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The 65th Annual Meeting of Korean Physiological Society

# KPS 2013

# Discovery of Colorful Life

**22-24 | 10 | 2013**

Chung-Ang University Art Center

## Chung-Ang University **Tuesday, October 22**

| | |
|---|---|
| 17:30-18:30 | **Opening Lecture** : Stem cell and physiology<br>*Hong Jun Lee (Chung-Ang University, Korea)* |

## Art center of Chung-Ang University **Wednesday, October 23**

| Time | Hall A | Hall B |
|---|---|---|
| 09:30-13:20 | **Registration** | |
| 10:00-12:00 | **Satellite Symposium**<br>: The roles of cation channels in pathophysiology of the human bodies<br>*Chair : Insuk So* | |
| 11:40-12:00 | **Advisory Board Meeting** (11F R&D Center University Club) | |
| 12:00-13:20 | **Board of Directors Meeting** (11F R&D Center University Club) | |
| 13:20-13:30 | **Opening Ceremony** | |
| 13:30-14:30 | **Plenary Lecture** : Dynamic aspects of the function and stoichiometry of ion channel complexes<br>*Chair : Byung-Il Min / Yoshihiro Kubo (NIPS, Japan)* | |
| 14:30-16:30 | **Symposium I** : Learning and memory<br>*Chair : Sang Jeong Kim* | **Special Focus Session I** : Ion channels in mechanotransduction<br>*Chair : Young Min Bae* |
| 16:30-18:00 | **Poster oral presentation I**<br>: Ion channels, muscles, and molecular physiology | **Poster Presentation** |
| 18:00 | **Standing Reception** (Faculty office building / Gymnasium) | |

## Art center of Chung-Ang University **Thursday, October 24**

| Time | Hall A | Hall B |
|---|---|---|
| 09:00-09:30 | **Youdang Scholarship Award Lecture** | |
| 09:30-12:00 | **Symposium II** : Integrative and computational physiology<br>*Chair : Chae Hun Leem* | **Special Focus Session II** : Physiological studies using Drosophila melanogaster : from molecules to behaviors<br>*Chair : Ji hye Lee* |
| 12:00-12:30 | **General Assembly** | |
| 12:30-13:30 | **Photograph and Lunch** (Collge of Business Administration) | |
| 13:30-15:00 | **Poster oral presentation II** :<br>Neuronal, Systemic, and Integrative physiology | **Poster Presentation** |
| 15:00-17:00 | **Symposium III** : Disease animal models<br>*Chair : Tong Mook Kang* | **Special Focus Session III** : Inner Ear Physiology<br>*Chair : Min Sun Kim* |
| 17:00-17:30 | **Poster Prize Award and Closing Ceremony** | |

Korean Physiological Society
Chung-Ang University

Supported by Korean Federation of Technology Societies, Neuro-immune Information Storage Network Research Center and Seoul National University TRP Research Lab

# 2013 대한생리학회 임원명단

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

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

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

# Acknowledgement


**Supported by**

Supported by Korean Federation of Technology Societies,
Neuro-immune Information Storage Network Research Center,
Seoul National University TRP Research Lab and Department of Physiology
College of Medicine Chung-Ang University

**Exhibited by**

싸이텍코리아
상정상사

에스엔텍

# Contents

# Invitation (초대의 글)

회원 여러분, 안녕하십니까?
올해는 유난히도 무더웠던 여름이었습니다. 건강하게 잘 지내셨는지요?

우리 학회의 금년 학술활동은 지난 4월 26일에 춘천 한림대학교에서 열린 제21회 기초의학 학술대회를 시작으로 7월 21～26일까지 영국 버밍엄에서 열린 IUPS 2013, 그리고 9월 4～7일까지 일본 하마마츠시에서 열린 제11회 한일공동 뇌 과학, 심근과 평활근 심포지엄이 있었습니다. 상기 대회에 참석하여 주신 회원 여러분들께 지면을 통하여 감사의 말씀을 드립니다.

오는 10월 22～24일에는 중앙대학교에서 제65회 대한생리학회 학술대회가 개최됩니다. 우리 실행이사회는 지난 1년간 모든 회원이 만족할 수 있는 학술프로그램을 만들기 위하여 주제를 다양화하여 훌륭한 발표자를 초청하고, 또한 많은 회원들이 발표할 수 있는 기회를 주기 위하여 최선의 노력을 하였습니다. 이번 대회에 계획된 학술프로그램은 Plenary Lecture 1회, Symposium 3회, Special Focus 3회, Poster Presentation 2회 그리고 Satellite Symposium으로 구성되어 있습니다.

최근에 우리나라뿐만 아니라 세계적으로도 생리학회의 활동이 위축되는 경향이 있습니다만, 제65회 대한생리학회 학술대회는 그 숫자가 의미하듯이 역사와 전통을 자랑하고 있습니다. 따라서 선배들이 이룩한 이 훌륭한 업적을 우리는 계승발전 시켜야 할 것입니다.

저는 우리회원 모두가 이번 학술대회에 적극적으로 참여하여, 자신들의 연구결과의 발표는 물론 동료들의 연구발표도 경청하기를 희망합니다. 또한 발표된 연제에 대한 토론의 활성화를 통한 지식정보의 공유와 개인 간의 긴밀한 교제를 통하여 우정을 돈독히 하는 기회가 되었으면 합니다. 결과로 우리회원 개인의 발전과 학회의 발전이 함께하는 제65회 대한생리학회 학술대회가 되기를 간절히 바라는 바입니다.

끝으로, 회원 여러분들의 건강과 가정의 행복이 함께하기를 기원합니다.

감사합니다.

대한생리학회 회 장 **민 병 일**
대한생리학회 이사장 **조 양 혁**

# Schedule (일정표)

## Tuesday, October 22

Chung-Ang University

| Time | Contents |
|---|---|
| 17:30～18:30 | **Opening Lecture:** Human Stem Cell Based Cell- and Gene- Therapy in Diseases<br>*Hong Jun Lee (Chung-Ang University, Korea)* |

## Wednesday, October 23

Art Center of Chung-Ang University

| Time | Hall A | Hall B |
|---|---|---|
| 09:30～13:20 | **Registration** | |
| 10:00～12:00 | **Satellite Symposium**:<br>The Roles of Cation Channels in Pathophysiology of the Human Bodies<br>***Chair:*** *Insuk So* | |
| 11:40～12:00 | **Advisory Board Meeting** (11F R&D Center University Club) | |
| 12:00～13:20 | **Board of Directors Meeting** (11F R&D Center University Club) | |
| 13:20～13:30 | **Opening Ceremony** | |
| 13:30～14:30 | **Plenary Lecture:** Dynamic Aspects of the Function and Stoichiometry of Ion Channel Complexes<br>***Chair:*** *Byung-Il Min* | |
| 14:30～16:30 | **Symposium I:**<br>Learning and memory<br>***Chair:*** *Sang Jeong Kim* | **Special Focus Session I :**<br>Ion Channels in Mechanotransduction<br>***Chair:*** *Young Min Bae* |
| 16:30～18:00 | **Poster oral presentation I:**<br>Ion Channels, Muscles, and Molecular Physiology | **Poster Presentation** |
| 18:00 | **Standing Reception** (Faculty Office Building / Gymnasium) | |

## Thursday, October 24

Art Center of Chung-Ang University

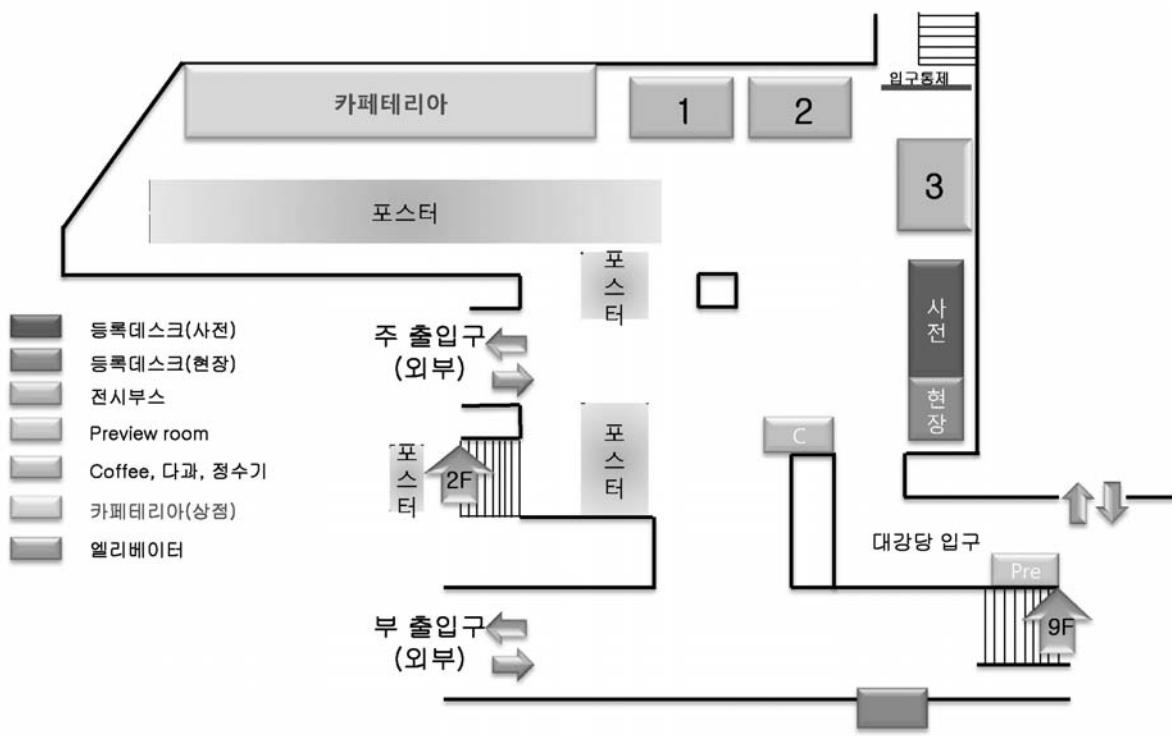
| Time | Hall A | Hall B |
|---|---|---|
| 09:00～09:30 | **Youdang Scholarship Award Lecture** | |
| 09:30～12:00 | **Symposium II :**<br>Integrative and Computational Physiology<br>***Chair:*** *Chae Hun Leem* | **SF II :** Physiological Studies Using *Drosophila melanogaster*: from Molecules to Behaviors<br>***Chair:*** *Ji Hye Lee* |
| 12:00～12:30 | **General Assembly** | |
| 12:30～13:30 | **Photograph and Lunch** (Collge of Business Administration) | |
| 13:30～15:00 | **Poster Oral Presentation II :**<br>Neuronal, Systemic, and Integrative Physiology | **Poster Presentation** |
| 15:00～17:00 | **Symposium III:** Disease Animal Models<br>***Chair:*** *Tong Mook Kang* | **Special Focus Session III:** Inner Ear Physiology<br>***Chair:*** *Min Sun Kim* |
| 17:00～17:30 | **Poster Prize Award and Closing Ceremony** | |

# Venue Guide (학술대회장 안내)

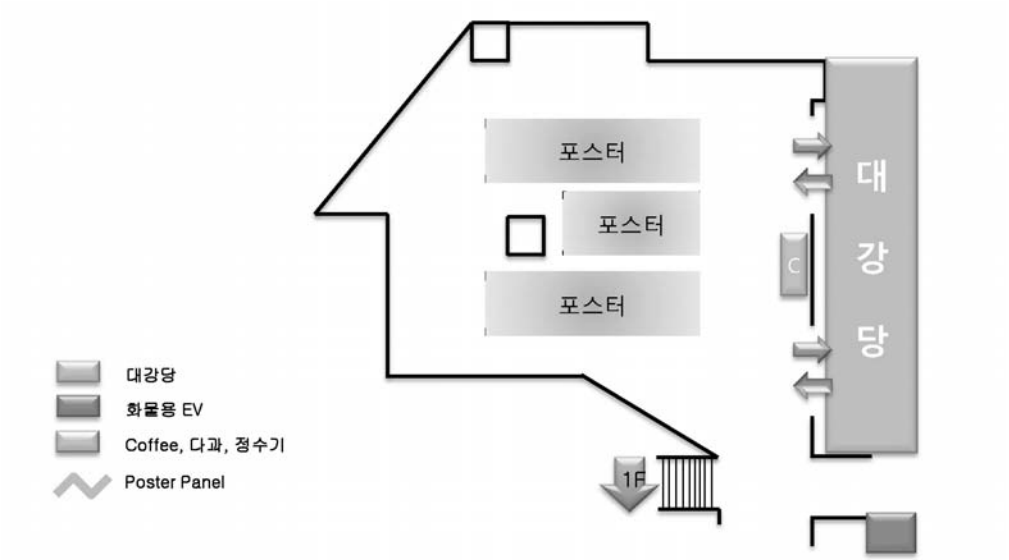
## 중앙대학교 아트센터


102관(R&D센터) - 23일 자문위원회, 이사회(11F University Club)
106관(제2의학관) - 22일 Opening Lecture(2F 205호)
205관(학생회관) - 24일 교수전용식당(B1)
301관(아트센터) - 23~24일 행사장(대강당, 9F 미디어실)
303관(법학관) - 24일 학생전용식당(B1)
305관(체육관) - 23일 Standing Reception(2F)

## 아트센터 1F

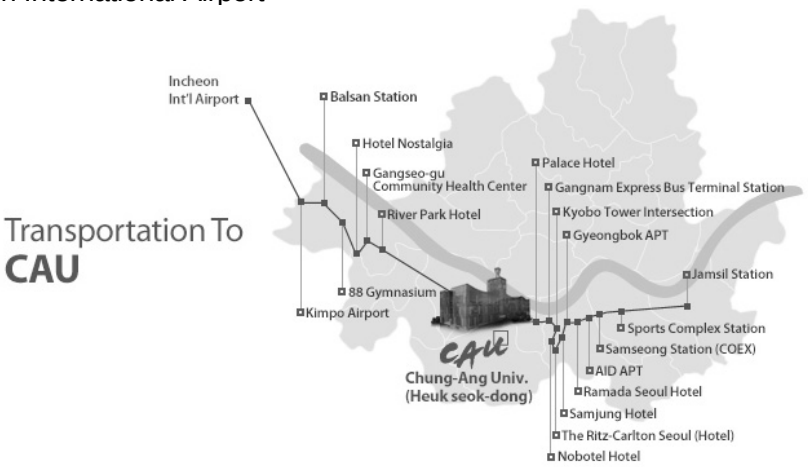



## 아트센터 2F



## ➜ 교통편

### ❶ From Incheon International Airport




- By Bus

  At Incheon International Airport, take Airport Limousine Bus #6016 and get off at Heukseok-dong stop. The fare is around 10,000 won (approx. $10). CAU is 10 minutes away from the stop, and easy to find by asking nearby passengers

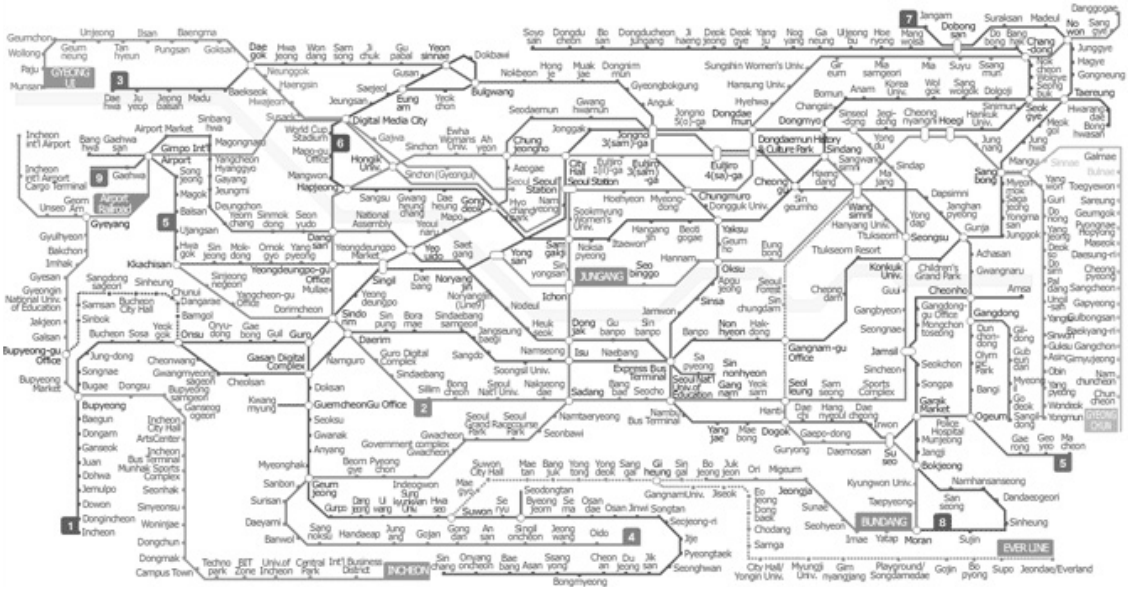
  - Details on Airport Limousine #6016

    Direction: from Incheon International Airport to Nambu Bus Terminal
    Interval: 30 minutes
    Fee: 10,000 won

- By Taxi

  The fare for a regular taxi from the airport to the school is about 70,000 won (approx. $70).
  If you take a deluxe taxi (Mobeom Taxi; colored black), the fare will be around 90,000 won (approx. $90).
  The fares of regular taxis start at 2,400 won (approx. $2), while those of deluxe taxis start at 4,500 won (approx. $4.5).
  Most taxi drivers know how to get to Chung-Ang University.

## ❷ From the School to the downtown area of Seoul



- By Subway
  Chung-Ang University is located in the heart of Seoul.
  Two subway lines link CAU with downtown Seoul.
  The subway stations near CAU are Sangdo Station on Line 7 and Heukseok Station on Line 9.
  By subway, downtown Seoul is 30 minutes away from CAU.

