALP, and TRAP activity, which are known as bone resorption indexes, were measured at the concentration of 1 mg/kg, 10 mg/kg, 100 mg/kg of *Parthenocissus tricuspidata* spray drying/ freeze drying powder. DPD acitivity was significantly changed at 100 mg/kg concentration of *Parthenocissus tricuspidata* spray drying or freeze drying powder. PYD, ALP and TRAP activities were significantly highest at 100 mg/kg concentration of *Parthenocissus tricuspidata* freeze drying powder.

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Key Words: *Parthenocissus tricuspidata,* Osteogenesis, Ovariectomy, osteoporosis

P12-14

The effect on osteogenesis of *paeonia lactiflora* extract in MC3T3-E1 cells

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Osteoporosis is a skeletal disorder in which the strength of the bone is weakened and fractures are easily caused. At present, the general principle of osteoporosis treatment is to inhibit osteoclast formation. We have focused on not only inhibiting osteoclast formation but also increasing osteoblast formation, thereby establishing the balance of formation and destruction of bone cell. Using the cells that can differentiate into osteoblasts, we have tested the efficacy of *Paeonia lactiflora* in oriental herbal ingredients, which are thought to be beneficial to bone health and high in food utilization. MC3T3-E1 cells were treated with differentiating medium to differentiate into osteoblasts, and bone mineralization was observed at a concentration of 25 μg/ml or more. Raw264.7 cells were treated with RANKL and then treated with *Paeonia lactiflora* to inhibit bone destruction at 25 μg/ml. Animal models ovariectomies were performed to create osteoporosis models and oral administration of *Paeonia lactiflora*. 100 mg/ kg, ALP activity 100 mg/kg, TRAP activity 100 mg/kg, and PYD activity in the osteoporosis animal model 100 mg/kg. *Paeonia lactiflora* extracts showed enhanced the

osteogenesis and inhibited the bone destruction at 25 μg/ml in vitro and 100 mg/kg osteoporosis relief in vivo.

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Key Words: mc3t3-e1, Osteogenesis, Paeonia lactiflora extract, Osteoporosis, Osteoblast

P12-15

Effect on osteoclast and osteoblast differentiation from *peanut sprout* extract

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Osteoporosis is a disease that requires attention because it is not easy to detect until fracture occurs. Currently available drugs are bisphosphonates, which reduce the number of osteoclasts that can lead to bone destruction and reduce the number of osteoclasts resulting in a small increase in bone mass. This study was conducted to investigate the effect of peanut sprout extracts on osteoblast-differentiating cell lines. In the last 3 weeks, it was confirmed that osteoblast formation was increased at 2.5-50 μg/ml concentration of peanut sprout extract according to Ethly acetate extraction method, and it was increased most at 25 μg/ml concentration. Ethanol extraction method showed the maximum increase of osteoblast at 3rd week at 25 μg/ml concentration of peanut sprout extract. The osteoclast cell was used in the same manner as the osteoblast experiment. It was confirmed that the number of ROC and the TRAP activity were decreased from 12.5 μg / ml, that is, the osteoclast differentiation was inhibited.

Acknowledgement: This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-Added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (31606203).

Key Words: Osteoclast, Osteoblast, Peanut sprout, Osteoporosis, Natural material

P12-16

The acute hypotension differently affects neuronal activities in medial vestibular nucleus of rats with time according to various types of their cells

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Medial vestibular nucleus (MVN) function has been known that it is deeply associated with the control of blood pressure including orthostatic hypotension. Especially, several studies showed that acute hypotension induces the excitation of neural activities in MVN. However, detailed properties of neuronal electrophysiological changes in MVN following acute hypotension have been unknown. Therefore, we observed changes of neuronal activities in MVN of rats through extracellular recording using two tetrode electrodes in order to investigate effects of acute hypotension on neuronal electrophysiology in MVN of rats. After we divided into three types according to shapes and firing rates of neurons, we monitored changes of neural firing rates for each neuron having characteristics of three types. Most of neurons showed excitatory responses for about 5 minutes after the induction of acute and then maintained inhibitory responses over about 30 minutes regardless of types. These electrophysiological observations suggest that medial vestibular nucleus may differently influence on controlling blood pressure with time.

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Key Words: Extracellular recording, Medial vestibular nucleus, Hypotension

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