- By Bus
  There are several buses that connect CAU with various parts of Seoul.
  Fares start at 1,000 won and increase according to distance.
  For more information on the Seoul bus system, visit http://english.seoul.go.kr

## ❸ Transportation card

Seoul has a distinctive and highly effective transportation card system.
Visit http://www.t-money.co.kr for more information.
A link to a Guide for foreigners is offered on the top of the homepage.

## ➜ 관광편

- Banpo Han River Park
  - Overview The Han River is Seoul's most iconic symbol. Banpo Han River Park is the very first outcome of the 'Han River Renaissance Project' and offers an impressive number of options for recreation and relaxation.
    The Rainbow Fountain, Moonlight Square, Floating Island, Seorae Island and Marina are now welcoming tourists.

# Scientific Program (학술프로그램)

## Wednesday, October 23

| | |
|---|---|
| 13:30-14:30 | **Plenary Lecture**<br>**Chair:** *Byung-Il Min* |
| | ▶ Dynamic Aspects of the Function and Stoichiometry of Ion Channel Complexes<br>*Yoshihiro Kubo (NIPS, Japan)* |
| **Hall A** | |
| 10:00-12:00 | **Satellite Symposium:** The Roles of Cation Channels in Pathophysiology of the Human Bodies<br>(Sponsored by TRP Research Lab)<br>**Chair:** *Insuk So* |
| 10:00-10:30 | ▶ TRPC, Orai1 and STIM1 in Physiological and Pathological $Ca^{2+}$ Influx<br>*Shmuel Muallem (NIH, USA)* |
| 10:30-11:00 | ▶ Sensing Fluid Flow by Ciliary Localized Pkd2 for Left-Right Patterning<br>*Hiroshi Hamada (Osaka University, Japan)* |
| 11:00-11:20 | ▶ TMC-1 is a Cation Channel for Sodium Sensation in C. elegans Nociceptor Neurons<br>*SunWook Hwang (Korea University, Korea)* |
| 11:20-11:40 | ▶ The Role of Cation Channels in Interstitial Cells of Cajal from the Murine Small Intestine<br>*Byung Joo Kim (Pusan University, Korea)* |
| 11:40-12:00 | ▶ Neurotoxic Action of Oxidized TRPC5 in Huntington Disease due to Abnormal Glutathione Homeostasis<br>*Chansik Hong (Seoul University, Korea)* |
| 14:30-16:30 | **Symposium I:** Learning and Memory<br>(Sponsored by Neuro-Immune Information Storage Network MRC)<br>**Chair:** *Sang Jeong Kim* |
| 14:30-15:00 | ▶ Synapse-to-Nucleus and Nucleus-to-Synapse Signaling:<br>Molecular Mechanisms of Arc/Arg3.1 Regulation and Its Role on Synaptic Function<br>*Hiroyuki Okuno (Medical Innovation Center Kyoto University, Japan)* |
| 15:00-15:30 | ▶ Structural and Molecular Remodeling of Single Dendritic Spines During Long-Term Potentiation<br>*Yasunori Hayashi (Brain Science Institute, RIKEN, Japan)* |
| 15:30-16:00 | ▶ Mechanism and Adult Treatment for the Memory Deficits Associated with Mouse Models of RASopathy<br>*Yong-Seok Lee (Chung-Ang University, Korea)* |
| 16:00-16:30 | ▶ Neuron-Glia Crosstalk under Pathological Condition of Alzheimer's Disease<br>*Inhee Mook-Jung (Seoul National University, Korea)* |
| **Hall B** | |
| 14:30-16:30 | **Special Focus Sessions I:** Ion Channels in Mechanotransduction<br>**Chair:** *Young Min Bae* |
| 14:30-15:00 | ▶ Revisiting Ion Channel Mechanosensitivity<br>*Young Min Bae (Konkuk University, Korea)* |
| 15:00-15:30 | ▶ The Mechanosensitivity of TREK-2 Channel: Role of PLC Activation by Membrane Stretch<br>*Joo Hyun Nam (Dongguk University, Korea)* |
| 15:30-16:00 | ▶ Segregation of TRP Channels in *Drosophila* Mechanosensory Cilia<br>*Yun Doo Chung (University of Seoul, Korea)* |
| 16:00-16:30 | ▶ Ectopic Pacemaking in Atrial Myocytes under Fluid Pressure<br>*Sun-Hee Woo (Chungnam University, Korea)* |
| 16:30-18:00 | **Poster Oral Presentation I (PO-1~8):** Ion channels, Muscles, and Molecular Physiology<br>**Chairs:** *Tong Mook Kang, Byeong-Hwa Jeon* |

## Thursday, October 24

| | |
|---|---|
| 09:00-09:30 | **Youdang Scholarship Award Lecture**<br>**Chair:** *Yang-Hyeok Jo* |
| | ▶ Angiotensin-(1-7) Stimulates ANP Secretion and Attenuates Hypertension and Cardiac Hypertrophy via Mas Receptor<br>*Suhn Hee Kim (Chonbuk National University, Korea)* |
| **Hall A** | |
| 09:30-12:00 | **Symposium II:** Integrative and Computational Physiology<br>**Chair:** *Chae Hun Leem* |
| 09:30-10:00 | ▶ Modelling for Better Understanding Physiological Phenomena<br>*Chae Hun Leem (Ulsan University, Korea)* |
| 10:00-10:30 | ▶ An Updated Model of Interstitial Cells of Cajal Reproducing Intestinal Pacemaker Activity<br>*Jae Boum Youm (Inje University, Korea)* |
| 10:30-11:00 | ▶ A Physiomic Model for the Analysis of Heart Mechanics and Blood Circulation<br>*Eun Bo Shim (Kangwon University, Korea)* |
| 11:00-11:30 | ▶ Comparisons of Clinical Catheter Ablation of Atrial Fibrillation and Virtual Ablation on the Personalized *in-silico* Left Atrial Electroanatomical Modeling<br>*Hui-Nam Pak (Yonsei University, Korea)* |
| 11:30-12:00 | ▶ Hemodynamics in Vascular Disease<br>*Kyehan Rhee (Myongji University, Korea)* |
| 15:00-17:00 | **Symposium III:** Disease Animal Models<br>**Chair:** *Tong Mook Kang* |
| 15:00-15:30 | ▶ The Power of Mouse Genetics: Genome Engineering in Mice by TALENs and RGENs<br>*Han-Woong Lee (Yonsei University, Korea)* |
| 15:30-16:00 | ▶ Prediction of Gene Function through Mouse Phenotype Analysis in Metabolic Syndrome<br>*Je Kyung Seong (Seoul National University, Korea)* |
| 16:00-16:30 | ▶ Mechanism and Therapy for Vascular Malformation<br>*S. Paul Oh (Gachon University, Korea)* |
| 16:30-17:00 | ▶ A Rat Model of Atopic Dermatitis<br>*Heung Sik Na (Korea University, Korea)* |
| **Hall B** | |
| 09:30-12:00 | **Special Focus Sessions II:** Physiological Studies Using *Drosophila melanogaster*: from Molecules to Behaviors<br>**Chair:** *Ji Hye Lee* |
| 09:30-10:00 | ▶ Why do We Study *Drosophila* Melanogaster?:<br>A Powerful Genetic Model System to Study Molecular Functions and Relevant Behaviors<br>*Ji Hye Lee (Pusan National University, Korea)* |
| 10:00-10:30 | ▶ Sensory Discrimination of Polymodal TRPA1 in *Drosophila*<br>*KyeongJin Kang (Sungkyunkwan University, Korea)* |
| 10:30-11:00 | ▶ Modeling and Studying Parkinson's Disease Using *Drosophila* Genetics<br>*Hyongjong Koh (Dong-A University, Korea)* |
| 11:00-11:30 | ▶ A Stress Hormone Regulates the Female Reproduction by Facilitating the Sperm Storage<br>*Young-Joon Kim (GIST, Korea)* |
| 11:30-12:00 | ▶ Role for dCLOCK in Behavioral Synchronization to Daily Temperature Cycle<br>*Eun Young Kim (Ajou University, Korea)* |
| 13:30-15:00 | **Poster Oral Presentation II (PO-9~16):** Neuronal, Systemic, and Integrative Physiology<br>**Chairs:** *Young-Ho Jin, Sung-Joon Kim* |
| 15:00-17:00 | **Special Focus Sessions III:** Inner Ear Physiology<br>**Chair:** *Min Sun Kim* |
| 15:00-15:30 | ▶ Electrophysiology Related Cochlear Implantation<br>*Jae Young Choi (Yonsei University, Korea)* |
| 15:30-16:00 | ▶ Noise Induced Hearing Loss and Hair Cell Regeneration<br>*Jong Woo Chung (Ulsan University, Korea)* |
| 16:00-16:30 | ▶ Vestibular Afferent Signaling and Homeostasis<br>*Gyu Cheol Han (Gachon University, Korea)* |
| 16:30-17:00 | ▶ Role of Vestibular System in Function of Basal Ganglia in Rats<br>*Min Sun Kim (Wonkwang University, Korea)* |

## Opening Lecture



## Satellite Symposium



## Youdang Scholarship Award Lecture



## Plenary Lecture



## Symposia

## Special Focus Session

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Min Sun Kim
Department of Physiology, Wonkwang University School of Medicine & Brain Science Institute at Wonkwang University, Iksan 570-749, Korea

## Poster Presentation (Poster Oral Presentation)

**Session I :** Ion Channels & Transporters

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IC-1 Curcumin Inhibits the Voltage- Dependent $K^+$ Channels in Rabbit Coronary Arterial Smooth Muscle Cells ····· S 49
Da Hye Hong, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon 200-701, Korea

IC-2 The Inhibitory Effect of $Ca^{2+}$ Channel Inhibitor Efonidipine On Voltage-Dependent $K^+$ Channel Activity in Coronary Arterial Smooth Muscle Cells of Rabbit ····· S 49
Youn Kyoung Son, Hongliang Li, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, South Korea

IC-3 The Inhibitory Mechanisms of LY294002, a PI3 Kinase Inhibitor on Voltage- Dependent $K^+$ Channels In Rabbit Coronary Arterial Smooth Muscle Cells ····· S 49
Da Hye Hong, Won Sun Park
Department of Physiology, Kangwon National University School of Medicine, Chuncheon, 200-701, South Korea

IC-4 Role of $Li^+$-permeable $Na^+/Ca^{2+}$ exchangers, NCLX, in the Exocytosis of Pancreatic $\beta$ Cells ····· S 49
Young Eun Han, Sun Hyun Park,Young Sun Ji, Suk Ho Lee, Shin Young Ryu, Won Kyung Ho
Department of Physiology and Biomembrane Plasticity Research Center, Seoul National University College of Medicine, Seoul 110-799, Korea

IC-5 *Trans*-Anethole Enhances Long-Term Potentiation Through Voltage-Dependent Calcium Channels Independent Pathway ····· S 50
SangYeop Shin[1], SeungHo Han[1], Jaeyong Yee[1], Chan Kim[1], Geun Hee Seol[2], Sun Seek Min[1]
[1]Department of Physiology and Biophysics, School of Medicine, Eulji University, Daejeon 301-832,
[2]Department of Basic Nursing Science, School of Nursing, Korea University, Seoul 136-705, Korea

IC-6 $Ca^{2+}$ Release via Ryanodine Receptors of ER Increase Somatic Excitability by Downregulating Somatic A-type $K^+$ Channels ····· S 50
Yoon-Sil Yang, Seon-Hee Kim, Jin-Ji Wu, Jee-Yun Park, Su-Yong Eun, Joo-Min Park, Sung-Cherl Jung
Department of Physiology, School of Medicine, Jeju National University, Jeju, Korea

IC-7 Alterations of Contraction and L-type $Ca^{2+}$ Current by Murrayafoline-A in Rat Ventricular Myocytes ····· S 50
Min-Jeong Son[1], Bojjibabu Chidipi[1], Joon-Chul Kim[1], Tran Thu Huong[3], Young Ho Kim[2], Nguyen Manh Cuong[3], Sun-Hee Woo[1]
Laboratory of [1]Physiology and [2]Laboratory of Natural Product, College of Pharmacy, Chungnam National University, Daejeon 305-764, South Korea,
[3]Department of Bioactive Products, Vietnam Academy of Science and Technology, Hoang Quoc Viet Rd., Hanoi, Vietnam

IC-8 Attenuated Benzodiazepine-Sensitive Tonic $GABA_A$ Currents of Supraoptic Magnocellular Neuroendocrine Cells in 24-h Water-Deprived Rats ····· S 51
Sudip Pandit, Yoon Hyung Pai, Hyun-Woo Kim, Byeong Hwa Jeona, Jin Bong Park
Department of Physiology, Brain Research Institute, School of Medicine, Chungnam National University, Daejeon 301-131, Republic of Korea

**Thursday, October 24**

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**Thursday, October 24**

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Seung Pil Yun, Sei-Jung Lee, Sang Yub Oh, Young Hyun Jung, Hyun Jik Lee, Ho Jae Han*
Department of Veterinary Physiology, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Korea

**Session III :** Muscle Cells

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[1]Laboratory of Health Science & Nanophysiotherapy, Department of Physical Therapy, College of Public Health & Welfare Yongin University, Yongin 449-714, Korea and [2]Department of Physiology, Institute of Functional Genomics, School of Medicine, Konkuk University, Choongju 380-701, Korea

MU-2 Role of TRPC3 in Endothelium- Dependent Relaxation ····· S 79
Soo-In Yeon, Mihwa Lim, Soo-kyoung Choi, Duck Sun Ahn, Young-Ho Lee
Department of physiology, College of Medicine, Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, 120-752, Korea

MU-3 Effect of Skin Regeneration and Composition of the Essential Oil from Artemisia Montana Pampan ····· S 79
Mi-So Yoon[1], Do-Yoon Kim[1], Joo-Hoon Park[1], Kyung-Jong Won[2], Bokyung Kim[2], Hwan-Myung Lee[1]
[1]Department of Cosmetic Science, College of Natural Science, Rsearch Institute for Basic Sciences, Hoseo University, Asan 336-795, Korea, [2]Departments of Physiology, School of Medicine, Konkuk University, Chungju 380-701, Korea

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Byung Joo Kim[1], Hyung Woo Kim[2], Guem San Lee[3], Seok Choi[4], Jae Yeoul Jun[4], Insuk So[5], Seon Jeong Kim[6]
[1]Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Yangsan 626-870, [2]Division of Pharmacology, Pusan National University School of Korean Medicine, Yangsan 626-870, [3]Wonkwang University College of Korean Medicine, Iksan 570-749, [4]Department of Physiology, Chosun University College of Medicine, Gwangju 501-759, [5]Department of Physiology, Seoul National University College of Medicine, Seoul 110-799, [6]Center for Bio-Artificial Muscle and Department of Biomedical Engineering, Hanyang University, Seoul 133-791, Korea

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Keon Jin Lee[1], Changdo Hyun[1], Jin Seok Woo[1], Chang Sik Park[2], Do Han Kim[2], Eun Hui Lee[1]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul 137-701, [2]School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

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Dong Jun Sung[3], Sang Woong Park[1], Hyun Ju Noh[1], Jae Gon Kim[2], Hana Cho[2], Young Min Bae[1]*
[1]Department of Physiology, Institute of Functional Genomics, and Research Institute of Medical Science, Konkuk University School of Medicine, [2]Department of Physiology and The Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, [3]Division of Sport Science, College of Science and Technology, Konkuk University

MU-7 Angiopoietin 1 Enhances the Proliferation and Differentiation of Skeletal Myoblasts ····· S 81
Eun Hui Lee[1], Jin Seok Woo[1], Ji-Hye Hwang[2], Jae-Hyeong Park[3], Chung-Hyun Cho[2]
[1]Department of Physiology, College of Medicine, The Catholic University of Korea, Seoul, [2]Department of Pharmacology and Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, [3]Division of Cardiology, Department of Internal Medicine, School of Medicine, Chungnam National University, Chungnam National University Hospital, Daejeon, Korea

**Session IV :** Neuronal Cells

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Sung-Min Hwang, Moon-Yong Park, Min-Kyung Kim, Kyungpyo Park
Department of Physiology, School of Dentistryand Dental Research Institute, Seoul 110-749, Korea

SC-2 Alteration of Regulated Exocytosis in Parotid Gland Acinar Cells from TRPML1$^{-/-}$ Mice ····· S 98
Soonhong Park[1,3], Min Seuk Kim[2], Shmuel Muallem[3], Dong Min Shin[1]
[1]Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, 120-752, Korea, [2]Department of Oral Physiology, College of Dentistry, Wonkwang University, Iksan, 570-749, Korea, [3]Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Bethesda, MD 20892, USA

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SC-3 Alteration of RANKL-Induced Osteoclastogenesis in Primary Cultured Osteoclasts from Homer2/3$^{-/-}$ Mice ····· S 98
Sung Jun Lee, Aran Son, Yu-Mi Yang, Dong Min Shin
Department of Oral Biology, BK21 PLUS Project, Yonsei University College of Dentistry, Seoul, 120-752, Korea

SC-4 $Ca^{2+}$ Signaling and Cytokine Release by Asthma Related Allergens in Human Gingival Epithelial Cells ····· S 99
Aran Son[1], Wonse Park[1], Dong Min Shin[1]
[1]Department of Oral Biology, BK21 PLUS Project, [2]Department of Advanced General Dentistry, Yonsei University College of Dentistry, Seoul, 120-752,Korea

SC-5(PO-9) Cytosolic and Mitochondrial Matrix Alkalinisation is Involved in Superoxide Generation and Cytotoxicity by High Phosphate in Insulin Secreting Cells ····· S 99
Tuyet Thi Nguyen, Xianglan Quan, Kyu-Hee Hwang, Seong-Kyung Choi, Seong-Woo Jeong, In Deok Kong, Seung-Kuy Cha, Kyu-Sang Park
Department of Physiology, Yonsei University Wonju College of Medicine, Wonju, 220-701, Korea

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Do-Yoon Kim[1], Mi-So Yoon[1], Joo-Hoon Park[1], Kyung-Jong Won[2], Bokyung Kim[2], Hwan-Myung Lee[1]*
[1]Department of Cosmetic Science, College of Natural Science, Research Institute for Basic Sciences, Hoseo University, Asan 336-795, Korea, [2]Departments of Physiology, School of Medicine, Konkuk University, Chungju 380-701, Korea

SY-2 Diluted Bee Venom Injection Reduces Ipsilateral Mechanical Allodynia in Oxaliplatin-Induced Neuropathic Mice ····· S 100
Ji-Hee Yeo[1], Seo-Yeon Yoon[2], Dae-Hyun Roh[1]
[1]Department of Maxillofacial Tissue Regeneration and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea, [2]Laboratory of Molecular Signal Transduction, Center for Neural Science, Korea Institute of Science and Technology (KIST), Seoul 136-791, Republic of Korea

SY-3 Angiotensin III Protects Ischemic Injury of Hearts through Mitochondrial KATP Channel and Antioxidant Pathway ····· S 100
Byung Mun Park[1], Seung Ah Cha[1], Shan Gao[1], Yi Wang[1], Byung Hyun Park[2], Suhn Hee Kim[1]
Departments of [1]Physiology and [2]Biochemistry, Diabetic Research Center, Chonbuk National University Medical School, Jeonju, Korea

SY-14 An Increase of $PIP_3$ by ROS is Involved in the Induction of Mechanical Hyperalgesia in a Rat Model of Peripheral Neuropathy ····· S 105
Se Jung Jung[1], Hyun Ah Kim[1], Jae Beom Jun[1,2], Joong Woo Leem[1,2]
[1]Department of Physiology, [2]Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea

SY-15 Effect of Opioid and Adrenergic Antagonists on Electroacupuncture- Induced Antinociception in Mice with Paclitaxel-Evoked Neuropathic Pain ····· S 105
Jung-Wan Choi, Dong-Wook Kang, Jae-Gyun Choi, Jin Bong Park, Hyun-Woo Kim
Department of Physiology and Institute of Brain Research, Chungnam National University School of Medicine, Daejeon, 301-747, South Korea

SY-16 Sigma-1 Receptor Contributes to Induction of Below-Level Mechanical Allodynia after Spinal Cord Injury via iNOS Activation ····· S 105
Sheu-Ran Choi[1], Ji-Young Moon[1], Soon-Gu Kwon[1], Hoon-Seong Choi[1], Suk-Yun Kang[1], Dae-Hyun Roh[2], Ho-Jae Han[1], Jang-Hern Lee[1]
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Jihoon Yang[1], Jeonghoon Oh[1], Hyunkyoung Lee[2], Taeho Han[2], Jaehee Lee[2], Heung Sik Na[2], Seung Keun Back[2]
[1]Gyeonggi Science High School, 440-800, Suwon, Korea, [2]Neuroscience Research Institute and Department of Physiology, Korea University College of Medicine, Seoul, 136-705, Korea

SY-18 Liquiritigenin, a Licorice Flavonoid, Ameliorates TNBS-Induced Colitis in Mice ····· S 106
Joon Ki Min, Chi Hoon Lee[1], Se-Eun Jang[2], Jae-Woo Park[3], Jae Myung Cha[1], Dong-Hyun Kim[2], Byung-Il Min[4]
[1]Department of Internal Medicine, Kyung Hee University Hospital at Gangdong, Kyung Hee University School of medicine, Seoul 130-701, Republic of Korea, [2]Department of Life and Nanopharmaceutical Sciences and Department of Pharmaceutical Science, Kyung Hee University, Seoul 130-701, Republic of Korea, [3]Department of Gastroenterology, College of Oriental Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea, [4]Department of Physiology, College of Medicine, Kyung Hee University, Seoul 130-701, Republic of Korea

SY-19(PO-10) Reduced Synaptic Nitric Oxide Function Contributes to Neuronal Excitation of Medullary-projecting PVN Neurons in Rats with Myocardial Infarction ····· S 107
Eun A Kang, Tae Hee Han, So Yeong Lee, Pan Dong Ryu
Laboratory of Pharmacology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea

SY-20 Repetitive Treatment with Diluted Bee Venom Relieves Mechanical Allodynia and Restores Intraepidermal Nerve Fiber Loss in Oxaliplatin-Induced Neuropathic Mice ····· S 107
Dae-Hyun Roh[1], Ji-Hee Yeo[1], Seo-Yeon Yoon[2], Soon-Keun Kwon[1]
[1]Department of Maxillofacial Tissue Regeneration and Research Center for Tooth and Periodontal Tissue Regeneration, School of Dentistry, Kyung Hee University, Seoul 130-701, Republic of Korea, [2]Laboratory of Molecular Signal Transduction, Center for Neural Science, Korea Institute of Science and Technology (KIST), Seoul 136-791, Republic of Korea

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Yujin Jang, Min Hee Lee, Hyung Seo Park, Se Hoon Kim, Hwang Ji Yeong, Eun Ji Kim, Dong Kwan Kim
Department of Physiology, College of Medicine, Konyang University, Daejeon,302-718, Korea

SY-22 CaMKII Contributes to NR2B Ser1303 Phosphorylation in the Early Phase of Neuropathic Pain after Nerve Injury ····· S 108
Youngkyung Kim, Jeonghwa Oh, Young Wook Yoon*
Department of Physiology, College of Medicine, Korea University

## OL

### Human Stem Cell based Cell- and Gene- Therapy in Diseases

Hong Jun Lee

*Chung-Ang University*

In the field of induced potency and fate reprogramming, it remains unclear what the best starting cell might be and to what extent a cell need be transported back to a more primitive state for translational purposes. Reprogramming a committed cell back to pluripotence to then instruct it toward a particular specialized cell type is demanding and may increase risks of neoplasia and undesired cell types. Precursor/progenitor cells from the organ of therapeutic concern typically lack only one critical attribute?the capacity for sustained self-renewal. We speculated that this could be induced in a regulatable manner such that cells proliferate only in vitro and differentiate in vivo without the need for promoting pluripotence or specifying lineage identity. As proof-of-concept, we generated and tested the efficiency, safety, engraftability, and therapeutic utility of “induced conditional self-renewing progenitor (ICSP) cells” derived from the human central nervous system (CNS); we conditionally induced self-renewal efficiently within neural progenitors solely by introducing v-myc tightly regulated by a tetracycline (Tet)-on gene expression system. Tet in the culture medium activatedmyc transcription and translation, allowing efficient expansion of homogeneous, clonal, karyotypically normal human CNS precursors ex vivo; in vivo, where Tet was absent, myc was not expressed, and self-renewal was entirely inactivated (as was tumorigenic potential). Cell proliferation ceased, and differentiation into electrophysiologically active neurons and other CNS cell types in vivo ensued upon transplantation into rats, both during development and after adult injury?with functional improvement and without neoplasia, overgrowth, deformation, emergence of non-neural cell types, phenotypic or genomic instability, or need for immunosuppression. This strategy of inducing self-renewal might be applied to progenitors from other organs and may prove to be a safe, effective, efficient, and practical method for optimizing insights gained from the ability to reprogram cells.

## Satellite S-1

### TRPC, Orai1 and STIM1 in Physiological and Pathological $Ca^{2+}$ Influx

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*Epithelial Signaling and Transport Section, Molecular Physiology and Therapeutics Branch, NIDCR, NIH, Bethesda MD 20892*

A critical component of the receptor-evoked $Ca^{2+}$ signal is $Ca^{2+}$ influx mediated by the TRPC and Orai channels and is regulated by the ER luminal $Ca^{2+}$ sensor STIM1. $Ca^{2+}$ influx is essential for all cell functions, yet excess $Ca^{2+}$ influx is highly toxic and is involved in numerous cellular pathologies. A key question is how STIM1 interacts and open these channels. Most aspects of STIM1 interaction with the TRPC and Orai channels have been studied in model system, while the behavior of the native proteins is not known, in particular in polarized cells. We used model system and gene deletion in mice to study regulation of Orai and TRPC channels by STIM1. We identified several critical domains in STIM1 that are required for regulation of the channels. In particular, the STIM1 SOAR domain mediates interaction of STIM1 with both channel type. The newly discovered CTID domain downstream of SOAR regulates access of the inhibitor SARAF to SOAR. SOAR also mediates interaction of STIM1 with the C terminus of TRPC channels to present the basic lysine-rich domain of STIM1 to the TRPC channels to gate them. The localization of the native Orai1, TRPCs and STIM1 in polarized cells provide further clues as to the regulation and potential role of TRPC channels in cellular pathology. Finally, deletion of TRPC channels in mice markedly affect acinar cells receptor and store mediated $Ca^{2+}$ influx and protects the cells from $Ca^{2+}$-mediated toxicity brought about by cell stress.

## Satellite S-2

### Sensing Fluid Flow by Ciliary Localized Pkd2 for Left-Right Patterning

Hiroshi Hamada

*Graduate School of Frontier Biosciences, Osaka University*

Cilia play central role in left-right asymmetry. Left-right symmetry breaking in the mouse and other vertebrates involves unidirectional fluid flow in the node or an equivalent region. The leftward flow in the mouse embryo is generated by clockwise rotation of cilia in the node. Rotational movement can generate leftward flow because rotational axis of cilia is posteriorly tilted. Despite recent progress, however, there remain several important questions. For example, it has remained unknown how the fluid flow works. In my talk, I will discuss how the fluid flow is sensed by mouse embryo for left-right patterning. In particular, I will show 1) role of immotile cilia and ciliary localized Pkd2 in flow sensing, 2) involvement of $Ca^{2+}$ signaling in flow sensing, 3) intra-cellular events after flow sensing.

**References:**

1. S. Yoshiba et al (2012) Science 338:226-231.
2. T. Nakamura et al (2012) Nat. Communic. 3:1322.

## Satellite S-3

### TMC-1 is a Cation Channel for Sodium Sensation in C. elegans Nociceptor Neurons

Sun Wook Hwang

*Department of Biomedical Sciences, Korea University College of Medicine*

Sodium detection of the sensory system is an essential biological function for pain modulation, ionic homeostasis, and taste perception. However, receptor molecules responsible for sensing sodium and for transducing the signal into electrical firing were largely unknown. Here we suggest one of cation channels encoded by a novel gene family for this role. Amphid single ciliated class H (ASH) neuron, a polymodal nociceptor neuron in Caenorhabditis elegans expresses diverse types of sensory receptor molecules to monitor and avoid harmful environmental changes. We show that TMC-1, a putative sensory protein expressed in ASH neuron is a cation channel activated by high concentrations of sodium. Mammalian cell lines transfected with tmc-1 gene generated cation-selective conductance upon extracellular increases in sodium concentrations. Details in electrophysiological profiles such as ionic selectivity and voltage-dependence are also observed. Sodium is not only an activator for TMC-1, but also the most permeable cation. Relatively low but significant anion permeation was also detected. Sodium sensitivity of TMC-1 occurred at over 150 mM, which indicates that TMC-1 may be a warning sensor for non-physiological ranges of sodium concentrations. ASH neuronal firing and behavioral aspects mediated by ASH neuron strongly support this hypothesis. ASK neuron natively lacks both in TMC-1 protein and in sodium responsiveness. Ectopic expression of TMC-1 by using extrachromosomal array in ASK neuron conferred the ability to fire in response to sodium exposure to the neuron, suggesting that TMC-1 is the necessary and sufficient component for cellular sodium responsiveness. Taken together, we found that tmc-1 generates a high sodium concentration-activated cation channel for sodium nociception, and this newly found information may give an insight for understanding defense mechanisms to abnormal sodium environment.

## Satellite S-4

### The Role of Cation Channels in Interstitial Cells of Cajal from the Murine Small Intestine

Byung Joo Kim

*Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Yangsan 626-870, Republic of Korea*

Interstitial cells of Cajal (ICCs) are the pacemakers in gastrointestinal (GI) muscles. Discovering the molecules involved in the generation of pacemaker activity in ICCs may lead to dramatic new therapies for chronic GI diseases that result in lifelong suffering. Electrophysiological and pharmacological properties of the nonselective cation channel (NSCC) in ICCs were the same as those of TRPM7 (Transient Receptor Potential Melastatin 7). Reverse-transcription polymerase chain reaction, western blotting, and immunohistochemistry all showed abundant and localized expression of TRPM7 messenger RNA and protein in mouse small intestine. Treatment of primary cultured ICCs with TRPM7-specific small interfering RNA resulted in inhibition of pacemaking activity. Therefore, TRPM7 is required for intestinal pacemaking and this protein is a likely potential target for pharmacological treatment of motor disorders of the gut.

Also, endogenous agents, such as, neurotransmitters, hormones, and paracrine substances modulate GI tract motility by influencing ICCs. Cholecystokinin (CCK) was one of the first GI hormones discovered, and is produced in specialized epithelial cells located in the mucosa of the small intestine.

CCK was found to induce the depolarization of pacemaking activity in a G-protein-, PLC-, PKC-, and PKA-dependent manner via CCK1 receptor. Under voltage clamp conditions at a holding potential of -60 mV, CCK induced inward currents. The features of this currents were similar to those of overexpressed TRPC5 (Transient Receptor Potential Classical 5) in HEK 293 cells. These results suggest that TRPC5 channel is a candidate for CCK induced inward currents in cultured ICCs from murine small intestine.

Substance P (SubP) is a member of the family of mammalian tachykinin peptides that are predominantly released by enteric neurons. Recently, the $Na^+$-leak channel (NALCN) has been characterized. NALCN is a member of the 24-transmembrane-spanning (24 TM) ion channel family and the only nonselective, voltage-independent channel. NALCN forms a background $Na^+$ leak conductance in neurons and is required for normal respiratory rhythm. Electrophysiological and pharmacological properties of SubP in ICCs pacemaking activity were similar to those of NALCN. NALCN messenger RNA and protein was expressed abundant. In the mutant mice, the pacemaking activity was generated and SubP marginally depolarized the pacemaking activity in cultured ICC clusters. In this study, we found that NALCN is not required for the basal pacemaking activity in ICCs and, however, NALCN is partly involved in the SubP-induced depolarization and the modulation of pacemaking activity in ICCs.

**Key Words:** Interstitial cells of Cajal, ICCs, Cholecystokinin, Substance P, pacemakers, Gastrointestinal

## Satellite S-5

### Neurotoxic Action of Oxidized TRPC5 in Huntington Disease due to Abnormal Glutathione Homeostasis

Chansik Hong

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Aberrant $Ca^{2+}$ signaling is a nodal point in many neurodegenerative disorders. $Ca^{2+}$-permeant, non-selective cation channel, play an important role in mediating the cellular response to a wide range of stimuli, including oxidants that cause $Ca^{2+}$ signaling dysregulation. However, the molecular basis of TRPC channel regulation by oxidants and their role in neurodegeneration are not known. Here, we report the molecular mechanism by which TRPC5 are activated by oxidants and the role of glutathionylated TRPC5 in Huntington's disease (HD). TRPC5 currents and $Ca^{2+}$ influx are activated by several chemical oxidants, and, most notable, by the physiological intracellular oxidized glutathione (GSSG) that was reversed by reduced glutathione (GSH) and by DTT. Extensive analysis revealed that regulation by oxidation is mediated by the conserved TRPC5 C176 and C178 that are directly glutathionylated by GSSG. Accordingly, TRPC5(C176S) and TRPC5(C178S) are resistant to activation by oxidation. TRPC5 are endogenously expressed in striatal cells from wild-type (ST*Hdh*$^{Q7/7}$) and mutant huntingtin knock-in (ST*Hdh*$^{Q111/111}$) mice. Oxidized GSSG activated TRPC5-like current in these cells. Depleting GSH to change the GSH/GSSG ratio resulted in cell death, which was more pronounced in HD cells due to a sustained $Ca^{2+}$ increase that induced apoptotic cell death by caspases-dependent calpains activation. Knockdown of TRPC5 and inhibition of TRPC5 by 10 $\mu$M ML204 significantly attenuated oxidation-dependent cell death. TRPC5 and TRPC1 function as heterodimer in neurons with TRPC1 attenuating TRPC5 activity. Accordingly, knockdown of TRPC1 was sufficient to increased necrotic and apoptotic cell death in ST*Hdh*$^{Q111/111}$ cells that was exacerbated by oxidation. These findings revealed the role of TRPC5 glutathionylation in response to oxidative stress that leads to degeneration of striatal cells. This system is upregulated in HD making the HD striatal cells more sensitive to oxidative stress and cell death that may account for the neurodegeneration in the disease.

## Youdang Scholarship

### Angiotensin-(1-7) Stimulates ANP Secretion and Attenuates Hypertension and Cardiac Hypertrophy via Mas Receptor

Amin Shah, Suhn Hee Kim

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Angiotensin-(1-7) [Ang-(1-7)], one of the bioactive peptides produced in renin-angiotensin system plays pivotal roles in cardiovascular physiology, counterbalancing Ang II functions. It is recently considered as a potential candidate for therapeutic use in various cardiovascular diseases. The aim of the present study is to explain the modulatory role of Ang-(1-7) in atrial natriuretic peptide (ANP) secretion using isolated perfused beating rat atria and cardiac hypertrophied rat model. Ang-(1-7) (0.01, 0.1, and 1 $\mu$M) increased the ANP secretion and ANP concentration in a dose-dependent manner at high atrial pacing (6.0 Hz) with an increased cGMP amount. However, Ang-(1-7) did not cause any changes in atrial parameters at low atrial pacing (1.2 Hz). Pretreatment with an antagonist of Mas receptor or with inhibitors of phosphoinositide 3-kinase (PI3K), protein kinase B (Akt), or nitric oxide synthase blocked the augmentation of high atrial pacing-induced ANP secretion by Ang-(1-7). The similar result was observed with the inhibition of $Na^+/H^+$ exchanger-1 (NHE-1) and calcium/calmodulin-dependent kinase II (CaMKII).
In trained spontaneous hypertensive rats, Mas expression and protein are upregulated in ventricular tissue. Therefore, we examined the role of Ang-(1-7) on cardiac hemodynamics, cardiac functions, and cardiac remodeling in trained two-kidney one-clip hypertensive (2K1C) rats. For this purpose, rats were divided into sedentary and trained groups. Each group consists of sham and 2K1C rats with and without Ang-(1-7) infusion. Swimming training was performed for 1 hr/day, 5 days/wk for 4 wks following 1 wk swimming training for acclimatization. 2K1C rats showed moderate hypertension and left ventricular hypertrophy without changing left ventricular function. Chronic infusion of Ang-(1-7) attenuated hypertension and cardiac hypertrophy only in trained 2K1C rats but not in sedentary 2K1C rats. Chronic Ang-(1-7) treatment significantly attenuated increases in myocyte diameter and cardiac fibrosis induced by hypertension in only trained 2K1C rats. The Mas receptor, Ang II type 2 receptor protein, and eNOS phosphorylation in ventricles were upregulated in trained 2K1C rats. These results suggest that Ang-(1-7) increased the ANP secretion at high atrial pacing via Mas/PI3K/Akt pathway and the activation of NHE-1 and CaMKII. Chronic infusion of Ang-(1-7) attenuates hypertension and cardiac remodeling via an increased Mas receptor expression induced by exercise in trained 2K1C rats. (supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (No. 2008-0062279).

## PL

### Dynamic Aspects of the Function and Stoichiometry of Ion Channel Complexes

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Ion channel studies were established 60 years ago by the works by Hodgkin & Huxley. With the cDNA isolation since 1980's, the molecular identity of ion channels has been elucidated. Also, the snapshots of ion channels in the crystal have been accumulated by the recent crystal structure analyses. It appears the current trend of ion channel studies are directed to the understanding of dynamic aspects of the function and structure, in other words, the movies of ion channels in function, towards the elucidation of functioning mechanisms. In this presentation, I would like to introduce two topics on the dynamic aspects of ion channels from our group's research.
ATP receptor channel P2X is known to function in various perceptions such as pain, taste and shear stress. It is a trimer of two transmembrane type subunits with no canonical voltage sensor domain. We previously observed that the pore properties of $P2X_2$ changes depending on the open channel density of $P2X_2$ (Fujiwara and Kubo, J Physiol 2004). We also observed that $P2X_2$ shows clear voltage dependent activation in spite of the absence of canonical voltage sensor and that the voltage dependence shifts in accordance with [ATP] (Fujiwara et al. J Gen Physiol 2009; Keceli and Kubo, J Physiol 2009). These unexpected features demonstrate situation dependent changes of $P2X_2$.
Voltage-gated $K^+$ channel KCNQ1 assembles with accessory subunit KCNE1 to form a KCNQ1/KCNE1 complex. Its ionic current is called $I_{Ks}$ in the cardiac muscle cells and the mutations in KCNQ1 or KCNE1 are known to cause arrhythmia named Long QT syndrome. The most striking effect of KCNE1 on the function of KCNQ1 is a deceleration of voltage dependent activation. We previously showed that the effect is due to the decrease of the rate of the upward movement of the voltage sensor (Nakajo and Kubo, J Gen Physiol, 2007). We also identified the structural background for the interaction of KCNQ1 with KCNE1 or with KCNE3 using KCNQ1 ortholog of tunicate, which does not interact with KCNE (Nakajo et al. 2011). The molar ratio of assembly (stoichiometry) of KCNQ1/ KCNE1 had been generally accepted to be 4:2 but not yet determined conclusively. We directly counted the number of KCNE1 in the complex at a single molecule level by monitoring the bleaching steps of attached fluorescent proteins. We observed a clear presence of the 4:4 complex, and demonstrated that the stoichiometry of KCNQ1/KCNE1 changes depending on the relative expression level (Nakajo et al. Proc Natl Acad Sci USA, 2009). Using tandem constructs of E1-Q1 (which results in 4:4) or E1-Q1-Q1 (which results in 4:2), we demonstrated that their phenotypes such as conductance-voltage (G-V) relationship clearly differ one another. We also observed that the G-V relationship of

E1-Q1, but not of E1-Q1-Q1, changes with the extension of the linker length, suggesting that the assembly is dynamic.
In summary, these studies demonstrate situation-dependent flexible changes of the structure and function of ion channels. We believe the provided information is critical towards the elucidation of dynamic aspects of functioning ion channels.

## CURRICULUM VITAE

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S-I-1

## Synapse-to-Nucleus and Nucleus-to-Synapse Signaling: Molecular Mechanisms of Arc/Arg3.1 Regulation and Its Role on Synaptic Function

Hiroyuki Okuno

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The neuronal immediate early gene *Arc* (also known as *Arg*3.1) is one of the most dynamically regulated genes in the brain and its expression is tightly linked to information processing in neuronal circuits. *Arc* plays critical roles in AMPA receptor (AMPA-R) trafficking, synaptic plasticity, experience-dependent cortical reorganization, and long-term memory formation. Over the past years, we have focused on investigating the molecular basis of the signaling from synapses to the nucleus that regulates *Arc* transcription. We systematically analyzed the promoter region of the *Arc* gene and identified a potent synaptic activity-responsive element named SARE. Strikingly, the SARE of the *Arc* gene consisted of a unique cluster of binding sites for CREB, MEF2 and SRF, each of which significantly contributing to converting synaptic responses into transcriptional activation. Current efforts are being focused to take advantage of the understanding of these mechanistic details to visualize and manipulate specific neuronal circuits in which the signaling from synapses to the nucleus has been enhanced.

We have also been interested in how the newly expressed genes in the nucleus modified the function of synapses. We thus investigated dynamics and function of *Arc* protein at postsynaptic sites and uncovered a preferred targeting of *Arc* protein to inactive synapses, via its interaction with an inactive form of the beta subunit of calcium/calmodulin-dependent kinase II (CaMKII$\beta$). Importantly, synaptic *Arc* accumulation correlated with removal of surface GluA1 from individual synapses. Our findings suggest a novel "inverse" synaptic tagging mechanism that enhances the clearance of surface AMPA-R at un-potentiated synapses, thereby helping to maintain the contrast of synaptic strength between strong and weak synapses.

S-I-2

## Structural and Molecular Remodeling of Single Dendritic Spines during Long-Term Potentiation

Yasunori Hayashi

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Synapses store information by long-lasting modifications of their structure and molecular composition, but the precise chronology of these changes has not been studied at single synapse resolution in real time. I will present a model describing how and when postsynaptic substructures are reorganized during long-term potentiation (LTP). We imaged protein trafficking during induction of LTP in single dendritic spines by 2-photon glutamate uncaging. We identified four distinct patterns of protein dynamics and three sequential phases. I will focus on two surprising and opposite findings: 1) the actin depolymerization factor cofilin was rapidly and persistently enriched in the spine, forming a stable complex with F-actin, necessary for the consolidation of the spine enlargement; and 2) the postsynaptic density (PSD) was independently remodeled, as PSD scaffolding proteins did not change their amount and localization until a delayed protein synthesis-dependent third phase. These findings may represent the molecular explanation for known phenomena of metaplasticity such as synaptic lability, saturation and tagging.

S-I-3

## Mechanism and Adult Treatment for the Memory Deficits Associated with Mouse Models of RASopathy

Yong-Seok Lee

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Noonan syndrome (NS) is a developmental disorder affecting 1 in 2,500 live births and 30% to 50% of NS patients show cognitive deficits. Mutations in the *Ptpn11* gene, which up-regulate Ras-ERK signaling, account for ~50% of NS. In this talk, I will present our recent data showing that heterozygous knock-in mice expressing NS-associated gain-of-function *Ptpn11* mutations show spatial learning impairments as well as deficits in synaptic plasticity. We found that the abnormal increase in ERK activity result in the changes in excitatory synaptic transmission, deficits in hippocampal CA1 LTP and consequently in spatial learning impairments. Moreover, we found that a brief treatment with an FDA approved drug, lovastatin, which reduces Ras-ERK activation in the brain, rescues both the LTP and learning deficits in adult NS mutant. Our results demonstrate that increases in basal levels of ERK activity and corresponding impairments in LTP are responsible for the learning deficits in mouse models of NS. Furthermore, these data suggest that lovastatin may be used in treating the cognitive deficits in NS.

S-I-4

## Neuron-glia Crosstalk Under Pathological Condition Of Alzheimer's Disease

Inhee Mook-Jung

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Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder that leads to cognitive dysfunction, memory impairment and emotional disturbance in elderly persons. Activated microglia and reactive astrocytes are commonly found in and around the senile plaques that are the central pathological hallmark of AD. Beta-amyloid peptide (Abeta) accumulates in these plaques. Astrocytes respond to neuronal activity through the release of gliotransmitters such as glutamate, d-serine and adenosine 5'-triphosphate (ATP). How gliotransmitters regulate neuronal activity, however, is not well defined and even controversial. Also, astrocyte secreted several proteins to the synapse, which modulate synaptic function including synaptogenesis and neurogenesis directly or indirectly to the neurons. In the present study, we examined the effect of one of gliotransmiitters, ATP on neurons damaged by Abeta42 peptides in both primary astrocytes and U373 astrocyte cell line. We found that exogenous ATP protects against Abeta42-mediated reduction in synaptic molecules, such as NMDA receptor 2A, PSD-9ATP5 and synaptophysin, through purinergic receptor P2X in primary hippocampal neurons. ATP also prevented Abeta42-induced spine reduction and impaired long-term potentiation in the hippocampal neurons. Our findings suggest that Abeta-induced gliotransmitter ATP plays a protective role against Abeta42-mediated synaptic plasticity disruption. As an astrocyte-secreted protein, thrombospondin-1 (TSP-1) was examined *in vitro* and in AD animal model (5XFAD mice). The release of TSP-1 from astrocytes was decreased by Abeta42 *in vitro*, and the reduced level of TSP-1 was observed in brains of AD animal models. Synaptic pathology caused by Abeta42 such as decreased dendritic density, impaired synaptic activity, and reduced long-term potentiation (LTP) were prevented by co-incubation with TSP-1 and Abeta. TSP-1 is a potential therapeutic component against the damaging effects caused by Abeta42 in AD pathogenesis.

S-II-1

## Modelling for Better Understanding Physiological Phenomena

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Model is used in various circumstances. The model is used the most frequently when the hypothesis is set to design the experiment. This is the prime role of the model. However, the model can do more, when it is combined with the natural basic sciences, that is, the prediction with the computational aid. The first successful model in physiological science is the nerve action potential (AP) model made by Hodgkin and Huxley in 1952. It could predict and explain virtually all aspects of AP. And also, it provoked the debate on the ion specific pathway in the cell membrane. Model can also be used to explain the unpredictable physiological phenomena in the experiments. When we studied pH regulation in cardiac myocytes, the rapid alkalization with $NH_4^+$ pre-pulse in $CO_2/HCO_3^-$ buffer induced the bizarre pH transient. From the first model of pH regulation in cardiac myocytes, we could explain this was caused by the out-of-equilibrium of the $CO_2/HCO_3^-$ buffer. Noma perfused the half of single isolated cardiac myocytes with the different osmolarity to the other half and could get the width changes of both side were opposite (Sasaki, 1999). The mechanistic explanation was not possible and we developed the model and it could be happened by the opposite movements of water and ion from each compartment. From the model, we could obtain the hydraulic conductivity of the cytoplasm, too. The basic concepts of model could be expanded the other situation. Vaughan-Jones group perfused the different concentrations of weak acid or alkali in the part of ventricular myocytes and could generate the opposite change of pH and produced a large pH gradient in single cardiac myocytes (Swietach et al., 2005). Model was developed and could explain these phenomena were occurred by the proton shuttle of weak acid or alkali in cardiac myocytes. The generated pH gradient could also generated intracellular $Ca^{2+}$ gradient. The developed model could explain these phenomena were explained by shuttling proton and $Ca^{2+}$ buffer. The shuttling movement of the buffers could act as a $Ca^{2+}$-$H^+$ exchanger in sarcoplasm (Swietach, 2013). Model could be reused and developed further to explain or to reconstruct the experimental data. We developed the electrical activities of cardiac myocytes in pulmonary vein. The spontaneous action potential, $Ca^{2+}$-activated Cl- channel, and the release dependent inhibition of L-type $Ca^{2+}$ channel were reconstructed and examined in the model (Leem et al., 2006; Seol et al., 2008; Ryu et al., 2012). The majority part of the model was come from the previous results. In this case, the model could be a database for the experimental results. Physiome which is based on the model with computational aid will be the new field in physiology to hypothesize and predict the experiments using the model, to explain the experimental data, to integrate the experimental results and to accumulate the physiological knowledge.

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S-II-2

## An Updated Model of Interstitial Cells of Cajal Reproducing Intestinal Pacemaker Activity

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There is a consensus that interstitial cells of Cajal play as a pacemaker in gastrointestinal system. Previously, we constructed a biophysically based model of interstitial cells of Cajal in mouse small intestine to reproduce pacemaker activity. Since ion channels contributing to the pacemaker activity have been substantially updated, we tried to improve our mathematical model by including those ion channels and updating pre-existing components. We incorporated 5 more ion channels into our previous model. They are voltage-gated $Na^+$ channel ($Na_v$ 1.5), $Ca^{2+}$-activated $Cl^-$ channel, ERG $K^+$ channel, $Ca^{2+}$-activated $K^+$ (BK) channels, and $Na^+$-leak channel (NALCN). Modeling of ion channels were achieved by data fitting to curves for time course of each current, steady-state activation or inactivation, and voltage-dependence of time constants. The $IP_3$-mediated $Ca^{2+}$ release is a key event to drive regenerating pacemaker potentials and was updated to reproduce its stochastic behavior. The stochastic currents were reproduced by simulating the random openings and closing of individual ion channel. The updated model was able to reproduce stochastic feature of pacemaker potentials in interstitial cells of Cajal. Pacemaker potentials were not uniform in size, duration, and frequency. The resting and overshoot potential were -72.42±0.51 (mean±S.D., n=10) and -3.45±0.27 (n=10), respectively. The frequency was about 32 $min^{-1}$ and the duration at 50% repolarization was 614.3±40.6 (n=10). The model suggests that the $Na^+$-leak channel contributes to depolarization about 10 mV in resting membrane potential. The model also suggests that $Ca^{2+}$-activated Cl- channel is more likely to stabilize membrane potential rather than to excite under the physiological condition. We conclude that this improved mathematical model could give an insight how ion channels and $IP_3$-mediated $Ca^{2+}$ release drives pacemaker activity in gastrointestinal system.

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S-II-3

## A Physiomic Model for the Analysis of Heart Mechanics and Blood Circulation

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An integrated model of heart coupling excitation-contraction (EC) coupling of cardiac cell, tissue mechanics, blood flow and cardiovascular hemodynamics is proposed for analyzing cardiac mechanics and hemodynamics. For this purpose, we developed a comprehensive ventricular model with multi-physics and multi-scale characteristics that simulates the physiological events from membrane excitation of a cardiac cell to contraction of the ventricle, blood circulation in ventricle, coronary arteries and aorta. A lumped parameter model is used to compute the systemic and pulmonary circulations interacting with the cardiac tissue mechanics. We used a finite element method and the Galerkin approximation to discretize the three-dimensional (3-D) domain spatially. Model of body surface potential mapping in torso surface was also implemented to reproduce pseudo-EKG waves. Using the model, we delineated the blood vortex phenomena of ventricular hemodynamics. Simulated physiological results such as EKG waves and blood pressures were compared with clinical observations.

S-II-4

## Comparisons of Clinical Catheter Ablation of Atrial Fibrillation and Virtual Ablation on the Personalized *in-silico* Left Atrial Electroanatomical Modeling

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**Purpose:** Although catheter ablation of atrial fibrillation (AF) is an effective rhythm control strategy, there are substantial recurrences. The purpose of this study is to develop a computational platform that can build patient-specific model of AF ablation, and compare the outcome of clinical ablation and 5 different designs of virtual ablation.
**Methods:** Finite element model of atrium was built using geometry information from NavX data for 20 patients. The time-dependent distribution of electrical potential in the wall of atrium was obtained by solving diffusion equation adopting a mathematical model of ion channels of human atrium. Five different protocols of virtual ablation were applied to each individual atrial fibrillation model: 1) circumferential pulmonary vein isolation (CPVI), 2) complex fractionated electrogram guided ablation (CFAE), 3) CPVI+posterior wall box isolation (Box), 4) CPVI+Box+anterior linear ablation (Ant), 5) CPVI+CFAE. The time duration for which fibrillation was sustained was recorded for each ablation protocol and for each patient. The simulation results were compared with clinical ablation strategies those were chosen by the operator's decision.
**Results:** 1. Among 5 virtual ablation protocols in each 20 patients, CPVI+Box+Ant resulted in the highest number of AF termination (55%; 11/20) within 3.3 sec, and accord to the operator's decision in 86% (6/7). 2. The most frequently selected clinical protocol was 4PVI+Box+Ant (9 out of 20) which also produced high AF termination tendency compared to other 4 protocols in-silico (56% vs. 22%, p=0.06). 3. The patients whose fibrillation terminated were significantly younger than those whose fibrillation did not terminate (55.4±9.3 vs. 67.7±7.0, p=0.015).
**Conclusion:** Virtual AF ablation on the personalized modeling of LA was feasible and showed relatively similar outcome to the clinical AF ablation. For the clear validation of personalized virtual AF ablation modeling, prospective randomized clinical trial for *in-silico* guided AF ablation will be warranted.

*Both authors contributed equally to this study.

S-II-5

## Hemodynamics in Vascular Disease

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Hemodynamics is a field of physiology, which deals with physics related to the cardiovascular system, while it implies blood fluid dynamics in biomedical engineering. Blood flow plays an important role of transporting gas and nutrients to the tissues by circulation as well as maintaining adequate pressure for organ function. Blood flow in the vascular system applies physical forces, such as pressure and wall shear stress, on the vessel wall, and they may change the functions of endothelium which influences genesis and progression of vascular diseases such atherosclerosis and aneurysm.
Blood flow characteristics will be introduced in order to help the understanding of hemodynamics in the blood vessel, and the computational fluid dynamic studies in hemodynamic analysis on the initiation, progress and rupture of cerebral aneurysms will be mentioned. Moreover, current researches on hemodynamic effects on the genesis and progression of atherosclerosis will be summarized and clinical significance of hemodynamic studies will be mentioned.

S-III-1

## The Power of Mouse Genetics: Genome Engineering in Mice by TALENs and RGENs

Han-Woong Lee

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Phenotypic analysis of gene-specific knockout (KO) mice has revolutionized our understanding of in vivo gene functions. As the use of mouse embryonic stem (ES) cells is inevitable for conventional gene targeting, generation of knockout mice remains a very time-consuming and expensive process. To accelerate the large-scale production and phenotype analyses of KO mice, international efforts has organized global consortium such as the International Knockout Mouse Consortium (IKMC) and International Mouse Phenotype Consortium (IMPC), and they are persistently expanding the KO mouse catalogue that is publically available for the researches studying specific genes of interests *in vivo*. In addition, new technologies, adopting Transcription Activator-Like Effector (TALE) Nucleases (TALENs) and RNA-guided endonucleases (RGENs) to edit the mouse genome, are now emerging as valuable and effective shortcuts alternative for the conventional gene targeting using ES cells. Here I describe the establishment of gene-knockout mice by the injection of RGENs as Cas9 protein:guide RNA complexes or Cas9 mRNA plus guide RNA into one-cell stage embryos of both species. RGENs efficiently generated germ-line transmittable mutations in newborn mice with minimal toxicity. RGEN-induced mutations in the mouse *Prkdc* gene both in $F_0$ and $F_1$ mice. We propose that RGEN-mediated mutagenesis in animals will greatly expedite the creation of genetically-engineered model organisms, accelerating functional genomic research.

S-III-2

## Prediction of Gene Function Through Mouse Phenotype Analysis in Metabolic Syndrome

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Mouse models are crucial for the functional annotation of human genome. Gene modification techniques including gene targeting and gene trap in mouse have provided powerful tools in the form of genetically engineered mice (GEM) for understanding the molecular pathogenesis of human diseases. Several international consortium and programs are under way to deliver mutations in every gene in mouse genome. The information from studying these GEM can be shared through international collaboration. However, there are many limitations in utility because not all human genes are knocked out in mouse and they are not yet phenotypically characterized by standardized ways which is required for sharing and evaluating data from GEM. The recent improvement in mouse genetics has now moved the bottleneck in mouse functional genomics from the production of GEM to the systematic mouse phenotype analysis of GEM. Enhanced, reproducible and comprehensive mouse phenotype analysis has thus emerged as a prerequisite for effectively engaging the phenotyping bottleneck. In this review, current information on systematic mouse phenotype analysis and an issue-oriented perspective will be provided.

S-III-3

## Mechanism and Therapy for Arteriovenous Malformation

S. Paul Oh

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Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant vascular disorder, characterized by spontaneous recurrent nosebleeds, mucocutaneous telangiectases, and arterio-venous malformations (AVMs) in the brain, lung, liver or GI tract. While reduced expression of either endoglin (*ENG*) or activin receptor-like kinase 1 (*ALK1*) has shown to be associated HHT, the precise pathogenetic mechanisms underlying HHT remain elusive; and thus, while management options for HHT patients are well established, treatment options for this malady is currently lacking. The ultimate goal of my laboratory is to develop novel therapeutic reagents for treating HHT. To reach this goal we set out the following five stepwise goals: 1) development of mouse models that reproduce clinical features of vascular malformations found in HHT patients; 2) elucidation of pathogenetic mechanisms that underlie the vascular malformations using the animal model; 3) discovery of potential therapeutic target that can prevent or reverse the vascular malformations based on the mechanism; 4) preclinical validation of effects of the potential therapies using the animal models; 5) clinical trials of validated therapies through multi-HHT centers of excellence. I will present recent progress in the development of reliable animal models for HHT study. With this model, we unveiled novel pathogenetic mechanisms of disease. Potential therapeutic targets, preliminary data from preclinical studies, and future plans will be discussed.

S-III-4

## A Rat Model of Atopic Dermatitis

Seung Keun Back, Keun-Young Jeong, Chengjin Li, Jaehee Lee, Sat-Byol Lee, Heung Sik Na

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**Purpose:** The pathophysiological mechanisms underlying chronic pruritic skin diseases, e.g. atopic dermatitis (AD) remain elusive due to the paucity of animal models. Recently, we rediscovered that injection of capsaicin into rat pups resulted in vigorous scratching behavior and chronically relapsing AD-like cutaneous lesions well into adulthood. In the present study, we performed to characterize the chronic pruritic dermatitis induced by neonatal capsaicin treatment.
**Methods:** Capsaicin (50 mg/kg) was given to rat pups subcutaneously within 48 h after birth, and then scratching behavior, dermatitis and pathophysiological changes of rat skin were investigated chronologically.
**Results:** Neonatal capsaicin treatment led to not only severe scratching and cutaneous lesions but also a large number of pathophysiological changes in the skin, such as histopathological changes including the deficiency of epidermal filaggrin expression, increases in the number of mast cells, levels of tissue NGF and Th2 cytokine mRNA, impaired skin barrier function and colonization with Staphylococcus aureus. In addition, we observed the hyperproduction of serum IgE, which is clinically similar to the pathophysiology seen in the patients with atopic dermatitis. During the follow-up observation, the rats showed the alternative periods of relapsing and remitting skin lesions. Injection of capsaicin into rat pups results in chronically relapsing pruritic dermatitis, similar to human AD.
**Conclusion:** The rat model induced by neonatal capsaicin treatment could be useful for studying human AD and for the development of novel therapeutic drugs.
The Public Welfare & Safety research program through the NRF funded by the Ministry of Education, Science and Technology (NRF-2012-0009675).

## SFS-I-1

### Revisiting Ion Channel Mechanosensitivity

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Mechanotransduction refers to the conversion of mechanical force into biologic signals such as membrane depolarization. Activations of mechanosensitive ion channels such as stretch-activated $Ca^{2+}$-permeable non-selective cation channels are usually involved in the mechanotransduction process. However, only few mechanosensitive and/or mechanically activated (MA) channels have been identified to date. Moreover, mechanosensitivity of the previously-believed MA channels such as TRPC1 and TRPC6 was questioned. Besides ion channels, critical roles of some non-ion channel proteins that are linked to kinase activities such as G-protein-coupled receptors, epidermal growth factor receptors, and membrane nanostructures such as caveolae are now emerging in mechanotransduction process. In this talk, roles of these non-ion channel MA proteins in addition to MA ion channels during mechanotransduction process will be discussed.

## SFS-I-2

### The Mechanosensitivity of TREK-2 Channel: Role of PLC Activation by Membrane Stretch

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TREK-2 is a member of the two-pore domain $K^+$ channel family, and sets the resting membrane potential in many types of cells. TREK-2 controls diverse functions in several physiological processes including providing the electrical driving force that is required for calcium ion ($Ca^{2+}$) influx, cell volume regulation, and apoptotic volume decrease. TREK-2 is regulated by various chemical and physical stimuli such as polyunsaturated free fatty acids, intracellular pH, membrane stretches, and etc.
TREK-2 channels are inhibited by $PIP_2$ under physiological conditions. In inside-out patch clamp studies, the application of ATP (1 mM) inhibited TREK-2 due to the generation of $PIP_2$ by phosphoinositide (PI)-kinases. Consistently, ATP-free condition induced tonic activation of TREK-2. In the presence of ATP, membrane stretch induced by negative pipette pressure activated TREK-2, and this was completely inhibited by $PIP_2$. An application of methyl-$\beta$ cyclodextrin, a cholesterol scavenger disrupting lipid rafts, activated TREK-2 and facilitated the activation by stretch. In cell-attached patches, TREK-2 was activated by hypotonic swelling of cells as well as by negative pressure. The mechano-activation of TREK-2 was blocked by U73122, a PLC inhibitor. Neither actin depolymerization nor the inhibition of lipid phosphatase blocked the mechanical effects. Western blot analysis and confocal microscopy showed that the hypotonic swelling induces tyrosine phosphorylation of PLC$\gamma$2 and $PIP_2$ hydrolysis of plasma membrane. From the above results, we propose that the degradation of $PIP_2$ caused by stretch-activated PLC releases TREK-2 from the tonic inhibition by $PIP_2$.

SFS-I-3

## Segregation of TRP Channels in *Drosophila* Mechanosensory Cilia

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The *Drosophila* TRPN homolog, NOMPC, is required to generate mechanoreceptor potentials and currents in tactile bristles. NOMPC is also required, together with a TRPV channel, for transduction by chordotonal neurons of the fly's antennal ear, but the TRPN or TRPV channels have distinct roles in transduction and in regulating active antennal mechanics. Here we determined subcellular location-key for understanding there exact role in transduction-of TRP channels. Immunostaining showed that NOMPC is localized at the tips of mechanosensory cilia in both external and chordotonal sensory neurons, as predicted for a mechanotransducer channel. In chordotonal neurons, the TRPN and TRPV channels are respectively segregated into distal and proximal ciliary zones. This zonal separation is demarcated by and requires the ciliary dilation, an intraciliary assembly of intraflagellar transport (IFT) proteins.
Our results provide a strong evidence for NOMPC as a primary transduction channel in *Drosophila* mechansensory organs. The data also reveals a structural basis for the model of auditory chordotonal transduction in which the TRPN and TRPV channels play sequential roles in generating and amplifying the receptor potential, but have opposing roles in regulating active ciliary motility.

SFS-I-4

## Ectopic Pacemaking in Atrial Myocytes under Fluid Pressure

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Atrial fibrillation (AF) is the most common cardiac arrhythmia and occurs when several ectopic sites in the atrium fire. Although atrial enlargement is linked to AF, clinical evidence demonstrates that a regurgitant blood-jet or catheter whip against the atrial wall directly causes AF. Here we show that the application of fluid pressure to single atrial myocytes generates an arrhythmic beat through the activation of stereotyped local $Ca^{2+}$ waves originating from dense, disordered ryanodine receptor clusters. This arrhythmogenic local $Ca^{2+}$ wave is mediated by the activation of phospholipase C and inositol 1,4,5-trisphosphate receptors. When FP-induced local $Ca^{2+}$ waves are relatively fast, they elicit action potential via the forward-mode $Na^{+}$-$Ca^{2+}$ exchanger. In contrast, when the local $Ca^{2+}$ waves are slower, they trigger premature contraction without action potential. Our data provide $Ca^{2+}$-dependent arrhythmogenic mechanisms for AF initiation under fluid pressure.

SFS-II-1

## Why Do We Study Drosophila Melanogaster?: A Powerful Genetic Model System to Study Molecular Functions and Relevant Behaviors

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Studying correlations between functions of a certain molecular network and relevant behaviors from a single organism, especially in mammals, can often be challenging due to their complex anatomical structures and concurrent technical difficulties. Thus, it requires a development of alternative experimental models that offer simpler networks at a system level without compromising essential components of cellular functions. *Drosophila* melanogaster is one such model system that presents the following advantages. First of all, its fully sequenced genome is represented by highly conserved genes in that more than 50% of *Drosophila* proteins, including those involved in regulation of key cellular signaling pathways, are matched with mammalian homologs. Furthermore, well-advanced genetics, best characterized by highly sophisticated tools for genetic manipulations, empowers researchers with such high spatial and temporal controls of gene expression. In addition, its stereotypic, yet highly plastic behaviors allow systematic analyses of behavioral consequences following gene manipulations at a single-cell level. Lastly, the short life span of *Drosophila* with a large number of progeny produced in each cycle makes monitoring of cellular and behavioral changes very efficient. In line with these statements, we will present you today fascinating studies in *Drosophila* conducted by young Korean investigators and hope to make you believe at the end that *Drosophila melanogaster* still stands firm as a powerful model system to study molecular functions and relevant behaviors.

SFS-II-2

## Sensory Discrimination of Polymodal TRPA1 in *Drosophila*

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From flies to humans, Transient Receptor Potential (TRP) channels mediate the detection of various sensory stimuli, including temperature, chemicals, force and light. Individual sensory TRPs often maximizes the range of stimuli an animal can detect by acting as polymodal sensors; for example, many temperature-activated TRPs also respond to chemical cues. However, it sacrifices discriminatory power, which can be deleterious should one channel agonist elicit a behavior that is only appropriate for another agonist. Here we uncover a molecular mechanism for resolving such potential conflicts through cell-type specific restriction of TRP polymodality. In Drosophila, TRPA1 is a molecular sensor of both warmth (above ~25-27$^{o}$C) and noxious reactive chemicals. Therefore, warmth activation of TRPA1-expressing chemosensors would disrupt feeding at relatively moderate temperatures otherwise compatible with fly survival (~27-31$^{o}$C).However, we show that TRPA1- expressing taste cells respond to reactive chemicals, surprisingly not to warming. At the molecular level, this arises from tissue-specific expression of TRPA1 isoforms that contain distinct N-terminal modules which confer dramatically different temperature-responsiveness upon the channel. Thus, tissue-specific expression of distinct TRPA1 isoforms uncouples chemical nociception from thermosensation. We find similar functional diversity among TRPA1s from malaria transmitting mosquitoes, providing a potential explanation for the ability of these disease vectors to discriminate the warm-blooded host and reactive chemicals. The ability to cleanly restrict TRP polymodality in a cell-specific manner provides a potentially generalizable way to exploit polymodality to maximize the range of stimuli that can be detected without sacrificing discriminatory power.

SFS-II-3

## Modeling and Studying Parkinson's Disease Using *Drosophila* Genetics

Hyongjong Koh

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Parkinson's disease (PD) is the most common neurodegenerative disorder characterized by a selective loss of dopaminergic (DA) neurons. PD mainly occurs sporadically but can also occur genetically. After a mutation in alpha-synuclein was identified in a few families with PD, more than 20 PD-associated genes have been discovered. The cloning and characterization of these PD-associated genes initiated our understanding of the molecular mechanisms of the pathology of familial PD, facilitating the study of the underlying pathological mechanisms of sporadic PD. In developing PD model animals, mitochondrial toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat could induce parkinsonism accompanied by a loss of DA neurons, implicating the close relationship between mitochondrial dysfunction and PD pathogenesis. In recent Drosophila genetic studies, a deletion of PINK1, the PD-linked gene which encodes a mitochondrial kinase, results in a severe loss of motor activities and DA neurons accompanying with mitochondrial damages, suggesting PINK1 as the molecular link between PD and mitochondrial dysfunction. Further genetic and cell biological studies demonstrated that PINK1 translocates Parkin, an E3 ligase encoded by another PD gene parkin, and subsequently regulate mitochondrial remodeling process by inducing the Parkin-mediated ubiquitination of mitochondrial proteins, demonstrating that these two PD-liked genes have critical roles in maintaining mitochondrial integrity and function. In addition to investigating in vivo roles of PD-linked genes, Drosophila genetics also efficiently discover their molecular partners in preventing DA neuron loss, showing Drosophila as a valuable tool in modeling and studying human disease.

SFS-II-4

## A Stress Hormone Regulates the Female Reproduction by Facilitating the Sperm Storage

Young-Joon Kim

*GIST, Korea*

Modulatory substances such as neuropeptides and amines are ancestral molecules conserved across wide range of animal species, and many of them are implicated in physiological processes conserved throughout wide range of animal species, such as reproduction, feeding, and learning and memory. Here, we report a neuropeptide diuretic hormone (Dh44) orthologous to a vertebrate stress hormone corticotrophin releasing factor (CRF) regulates female reproduction by facilitating the sperm uptake. Suppression of Dh44 expression in the nervous system kept females from developing the post-mating behaviors characterized by increased egg-laying and decreased secondary mating. Using males producing GFP-tagged sperms, we showed that females lacking Dh44 could not uptake sperms from a copulation. The sperm uptake function of Dh44 is mapped to three pairs of central neurons expressing a stress protein, salt-induced kinase 2 (SIK2) in the pars intercerebralis (PI) region of the brain, suggesting a possible connection between stress responses and the sperm uptake. Dh44-R1, one of two Dh44 receptors appears to mediate the Dh44 action, because female lacking Dh44-R1 but not Dh44-R2 also failed to uptake sperms. Dh44-R1 is expressed in the central neurons sending efferent processes to the female reproductive organs, particularly involved in the sperm storage. Furthermore, by adopting GFP-reconstitution across synaptic partner (GRASP) technique, we demonstrated that Dh44 neurons in the PI made synaptic contacts with Dh44-R1 neurons. Together, our results indicate that the centrally release Dh44 activates Dh44-R1 neurons that innervates the reproductive organs, and facilitates the sperm uptake. We now are asking whether stress affects the sperm uptake and storage, and how Dh44 translates the stress signal to modulate sperm uptake.

SFS-II-5

## Role for dCLOCK in Behavioral Synchronization to Daily Temperature Cycle

Eun Young Kim

*Ajou University, Korea*

By means of circadian clock, all the living organisms on Earth can anticipate daily changes of light and temperature and manifest timely appropriate behavior and physiological processes. In general, light-dark cycles are the most strong synchronizing signal, whereas temperature cycles are the second most powerful.
Our current understanding of circadian clock mechanism has been strongly based on findings using Drosophila as a model system. Molecular clockwork is highly conserved across animal kingdoms and established via transcriptional/translational feedback loop (TTFL) composed of positively acting transcription factors (dCLOCK and CYCLE) and negatively acting clock proteins (PERIOD and TIMELESS). TTFL enables rhythmic expression of not only clock genes but also clock controlled genes involved in diverse physiological processes. Although TTFLs constitute the overall architecture of circadian timing mechanisms, several post-translational modifications of clock proteins are essential to generate 24-h. Most notably, time-of-day-dependent phosphorylation of clock proteins has been shown to be critical for setting the pace of circadian rhythms.
dCLK also undergoes circadian changes in phosphorylation state, present in a mostly intermediate phosphorylated state throughout the day, converting to largely hyper-phosphorylated isoforms in the late night/early day. In this talk, I will discuss about the role for dCLK phosphorylation in Drosophila circadian clock.

SFS-III-1

## Electrophysiology Related Cochlear Implantation

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Cochlear implant (CI) could partially restore hearing. This device artificially stimulates the spiral ganglion in the cochlea using electrodes called channel. Doing relative electrophysiologic study before and after cochlear implant is important for evaluating hearing function and objectively adjusting processor post-operatively, or electing candidates of cochlear implant pre-operatively. The stimulation mode describes the location of the reference electrode relative to the active electrode. There are two stimulation modes: monopolar (MP) and common ground (CG) stimulation. The ECAP represents a synchronous response from electrically stimulated auditory nerve fibers and is essentially the electrical version of Wave I of the auditory brainstem response (ABR). The ECAP is recorded as a negative peak (N1) at about 0.2-0.4 ms following stimulus onset, followed by a much smaller positive peak or plateau (P2) occurring at about 0.6-0.8 ms The amplitude of the ECAP can be as large as 1-2 mV, which is roughly larger in magnitude than the electrically evoked ABR (EABR; Brown et al., 1998). ECAP measures have become a popular alternative to clinical EABR testing due to ease of recording and reduced testing time. In contrast to the EABR, ECAP measures do not require surface recording electrodes, sleep/sedation, or additional averaging equipment. The ECAP is recorded via the intracochlear electrodes of the implant; therefore, the neural potential is larger than the EABR and thus fewer averages are needed, which significantly reduces testing time. EABR refers to electrically Evoked Auditory Brain stem Responses in reference to cochlear implants. EABR results are used in the development and refinement of the cochlear implant technology. After cochlear implant, there are two aspects that should be mainly focus on. First, assessing the function of auditory conductive system by predict the electrical response of surviving spiral ganglion nerve fiber populations to a cochlear implant. Second, adjusting cochlear implant processor periodically.

SFS-III-2

## Noise Induced Hearing Loss and Hair Cell Regeneration

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Loud sound can damage the inner ear and induce hearing loss. According to the level and spectrum of energy of the noise, hearing loss can be temporary or permanent. Permanent shift of hearing threshold (permanent threshold shift, PTS) is related to the permanent damage of the inner ear structure, including sensory hair cells, supporting cells, ganglion cells, and supporting structure of the inner ear.
On the contrary, temporary threshold shift (TTS) is characterized by the elevation of hearing threshold after insult and the recovery of hearing level within 1 or 2 weeks. After recovery of hearing, there is no degeneration of the cells in the inner ear, except ribbon synapse.
The studies of ribbon synapse of the inner ear of TTS have been recently reported. The number of the ribbon synapse decreased after TTS even with the full recovery of the hearing level. Because percentage of the survived number of ribbon synapse is related to the hearing level, there may be a threshold of the number of survived ribbon synapse.
Even though there is no hearing loss in TTS, TTS is related to the early development of age-related hearing loss (ARHL, presbycusis). The main research area in above topic is the change of the ribbon synapse and ganglion cells of auditory nerve. Relationship between noise induced TTS and ARHL is popularizing the researches on the central area, such as brain stem, midbrain, and auditory cortex.
Regeneration of the inner ear sensory hair cells is a long history of research. Though many pioneer reports on the morphological regeneration have been published, functional regeneration of hearing was hard to be shown. After long study of many genes and signal molecules, there was a paper published recently showing the evidence of functional hearing recovery as well as morphological recovery.
In this session, I will talk on the updated reports on the noise induced hearing loss and inner ear regeneration.

SFS-III-3

## Vestibular Afferent Signaling and Homeostasis

Gyu Cheol Han

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The electrical signal, produced through mechano-electric transductions (MET) in the vestibular end organ, is transferred through the vestibular afferent pathway, in the bipolar neurons, to the vestibular nucleus. The signaling occurred in the vestibular end organ is information responding to the acceleration of head movement. However, as it passes through the vestibular nucleus, it is transformed into velocity information toward rotational or linear movement and obtains tonic or phasic frequency-specific characteristics. An afferent signal directly affects eye movement and posture; however, the vestibular information passed through hippocampus and locomotor center is used in various bio-regulations associated with autonomic nervous system and gait respectively. The information generated in the vestibular system not only reflects the human head or body dynamics but also requires the maintenance of constant tonic status. Hence, the vestibular end organ maintains constantly-patterned vestibular information through homeostasis and allows for highly sensitive detection of excitation and inhibition. This homeostasis involves potassium recycling process and various sodium channel activations, affecting the MET and nerve conduction.
This talk organizes the overall process of vestibular signal occurrence, homeostatic transmission, and bodily adjustments by the upper central nervous system.

SFS-III-4

## Role of Vestibular System in Function of Basal Ganglia in Rats

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The main function of the vestibular system is the reflex control of posture and eye position during body movement. The central vestibular nuclei in the brain stem have a massive neural network in the brain, suggesting that vestibular afferent information can affect brain functions. Several clinical studies have demonstrated that peripheral vestibular activation has a transient therapeutic effect on central pain, post-stroke hemineglect, and phantom limb illusion in human being. Recent clinical trials also reported that vestibular stimulation showed the possibility as therapeutic options for alleviating symptoms of Parkinson's disease recently. However, there is a little information about underlying possible mechanisms for therapeutic effects of vestibular activation in Parkinson's disease (PD). This study was designed to elucidate basic phenomena and mechanisms for role of vestibular system in change of basal ganglia (BG) neural activity in hemi-Parkinson model of rats.

Bilateral deprivation of peripheral vestibular end-organs aggravated apomorphine-induced turning behavior in hemi-Parkinson's rats. In contrary to, electrical stimulation to the ipsi-vestibular nuclei reduced significantly turning movement by apomorphine was decreased in hemi-Parkinson's rats. The parafascicular nucleus (PF) of thalamus is an essential structure in the feedback circuits of basal ganglia-thalamo-cortical systems and has anatomical connection with central vestibular nuclei. The electrophysiological recording revealed that short-latency neuronal excitation of PF neuron was noted by vestibular stimulation. Single unit activity of PF neuron was increased with a frequency-dependent modulation in response to electrical stimulation of vestibular nuclei.

Peripheral vestibular inputs also reached major components of the basal ganglia such as striatum and substantia nigra pars reticulata (SNr), a main output center of BG with polysyanptic nature. The short-term stochastic galvanic vestibular stimulation (sGVS) reduced a slow oscillation ($<$1 Hz) of spike trains induced by dopamine cell lesion in SNr neurons. Recording the single-unit activity and local field potentials revealed that sGVS leaded to a mild suppression of beta rhythm (13~35Hz) power of subthalamic nucleus (STn), a major output of BG and the motor cortex. This rhythm was considered as abnormal neuronal activities caused by PD as well as the decreased correlation between the STn and the motor cortex. Furthermore, sGVS tended to decrease neuronal activities and irregularity of STn. These results of present study suggest that the alteration of neuronal activities in BG component and PF neurons by vestibular stimulation can be a possible mechanism for vestibular neuromodulation for alleviating symptoms of Parkinson's disease.

This study was supported by Korea Basic Science Institute's high field NMR research program grant T3022B.

## IC-1

### Curcumin Inhibits the Voltage-Dependent $K^+$ Channels in Rabbit Coronary Arterial Smooth Muscle Cells

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We investigated the effects of curcumin, the principal active compound of turmeric, on voltage-dependent $K^+$ (Kv) channels in freshly isolated rabbit coronary arterial smooth muscle cells using the voltage-clamp technique. Curcumin reduced the Kv current in a dose-dependent manner with an apparent Kd value of 1.07±0.03 $\mu$M. Although curcumin did not alter the kinetics of Kv current activation, it predominantly accelerated the decay rate of channel inactivation. The association and dissociation rate constants of curcumin were 1.35±0.05 $\mu$M-1s-1 and 1.47±0.17 s-1, respectively. Curcumin did not alter the steady-state activation or inactivation curves. Application of train pulses (1 or 2 Hz) increased curcumin-induced blockade of the Kv current, and the recovery time constant also increased in the presence of curcumin suggesting, that the inhibitory action of Kv currents by curcumin was use-dependent. From these results, we concluded that curcumin inhibited vascular Kv current in a state-, time-, and use-dependent manner.

**Key Words:** Curcumin, Voltage-Dependent $K^+$ channel, Coronary artery

## IC-2

### The Inhibitory Effect of $Ca^{2+}$ Channel Inhibitor Efonidipine On Voltage-Dependent $K^+$ Channel Activity in Coronary Arterial Smooth Muscle Cells of Rabbit

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The effect of efonidipine, a commercially available antihypertensive drug and $Ca^{2+}$ channel inhibitor, on voltage-dependent $K^+$ (Kv) channels was studied in freshly isolated rabbit coronary arterial smooth muscle cells using the whole-cell patch clamp technique. The amplitude of Kv current was decreased by application of efonidipine in a dose-dependent manner, with IC50 of 0.26 $\mu$M and a Hill coefficient of 0.91, which suggests 1:1 binding stoichiometry. Efonidipine did not affect voltage-dependent activation of the Kv channel, but shifted the inactivation curve by -8.87 mV. The inhibitory effect of efonidipine was not significantly changed by depletion of extracellular $Ca^{2+}$ or intracellular ATP, which indicated no involvement of the $Ca^{2+}$ channel or intracellular protein kinase-dependent cascades. We conclude that efonidipine dose-dependently inhibits Kv current in a phosphrylation- and $Ca^{2+}$ channel-independent manner.

**Key Words:** Efonidipine, Voltage-Dependent $K^+$ channels, Coronary artery

## IC-3

### The Inhibitory Mechanisms of LY294002, a PI3 Kinase Inhibitor on Voltage-Dependent $K^+$ Channels In Rabbit Coronary Arterial Smooth Muscle Cells

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We examined the effect of LY294002, a phosphatidylinositol 3-kinase (PI3K) inhibitor, on voltage-dependent $K^+$ (Kv) channels in smooth muscle cells from freshly isolated rabbit coronary arteries using the whole-cell patch clamp technique. The Kv current amplitude was inhibited by LY294002 in a dose-dependent manner, with a $K_d$ value of 1.48 $\mu$M. Without alteration of the kinetics of activation, LY294002 accelerated the decay rate of Kv channel inactivation. The rate constants of association and dissociation for LY294002 were 1.83±0.01 $\mu M^{-1}s^{-1}$ and 2.59±0.14 $s^{-1}$, respectively. Application of LY294002 had no significant impact on the steady-state activation or inactivation curves. In the presence of LY294002, the recovery time constant from inactivation was increased, and Kv channel inhibition increased under train pulses (1 or 2 Hz). This indicates that LY294002-induced Kv channel inhibition is use-dependent. Furthermore, pretreatment with another PI3K inhibitor, wortmannin (10 $\mu$M), did not affect the Kv current, and did not change the inhibitory effect of LY294002. Based on these results, we suggest that LY294002 directly blocks Kv current irrespective of PI3K inhibition.

**Key Words:** LY294002, Voltage-Dependent $K^+$ channel, Coronary artery

## IC-4

### Role of $Li^+$-permeable $Na^+/Ca^{2+}$ exchangers, NCLX, in the Exocytosis of Pancreatic $\beta$ Cells

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The $Na^+/Ca^{2+}$ exchangers are key regulators for $Ca^{2+}$ homeostasis in pancreatic $\beta$ cells, but their exact role in insulin secretion is not fully understood. In the present study, we investigated the role of $Na^+/Ca^{2+}$ exchangers on cytosolic $Ca^{2+}$ dynamics and exocytosis in INS-1 cells. We newly discovered that the Li+-permeable $Na^+/Ca^{2+}$ exchangers (NCLX), which are known as mitochondrial $Na^+/Ca^{2+}$ exchangers, contribute to plasma membrane $Na^+$-dependent $Ca^{2+}$ transport, and confirmed the presence of

NCLX in the plasma membrane using immunocytochemistry and cell surface biotinylation experiments. We further investigated the role of NCLX on exocytosis function by measuring depolarization-induced capacitance increase in siNCLX-transfected INS-1 cells. Downregulation of NCLX significantly suppressed the second phase capacitance increase without affecting the first phase, but inhibition of mitochondrial $Na^+/Ca^{2+}$ exchange alone by removing intracellular $Na^+$ did not cause a significant effect. These results suggest that normal surface NCLX activity is required for vesicle recruitment in the second phase. In addition, we discovered that oligomycin inhibited the second phase capacitance increase in the presence of intracellular ATP, suggesting that local metabolic control by mitochondria is critical for this phase. Our data indicate that the activity of surface NCLX may regulate local $Ca^{2+}$ to optimize mitochondrial local metabolic control and thus contributes to vesicle recruitment for the normal secretory function in pancreatic $\beta$ cells.

**Key Words:** NCLX, Pancreatic $\beta$ cells, Exocytosis, Cytosolic $Ca^{2+}$ dynamics

## IC-5

### *Trans*-Anethole Enhances Long-Term Potentiation Through Voltage-Dependent Calcium Channels Independent Pathway

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*Trans*-anethole is aromatic compound that widely used as a flavoring substance and essential oils. *Trans*-anethole derives from anis and fennel and it is also abundant in tarragon and basil. *Trans*-anethole has been used for the relief of pain and anxiety, and for the treatment of gastrointestinal disturbances in folk medicine. However, the effects of *trans*-anethole in learning and memory have not been studied. The aim of this study was to investigate the effects of *trans*-anethole on the induction of NMDA receptor-dependent long-term potentiation (LTP) and NMDA receptor-independent LTP. As results, *trans*-anethole promoted both LTP induced by one theta burst stimulation (TBS) and 4 TBSs which was a protocol to induce NMDA receptor dependent LTP and NMDA receptor independent LTP, respectively. Furthermore, *trans*-anethole exerts its facilitatory effects on NMDA receptor-independent LTP in condition of nifedipine (L-type voltage dependent calcium channels antagonist) application. These results suggest that *trans*-anethole may affect learning and memory.

**Key Words:** *Trans*-anethole, Long-Term potentiation, NMDA receptor dependent LTP, NMDA receptor independent LTP

## IC-6

### $Ca^{2+}$ Release via Ryanodine Receptors of ER Increase Somatic Excitability by Downregulating Somatic A-type $K^+$ Channels

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Internalization of A-type $K^+$ channels ($I_A$ channels) which are targeted by regulating both somatic excitability and synaptic plasticity in neurons, depends on $Ca^{2+}$ influx via NMDA receptors (NMDARs). In the present study, we tested if the downregulation of somatic $I_A$ channels is affected by $Ca^{2+}$-induced $Ca^{2+}$ release (CICR) from ER containing $Ca^{2+}$ channels such as Ryanodine receptors (RyRs) and $IP_3$ receptors ($IP_3$Rs) in primary hippocampal neurons. In results, the reduction of $I_A$ peak amplitude in soma by high $Ca^{2+}$ was abolished by antagonists of NMDARs or voltage-dependent $Ca^{2+}$ channels (VDCCs), indicating that the downregulation of $I_A$ channels requires the activation of presynaptic VDCCs to release endogenous glutamate as well as postsynaptic NMDARs activation. Moreover, the RyRs of $Ca^{2+}$ store dominantly participates in this downregulation of $I_A$ channels by releasing rapidly more $Ca^{2+}$ than $IP_3$Rs. The released $Ca^{2+}$ from the RyRs can independently change somatic excitability by regulating $I_A$ channels via PKA activation without extracellular $Ca^{2+}$ influx. We here suggest that RyRs of $Ca^{2+}$ store which are targeted by synaptic activities, are crucial to regulate neuronal excitability via $I_A$ channels trafficking. The research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. NRF-2012R1A1A2041935).

**Key Words:** $I_A$ channel, Ryanodine receptor, Somatic excitability, Synaptic modification

## IC-7

### Alterations of Contraction and L-type $Ca^{2+}$ Current by Murrayafoline-A in Rat Ventricular Myocytes

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In the present study, we examined the effects of murrayafoline-A (Mu-A), a monomeric carbazole alkaloid, on ventricular myocyte contractility and possible mechanisms underlying these effects. Cell shortenings and L-type $Ca^{2+}$ current ($I_{Ca,L}$) were measured in isolated adult rat ventricular myocytes using video edge detection and patch

clamp techniques, respectively. Mu-A increased contraction in a dose-dependent manner with an $EC_{50}$ of ~20 $\mu$M. The positive inotropic effect of Mu-A reached a maximum after about 2-min exposures, and then gradually decreased after ~1-min steady-state. Prolonged (>5-min) exposure to Mu-A increased the propensity of arrhythmic contraction, which was reversible. The rate of contraction, but not relaxation, was accelerated by Mu-A during the positive inotropic phase. Acute application of Mu-A dose-dependently increased $I_{Ca,L}$ ($EC_{50}$ of ~20 $\mu$M). The stimulatory effect of Mu-A on $I_{Ca,L}$ was followed by a reversal of the effect after ~2-min exposure under a whole-cell patch clamp. The enhancement of $I_{Ca,L}$ by Mu-A was larger and more prolonged under a perforated patch configuration than in the whole-cell mode, indicating a role of intracellular components in the enhancement of $I_{Ca,L}$. Intracellular application of Mu-A through the patch pipettes did not alter $I_{Ca,L}$. The positive inotropic effect by Mu-A was significantly attenuated by a partial inhibition of $I_{Ca,L}$, but it was not affected by the protein kinase A (PKA) inhibitors. Our data suggest that Mu-A induces an acute increase in the contractility via enhancement of $I_{Ca,L}$, and that this effect may be mediated by PKA-independent intracellular signaling activated by external Mu-A.

**Key Words:** Murrayafoline-A, Cell shortening, L-type $Ca^{2+}$ current, Positive inotropic effect, Rat ventricular myocytes

## IC-8

### Attenuated Benzodiazepine-Sensitive Tonic $GABA_A$ Currents of Supraoptic Magnocellular Neuroendocrine Cells in 24-h Water-Deprived Rats

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In supraoptic nucleus (SON) magnocellular neurosecretory cells (MNCs), $\gamma$-aminobutyric acid (GABA), through activation of $GABA_A$ receptors ($GABA_A$Rs), mediates persistent tonic inhibitory currents ($I_{tonic}$), as well as conventional inhibitory postsynaptic currents (IPSCs, $I_{phasic}$). Here, we examined the functional significance of $I_{tonic}$ in SON MNCs challenged by 24-h water deprivation (24WD). While the main characteristics of spontaneous IPSCs were similar in 24WD compared with euhydrated (EU) rats, $I_{tonic}$, indicated by bicuculline-induced $I_{holding}$ shifts, were significantly smaller in 24WD compared with EU rats ($p<0.05$ in all cases). Propofol and diazepam prolonged IPSC decay time to a similar extent in both groups, but induced less $I_{tonic}$ in 24WD compared with EU rats, suggesting a selective decrease in $GABA_A$ receptors mediating $I_{tonic}$ over $I_{phasic}$ in 24WD rats. THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol), a preferential $\delta$ subunit agonist, and L-655,708, a $GABA_A$ receptor $\alpha_5$ subunit selective imidazobenzodiazepine, caused a significantly smaller inward and outward shift in $I_{holding}$, respectively, in 24WD compared with EU rats ($p<0.05$), suggesting an overall decrease in extrasynaptic $GABA_A$ receptors mediating $I_{tonic}$ in 24WD animals. Consistent with a decrease in 24WD $I_{tonic}$, bath application of GABA induced significantly less inhibition of the neuronal firing activity in 24WD SON MNCs compared with EU SON MNCs ($p<0.05$ in all cases). Taken together, our results showed a selective decrease in $GABA_A$Rs functions mediating $I_{tonic}$ as opposed to those mediating $I_{phasic}$ in SON MNCs, demonstrating the functional significance of $I_{tonic}$ in increased neuronal excitability and hormone secretion in 24WD rats.

**Key Words:** Tonic $GABA_A$ current, Supraoptic nucleus, Water deprivation, Vasopressin

## IC-9(PO-2)

### De Novo KCNQ1 Mutation Responsible for Age-Dependant Bradycardia and Persistent Atrial Fibrillation

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Atrial fibrillation (AF) is the most common arrhythmia. Gain-of-function mutations in KCNQ1, the pore-forming $\alpha$-subunit of the slow delayed rectifier K current ($I_{Ks}$) channel, have been associated with AF. The purpose of this study was functional assessment of a mutation in KCNQ1 identified in a family with persistent AF and sinus bradycardia. We investigated whether this KCNQ1 missense mutation could form the genetic basis for AF and bradycardia simultaneously in this family. Sanger sequencing in a family with hereditary persistent AF identified a novel KCNQ1 variant (V241F) in a highly conserved region of S4 domain. The proband and her son developed bradycardia and persistent AF in an age-dependent fashion. The other son was a mutation carrier but he showed sinus bradycardia and not AF. Whole-cell patch-clamp electrophysiology showed that V241F mutation in KCNQ1 shifted the activation curve to the left and dramatically slowed deactivation, leading to a constitutively open-like phenotype. Computer modeling showed that V241F would slow pacemaker activity. Also, simulations of atrial excitation predicted that V241F results in extreme shortening of action potential duration, possibly resulting in AF. Our study indicates V241F might cause sinus bradycardia by increasing $I_{Ks}$. Additionally, V241F likely shortens atrial refractoriness to promote a substrate for reentry. KCNQ1 mutations have previously been described in AF, yet this is the first time a mutation in KCNQ1 is associated with age dependent bradycardia and persistent AF. This finding further supports the hypothesis that sinus

node dysfunction contributes to the development of AF.
**Key Words:** KCNQ1, Atrial fibrillation, Bradycardia

## IC-10

### Defective Arginine Methylation of KCNQ Induces Channel Dysfunction and Neuronal Hyperexcitability

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Arginine methylation is a widespread posttranslational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). It is well known that PRMT1, the predominant arginine methyltransferase, is implicated in various cellular processes, but their physiological roles in central nervous system (CNS) remain unclear. We found genetic haploinsufficiency of PRMT1 caused spontaneous seizures and increased locomotor activity in mouse models. To identify the neural circuits mediating this phenotype, we examined neural activity in acute hippocampal slices. Neurons from PRMT1+/- mice showed a substantial reduction of firing threshold and enhanced repetitive firing without change in spike height and width. Pharmacological block of PRMT1 caused similar changes in neuronal excitability to those observed in PRMT1+/- mice. Using voltage clamp technique, we confirmed the reduction of KCNQ channel activity. Consistently, the changes in firing properties were not further modulated by KCNQ channel blocker XE991, indicating KCNQ current deficiency contributed to persistent hyper-excitability and spontaneous seizures observed in PRMT1+/- mice. Using molecular and electrophysiological tools, we demonstrated that blocking methylation greatly reduces channel's interaction with membrane lipid, $PIP_2$, which is a necessary cofactor for KCNQ channel activity. Finally, the addition of $PIP_2$ restored KCNQ currents and consequently, stabilized the synaptic strength and returned firing rate to the control level. Taken together, our data suggest that genetic deletion of PRMT1 shifts the excitability balance towards enhancement, and leads to over-excitability and seizures by closing KCNQ channels.
**Key Words:** KCNQ2, PRMT1, Methylation, $PIP_2$

## IC-11

### Origin of Atrial Arrhythmogenic $Ca^{2+}$ Wave Under Fluid Pressure: Role of Dense, Disoriented RyR Clusters Coupled with Membrane Invagination

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In atrial myocytes lacking t-tubules, fluid pressure (FP, ~16 dyn/cm$^2$) has been found to elicit global $Ca^{2+}$ waves: one with longitudinal propagation (L-wave) and the other with transverse propagation (T-wave). T-wave is associated with action potential, whereas L-wave often, but not always, triggers action potential involving T-wave during its propagation. Here we examined spatiotemporal characteristics, and structural and molecular basis for the FP-induced L-wave using two-dimensional (2-D) confocal $Ca^{2+}$ imaging, combined with three-dimensional (3-D) visualizations of cell membrane and type 2 ryanodine receptors (RyR2), in rat atrial myocytes. We found that FP-induced L-waves generally originated from central focal $Ca^{2+}$ release sites ("L-wave core") located at 15-25% of the cell length. The core releases had 3~6 signal peaks and grew faster in one-direction in 2-D measurements. They were more prolonged and much larger compared with the $Ca^{2+}$ sparks. Tetracaine (1 mM, 10 s) reversibly inhibited the FP-induced core release as well as the L-wave, suggesting both involve the RyRs. Immediately after recording FP-induced L-wave, the cell's membrane was further visualized with wheat germ agglutinin (WGA, 1.25 $\mu$g/ml). Overlapping the L-wave core and the cell membrane, and reconstructing the WGA confocal image stacks in 3-D, revealed membrane invagination at the centrally localized core or in the vicinity of the core site. Interestingly, in some atrial cells we found denser (~0.62-$\mu$m intervals) disoriented RyR alignments in the cell interior at ~20% of the cell length and nearby the cell end. We conclude that the crumpled invaginated surface membrane, associated with dense RyRs, may play an important role in transducing fluid pressure into the core $Ca^{2+}$ signal, resulting in arrhythmogenic L-wave.
**Key Words:** Atrial myocyte, Fluid pressure, $Ca^{2+}$ wave, Membrane invagination, Ryanodine receptor

## IC-12(PO-4)

### Expression and Functional Role of Store-operated $Ca^{2+}$ Channel in Podocytes Involving Diabetic Nephropathy

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Diabetic nephropathy is a progressive kidney disease that is characterized by albuminuria and abnormal glomerular

function. Podocyte dysfunctions including apoptosis, thickening of the glomerular basement membrane and effacement of the podocyte foot process are all typical features of diabetic nephropathy. Intracellular $Ca^{2+}$ regulation is critical for maintaining foot process of podocyte. Store-operated calcium entry (SOCE) is a principal $Ca^{2+}$ influx mechanism in non-excitable cells. However, expression and functional role of SOCE in podocyte remain elusive. Here, we examined expression and regulatory mechanism of Orai channels which are pore-forming unit of SOCE. All Orai proteins were expressed in both mouse and human podocyte. Among them, Orai1 was highly expressed in podocyte of diabetic nephropathy patients and diabetic *db/db* mouse. Orai1 was responsible for SOCE in cultured podocyte that was confirmed by siRNA Orai1. Orai1 activation was inhibited by a PI3K or Erk1/2 inhibitors indicating that both Akt and Erk1/2 signaling cascades are critical for channel regulation. Phosphorylation of Akt was increased in kidneys of diabetic db/db mouse supporting the notion that PI3K-Akt pathway activates Orai1 channel. Orai1-mediated $Ca^{2+}$ influx directly reorganized the actin cytoskeleton and disturbed the focal adhesion of podocyte. Cytoskeletal rearrangement and increased turnover rate of focal adhesion of this cell enhanced cell motility and increased podocyte permeability to albumin. Taken together, these data suggest that Orai1 is highly expressed in podocyte of diabetic kidneys and increased activation of this channel directly remodels the actin cytoskeleton causing albuminuria. These results provided a new perspective on the pathogenesis of diabetic nephropathy and shed light on the treatment of the proteinuric diseases. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010-0024789)].

**Key Words:** Orai, Podocyte, Diabetic nephropathy

## IC-13

### Inhibitory Effect of Glucocorticoids on hERG Channels Expressed in Xenopus Oocytes

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Cortisone is an important stress hormone, and its active version hydrocortisone could increase blood sugar, suppress the immune system and aid in fat, protein and carbohydrate metabolism. We investigated the effect of cortisone and hydrocortisone on human erther-a-go-go-related gene (hERG) $K^+$ channels expressed in *Xenopus* oocytes by using two-microelectrode voltage-clamp. Both cortisone and hydrocortisone had an increasing effect on the amplitude of hERG $K^+$ channel currents at lower concentration (10-20 $\mu$M for both cortisone and hydrocortisone) and inhibiting effect at higher concentration. Also their block on hERG $K^+$ channels decreased progressively relative to the degree of depolarization. Cortisone and hydrocortisone inhibits the hERG $K^+$ channels mainly in the open and inactivated state rather than in the closed state. These research indicated that cortisone and hydrocortisone blocked channels with a similar mechanism.

**Key Words:** Cortisone, Hydrocortisone, hERG $K^+$ channels, Heart

## IC-14

### Membrane Trafficking and $PIP_2$-Dependent Regulation of Kir2.2 via TLR4 in THP-1 Human Monocyte

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Lipopolysaccharide (LPS) is widely used as an effective agonist for Toll-like receptor 4 (TLR4) in innate immune cells. However, investigation on the changes of ion channel activity induced by LPS in the macrophage is lacking. In THP-1 human monocyte cells stimulated by LPS (1 $\mu$g/ml), time-dependent changes of $K^+$ channel activity were investigated. Voltage-gated $K^+$ channel current (IKv) was decreased while $Ca^{2+}$-activated $K^+$ channel (IKCa) currents was increased after 6 h of LPS stimulation. Inwardly rectifying $K^+$ current (IKir) was absent in the control cell, whereas it was increased dramatically by LPS stimulation; reached a peak level at 4 h and slowly decayed to 25% of peak at 24 h. Single channel conductance of the LPS-induced Kir was 38 pS, and their $Ba^{2+}$-sensitivity was relatively high ($IC_{50}$, 1.42 $\mu$M), which were consistent with the known properties of Kir2.2. Both Kir2.1 and Kir2.2 proteins were expressed in THP-1 cells, and their total amounts were not changed by LPS. However, confocal microscopy revealed that plasma membrane expression of Kir2.2 was significantly increased whereas Kir2.1 expression is mostly limited to cytosol. The functional upregulation of Kir2.2 was also confirmed in freshly isolated primary human monocytes. The IKir increase was attenuated by Exo-1 (vesicular trafficking inhibitor). However, the spontaneous decay after 4 h of LPS was not prevented by dynamin (a GTPase responsible for endocytosis) inhibitor. Interestingly, the decayed IKir was reversed by wortmannin and LY294002, PI3 kinase (PI3K) inhibitors. In contrast, the LPS-induced IKir was largely abolished by 10 $\mu$M bpV (Phen), an inhibitor for PTEN (phosphatase of $PIP_3$). The LPS-induced IKir was not affected by inhibitors for Akt signaling pathways that are downstream of $PIP_3$-dependent mechanisms. Taken together, we firstly report that Kir2.2 is functionally upregulated by LPS via membrane trafficking, not *de novo* synthesis. The tonic stimulation of PI3K may have reduced the availability of $PIP_2$ that is known to be critical for Kir2 activity.

**Key Words:** Monocyte, THP-1, LPS, Kir2.2, $PIP_2$

## IC-15

### Delayed Calcium Activation of Anoctamin 6 (ANO6) Enhanced by Serotonin-Specific Reuptake Inhibitors (SSRIs)

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$Ca^{2+}$-activated $Cl^-$ channels (CaCCs) control diverse functions in several physiological processes. Anoctamins (ANOs) have been identified as a family of putative $Cl^-$ channels, and ANO1 and ANO2 have been identified as essential components of the CaCCs. Recently, several research groups have reported that ANO6 generates outward-rectifying $Cl^-$ currents by increasing intracellular $Ca^{2+}$ levels, which was activated by a delay of several minutes. However, the reason for the very slow activation of the $Ca^{2+}$ current remains unexplained. Selective serotonin reuptake inhibitors (SSRIs) are a class of widely used antidepressants that act through inhibition of various ion channels, including voltage-activated $K^+$ channels, two-pore $K^+$ channels, and CaCCs. In particular, ANO1 is sensitively inhibited by the SSRI fluoxetine. Using the whole-cell patch-clamp technique, we examined the effects of SSRIs, including fluoxetine, paroxetine, and sertraline, on cloned human ANO6 expressed in HEK293 cells. In contrast to its inhibitory effect on ANO1, SSRI reduced the latency time for the activation of ANO6 currents in a concentration- dependent manner and enhanced the peak current size resulting from ANO6 $Ca^{2+}$-dependent activation. However, ANO6 currents were inhibited in recordings by using an inside-out patch-clamp. According to this result, SSRI might be involved in ANO6 activation via an indirect pathway. However, the mechanism underlying SSRI action requires further study.

**Key Words:** ANO6, TMEM16F, Fluoxetine, SSRI, CaCC

## IC-16

### Mitochondria Depletion Modulates Inward-Rectifying $K^+$ Currents in L6 Myoblasts

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Mitochondria produce most of the cell's energy in the form of ATP and regulate cellular metabolism at different levels. Mitochondrial diseases result from genetic defects in mitochondrial DNA (mtDNA) or nuclear genes that encode mitochondrial proteins. A profound reduction in mtDNA copy number is the molecular hallmark of mtDNA depletion syndromes (MDSs). In the past decade, an increasing number of syndromes associated with the tissue-specific reduction of mtDNA copy number have been reported. For example, patients with muscle-specific MDS show severe muscle hypotonia and muscle atrophy. A multinucleated skeletal muscle fiber is formed by the fusion of mononucleated myoblasts; this process is essential for skeletal muscle development, growth, and repair. Myoblast fusion is strictly dependent on $Ca^{2+}$ levels. Intracellular $Ca^{2+}$ levels are tightly regulated by different types of ion channels. In this study, we compared various ionic currents associated with intracellular $Ca^{2+}$ influx in normal L6 myoblasts and mitochondria-depleted L6 myoblasts developed through long-term treatment with ethidium bromide. Interestingly, in the mitochondria-depleted L6 myoblasts, we found substantial decrease in the inward-rectifying $K^+$ current that normally hyperpolarizes the membrane to generate the electromotive force required for $Ca^{2+}$ influx. However, western blot analysis revealed that the expression levels of Kir 2.1, a classical inward rectifying $K^+$ channel, were identical in both mitochondria-depleted myoblasts and normal myoblasts. The mechanisms underlying the downregulation of $I_{kir}$ in mitochondria-depleted myoblasts need to be elucidated.

**Key Words:** Mitochondria, $I_{kir}$, $K^+$ channel, Myoblast

## IC-17

### RASD1 Activates TRPC4 through G $\alpha$ i Independently of GPCR

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

**Key Words:** TRPC4, Ionchannel

## IC-18

### Inhibition of hERG Potassium Channels by Escitalopram

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Escitalopram, which is a selective serotonin reuptake inhibitor (SSRI), is the pharmacologically active S-enantiomer of the racemic mixture of RS citalopram and is widely used in the treatment of depression. The effects of citalopram and escitalopram on the human ether-a-go-go related gene (hERG) channels expressed in human embryonic kidney (HEK) cells were investigated using the whole cell patch-clamp technique. Both citalopram and escitalopram blocked hERG current in a concentration-dependent manner with an $IC_{50}$ value of 3.2 $\mu$M and a Hill coefficient of 1.1 for escitalopram, and an $IC_{50}$ value of 2.7 and a Hill coefficient of 1.1 for citalopram. The potencies of citalopram and escitalopram in blocking hERG were very similar, indicating that the channel block by these enantiomers did not display a stereoselectivity. The block of hERG by escitalopram was voltage-dependent with a steep increase across the voltage range of channel activation. However, voltage independence was observed over the full activation range. The blocking by escitalopram was frequency dependent. A rapid application of escitalopram induced a rapid and reversible block of the tail current of hERG. The extent of the block by escitalopram during the depolarizing pulse was less than that during the repolarizing pulse, suggesting that escitalopram has a high affinity for the open state of the hERG channel with a relatively lower affinity for the inactivated state. Both escitalopram and citalopram produced a reduction of hERG channel protein trafficking to the plasma membrane but did not affect the short-term internalization of the hERG channel. These results suggest that escitalopram blocked hERG currents at a supratherapeutic concentration and that it did so by preferentially binding to both the open and the inactivated states of the channels.

**Key Words:** hERG, Escitalopram, Citalopram, SSRI

## IC-19(PO-5)

### Effects of Primaquine on Action Potentials of Human Stem Cell-derived Cardiomyocytes and Cardiac Monophasic Action Potentials of Rats

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Human induced pluripotent stem cells (hiPSC-CMs) can provide cardiomyocytes originated from human for in vitro assays on drug discovery and safety pharmacology. Using hiPSC-CMs to toxicity testing would overcome limitations of cross-species predictions and ethical concerns of human embryonic stem cells. Although primaquine is the first-line drug for the treatment of relapsing malaria and in the prophylaxis of many forms of latent parasitic infections, it have been reported to have undesirable cardiovascular events due to blocking of cardiac ion channels. In this study, we examined the effect of primaquine on spontaneous action potentials (APs) of human iPSC-CMs (ventricular-type). In addition, cardiac monophasic action potentials (MAP) recordings were conducted in Langendorff-perfused rat hearts. MAP recordings were obtained from left ventricular endocardial surfaces during 3 Hz pacing, before and following superfusion with various concentrations of primaquine (1, 30, and 100 $\mu$M). According to the results, primaquine at 1 uM (i) decreased the maximum rate of depolarization (Vmax), (ii) depressed the total amplitude of APs (APA), (iii) reversibly prolonged the action potential duration (APD) at 60% and 90% levels of repolarization (APD60 and APD90), and (iiii) evoked the early afterdepolarization (EAD) on spontaneously firing APs of hiPSC-CMs. In these cells, primaquine blocked inward Na+ currents in a dose-dependent manner, with the IC50 of 15.8±0.046 $\mu$M (n=4). Cardiac MAP data were analyzed using the basic parameters for AP shape and the powerful proarrhythmic predictors, triangulation (the duration of APD90 to APD50) and instability of APD using short term variability (STV = $\Sigma$ | APD90n+1 − APD90n | /[30$\sqrt{2}$]). Primaquine at 100 $\mu$M exhibited triangulation but not affected on instability. In these experiments, primaquine at 100 $\mu$M significantly decreased APA but not Vmax. Unlike in hiPSC-CMs, primaqine did shorten the APD60 and APD90 at the concentration of 100 $\mu$M. To understand the different effects of primaquine on cardiac APs between hiPSC-CMs and Langendorff-perfused rat hearts, we need to perform further studies for the effects of primaquine on cardiac ion channels.

**Key Words:** Primaquine, Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), Cardiac action potentials, Cardiotoxicity

## IC-20

### Homer2 Acts as a Regulator of PMCA-Mediated $Ca^{2+}$ Signals in Mouse Parotid Acinar Cells

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Homer proteins are scaffold molecules with a domain structure that consists of an N-terminal Ena/VASP homology 1 (EVH) protein-binding domain and C-terminal leucine zipper/coiled-coil domain. The EVH domain recognizes a proline-rich motifs and binds many $Ca^{2+}$ signaling proteins, including G protein-coupled receptors, inositol 1,4,5-triphosphate receptors ($IP_3Rs$), ryanodine receptors, and transient receptor potential channels. However, their role in $Ca^{2+}$ signaling in non-excitable cells is not well un-

derstood. In the present work, we investigated the role of Homer2 in $Ca^{2+}$ signaling in parotid gland acinar cells using Homer2 deficient ($Homer2^{-/-}$) mice. Homer2 showed polarized luminal localization in wild-type acinar cells, and deletion of Homer2 did not affect $IP_3R$ localization or channel activity. The distribution of plasma membrane $Ca^{2+}$ ATPases (PMCA) and PMCA4 was especially increased in the apical region of $Homer2^{-/-}$ cells, which exhibited higher levels of PMCA protein but not sarco/endoplasmic reticulum $Ca^{2+}$ ATPase. Moreover, we found that Homer2 deficiency increased PMCA activity. Finally, co-immunoprecipitation showed that Homer2 interacted with PMCA in wild-type parotid and transfected HEK293 cells. The mutation of N-terminal PPXXF-like region in PMCA affected the interaction with Homer2 and PMCA and increased the ability of the PMCA to control the $Ca^{2+}$ homeostasis in cytosol and cytoplasm, but not affected interaction between Homer1a and PMCA and activities of $Ca^{2+}$ clearance by PMCA. The deletion of C-terminal PSD-95/Dlg/ZO-1 homology domain (PDZ)-binding domains in PMCA4 also changed the surface expressions of PMCA4 by Homer proteins. These findings suggest that Homer2 may play an important role in regulating PMCA expression and PMCA-mediated $Ca^{2+}$ signaling in parotid acinar cells.
**Key Words:** Plasma membrane $Ca^{2+}$ ATPase, Homer, Calcium signaling

## IC-21

## Extrasynaptic $GABA_A$ and Glycine Receptor-Mediated action of Taurine on the Substantia Gelatinosa Neurons of the Trigeminal Subnucleus Caudalis

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The substantia gelatinosa (SG) of the trigeminal subnucleus caudalis (Vc) has been known to be a key site in the processing of orofacial nociceptive information. Taurine, one of the most plentiful free amino acids in human, has proved to be involved in pain modulation. In this study, we used the whole-cell voltage and current clamp modes to investigate the direct membrane effects of taurine and the action mechanism behind taurine-mediated responses on the SG neurons of the Vc. Taurine induced non-desensitizing and repeatable membrane potential and holding current changes on SG neuron which remained in the presence of tetrodotoxin, a voltage dependent Na+ channel blocker, indicating that taurine acts on the postsynaptic SG neurons directly. Further, application of taurine at different doses ranging from 10 $\mu$M to 3 mM showed a concentration dependency of membrane depolarization and inward current with the $EC_{50}$ of 84.3 $\mu$M and 723 $\mu$M, respectively. Taurine-mediated responses were partially blocked by picrotoxin (50 $\mu$M) and completely blocked by strychnine (2 $\mu$M), a glycine receptor antagonist, suggesting it activates not only the synaptic heteromeric glycine receptors but also the homomeric extrasynaptic glycine receptors giving the tonic glycinergic inhibition on SG neurons. In addition, taurine (1 mM) activated extrasynaptic $GABA_A$ receptor-mediated currents. Taken together, our results indicate that taurine can be a target molecule for orofaction pain modulation through the activation of glycine or/and extrasynaptic $GABA_A$ receptor on the SG neurons of the Vc. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2003535).
**Key Words:** Whole cell patch clamp, Substantia gelatinosa neuron, Taurine, Glycine receptor, Extrasynaptic $GABA_A$ receptor

## IC-22(PO-3)

## Action of Glycine on Gonadotropin releasing Hormone (GnRH) Neurons

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Excitatory and inhibitory amino acid neurotransmission is an essential factor of the neuroendocrine transmission that regulates GnRH neurons along with hypothalamic pituitary gonadal axis. Here in this study the action of inhibitory neurotransmitter glycine and its role over NMDA neurotransmission was studied on GnRH neurons using whole cell patch clamp technique. Glycine at different doses ranging from 1 $\mu$M to 1mM showed dose dependent increase of inward current with an $EC_{50}$ of 98.5 $\mu$M and 114 $\mu$M on juvenile male and female and young adult male GnRH neurons under high $Cl^-$ pipette solution. Sensitivity to glycine in the presence of TTX and amino acid receptor blocking cocktail containing TTX (AABC) showed the postsynaptic action of glycine on the membrane or dendrites of GnRH neurons. Dose dependent activation of NMDA current exists with an $EC_{50}$ of 78.1 $\mu$M. In addition, glycine mediated potentiation of NMDA neurotransmission on GnRH neurons not only existed in the intact condition but also in the presence of strychnine a glycine receptor antagonist. Further, blocking of NMDA-glycine binding site by 7-CKNA completely inhibited the NMDA neurotransmission on GnRH neurons suggesting the importance of endogenous glycine on NMDA-GnRH neurotransmission. Further, the effect of glycine over postnatal development was investigated, in adult female. Glycine mediated responses did not exist in any of the estrous stages. In addition, glycine mediated responses were decreased in GnRH neurons from slices pretreated/ incubated with estrogen and progesterone. To confirm the steroid hormone mediated decrease, young juvenile mice were gonadectomized (GDX) and were analyzed electrophysiologically in adulthood, ~40% of the GnRH from the GDX mice showed response to glycine which was completely abolished in GDX mice injected with progesterone and estrogen. In conclusion, these results suggest that glycine may play an important role in regulation of GnRH neuronal activity postnatally and glycine mediated neurotransmi-

ssion on GnRH neurons are influenced by gonadal steroids. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2003535).
**Key Words:** Whole cell patch clamp, Gonadotropin releasing hormone neuron, Glycine

## IC-23

### Hypotaurine Actions on the Substantia Gelatinosa Neurons of the Trigeminal Subnucleus Caudalis through the Activation of Glycine and $GABA_A$ Receptors

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The substantia gelatinosa (SG) of trigeminal subnucleus caudalis (Vc) receives and integrates the nociceptive information from orofacial region. Hypotaurine, the immediate precursor of taurine, is known to be biosynthesized from cysteine in astrocytes responding to noxious dural and facial stimulation. However, the physiological role of hypotaurine in the central nervous system remains unclear. In this study, to understand the role of hypotaurine in the orofactial pain modulation, we investigated the direct membrane effects of hypotaurine and the action mechanism on the SG neurons of the Vc which are involved in orofaction pain processing. Hypotaurine (300 $\mu$M) induced repeatable membrane depolarization and inward currents under the condition of high chloride pipette solution. The hypotaurine-mediated actions were concentration dependent (10 $\mu$M to 3 mM) and persisted in the presence of tetrodotoxin, a voltage dependent sodium channel blocker. Hypotaurine-induced inward currents were partially blocked by picrotoxin (50 $\mu$M) which blocks homomeric glycine receptor, and completely blocked by trychnine (2 $\mu$M), a glycine receptor antagonist. In addition, at higher concentrations (1 mM), hypotaurine activated extrasynaptic GABAA receptor on SG neurons. These results indicate that hypotaurine affects SG neuronal activities by glycine or GABAA receptor activation on the SG neurons and suggest that hypotaurine can be a target molecule for orofacial pain modulation. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2003535).
**Key Words:** Hypotaurine, Orofacial pain, Patch clamp, SG neurons, Glycine receptor, Extrasynaptic $GABA_A$ receptor

## IC-24

### The Unexpected Effect of Mibefradil, a T-type $Ca^{2+}$ Channel Inhibitor on Voltage-Dependent $K^+$ Channels in Coronary Smooth Muscle Cells

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

## IC-25

### Effect of Glucocorticoids on Human Kv1.3 Channels Expressed in Xenopus Oocytes

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Cortisone, as an inactive version of hydrocortisone, is one of the main hormones released in response to stress and has a function of suppressing the immune system. One of the voltage-dependent potassium channels-Kv1.3 channels are expressed in the immune and nervous system generally. We examined what is the effect of cortisone and hydrocortisone on Kv1.3. Either cortisone or hydrocortisone reduced the amplitude of Kv1.3 channels current concentration-dependently with an IC50 values of 94.8 and 56.8 $\mu$M, respecrtively. Also their block on Kv1.3 remain stable relative to the degree of depolarization. What is more, cortisone and hydrocortisone shifted both activation curve and inactivation curve of Kv1.3 to a positive direction, but they shifted activation curves in more extent. Our results suggested that cortisone and hydrocortisone has a similar blocking effect on Kv1.3 channels.
**Key Words:** Cortisone, Hydrocortisone, Kv1.3, Immunoreaction

## IC-26

### Existence of $GABA_A$-alpha 5 Receptor Mediated Tonic Conductance on Gonadotropin Releasing Hormone (GnRH) Neurons

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Gonadotropin releasing hormone (GnRH) neurons are central regulator of hypothalamic pituitary gonadal axis governing reproductive physiology. Various neurotransmitters and neuroactive steroids play an important role in the regulation of GnRH neuronal physiology. Although facilitation of the conventional phasic inhibitory postsynaptic current (IPSCs, $I_{phasic}$) has been considered as the primary mechanism by which neurosteroids influence neuronal excitability, $GABA_A$ receptors mediate a sustained tonic current (Itonic) as well as $I_{phasic}$ in GnRH neurons. In addition, steroidal modulation on $I_{tonic}$ in GnRH neuron is unknown. Here, we studied the influence of prognane neurosteroid on GnRH neurons and tried to figure out the potential molecular configuration of $GABA_A$ receptors ($GABA_AR$) mediating neurosteroid sensitivity of $I_{tonic}$ in GnRH neurons. 3$\alpha$,5$\alpha$-THDOC induced the increase in inward currents on GnRH neurons and these inward current remain persisted in the presence of gabazine, a competitive synaptically mediated IPSCs blocker, and amino acid blocking cocktail containing (AP-5: NMDA receptor antagonist; CNQX: non-NMDA receptor antagonist; strychnine: glycine receptor antagonist; and TTX: Na+ channel blocker) providing the evidence that the tonic currents are purely postsynaptic events. Further 3$\alpha$,5$\alpha$-THP-induced inward current on the GnRH neurons remained in the presence of gabazine and were partially blocked by $GABA_A$-alpha5 inverse agonist L-655708 (10 $\mu$M). In addition, $GABA_A$-$\alpha$5 mRNA transcripts were detected on GnRH neurons. These results suggest that there exists a alpha5 and/or delta $GABA_A$ receptor-mediated tonic conductance via prognane neurosteroids on GnRH neurons and these tonic currents may affect the HPG axis and reproductive physiology. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2003535).

**Key Words:** Gonadotropin releasing hormone (GnRH), Tonic $GABA_A$ current, Whole cell recording

## IC-27

### Serum Starvation-Induced Kv7.5 Expression and Its Regulation by Sp1 in Canine Osteosarcoma Cells

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The KCNQ gene family, encoding Kv7 channels, belongs to the group of voltage-gated potassium (Kv) channels, and their roles have been widely investigated in nerve and muscle cells. In the present study, we investigated several characteristics of Kv7.5, which was strongly expressed in the canine osteosarcoma cell line, CCL-183. Serum starvation effectively upregulated Kv7.5 expression, and the Kv7 channel opener, flupirtine, attenuated cell proliferation by arresting cells in the $G_0/G_1$ phase. We also showed that Kv7.5 knockdown helps CCL-183 cells to proliferate. Transcription factor Sp1, found to be upregulated by serum deprivation, strongly mediated endogenous induction of Kv7.5 in CCL-183 cells. These results suggest that Kv7.5 may exert an anti-proliferative effect in canine osteosarcoma and Kv7.5 is therefore a possible molecular target for canine osteosarcoma therapy.

**Key Words:** Kv7.5, Serum starvation, Sp1, Flupirtine

## IC-28

### Cell-Cycle-Dependent Regulation of Mechanosensitive TREK1 and TREK2 in Human Bladder Carcinoma Cells

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Bladder cancer is the seventh most common cancer in men that smoke, and the incidence of disease increases with age. The mechanism of occurrence has not yet been established. Potassium channels have been linked with cell proliferation. Some two-pore domain $K^+$ channels (K2P), such as TASK3 and TREK1, have recently been shown to be overexpressed in cancer cells. We confirmed that K2P channels such as TASK1, TASK3, TWIK1, TREK1 and TREK2 were expressed in bladder cancer cell line by quantitative real-time PCR. Here we focused on the relationship between cell-cycle-dependent growth and the mechanosensitive K2P channels, TREK1 and TREK2, in the human bladder cancer cell line, 253J. Using the patch- clamp technique, the mechanosensitive TREK1 and TREK2 channel was recorded in the presence of symmetrical 150 mM KCl solutions. The TREK1 and TREK2 channel was activated by polyunsaturated fatty acids, intracellular acidosis at -60 mV and mechanical stretch at -40 mV or 40 mV. TREK2 had a higher single channel conductance than TREK1, and TREK2 showed a more inward rectifying I-V relationship than TREK1 in symmetrical 150 mM KCl solutions. Furthermore, small interfering RNA (siRNA)- mediated TREK1 or TREK2 knockdown decreased proliferation of 253J cells, compared to negative control siRNA. 253J cells treated with TREK1 siRNA or TREK2 siRNA showed a significant increase in the expression of cell cycle boundary proteins p21, P16 and p53 and also a decrease in protein expression of RB and E2F1. Taken together, the TREK1 and TREK2 channel is present in bladder cancer cell lines and may, at least in part, contribute to cell cycle-dependent growth.

**Key Words:** Bladder cancer, Proliferation, Mechanosensitive, Cell cycle, Small interfering RNA

## IC-29

### Glucocorticoids Blocked Human Kv1.5 Channels Expressed in Xenopus Oocytes

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Cortisone and hydrocortisone are glucocorticoids and they are released in response to stress, while cortisone is a inactive version of hydrocortisone. Kv1.5 is widely expressed in macrophages, dendritic cells and heart, skeletal muscles, as well as smooth muscles in vessels. We compared the effect of cortisone and hydrocortisone on human Kv1.5 channel. Both cortisone and hydrocortisone suppressed the amplitude of Kv1.5 channels current with IC50 vales of 71.8 and 47 $\mu$M, respectively. The inhibition degree of cortisone on Kv1.5 decreased progressively from -10 mV to +30 mV, and then remain relative stable from +30 mV to +60 mV, while hydrocortisone's inhibition degree did not change at the same range of voltage. Hydrocortisone shifted not only activation curves ($\Delta$V1/2 = 3.15 mV) but also inactivation curves ($\Delta$V1/2 = 3.03 mV) to a positive direction. However, cortisone shifted them in a less extent ($\Delta$V1/2 of activation = 1.47 mV, $\Delta$V1/2 of inactivation = 1.27 mV). The present data indicated that cortisone and hydrocortisone inhibited Kv1.5 channel currents in a similar mechanism, but the channel is more sensitive to hydrocortisone.

**Key Words:** Cortisone, Hydrocortisone, Kv1.5, Inflammatory reaction, Heart

## IC-30

### Hydrogen Peroxide Induces Vasorelaxation by Enhancing 4-AP Sensitive Kv Currents through S-Glutathionylation

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Hydrogen peroxide ($H_2O_2$) is an endothelium-derived hyperpolarizing factor. Because opposing vasoactive effects have been reported for $H_2O_2$ depending on the vascular bed and experimental conditions, the aim of this study was to assess whether $H_2O_2$ may act as a vasodilator in mesenteric artery and if so to determine the underlying mechanisms. On mesenteric arteries precontracted with norepinephrine, $H_2O_2$ elicited a dose dependent relaxation, which is markedly blunted by DTT. $H_2O_2$-elicited vasodilation was significantly reduced by blockade of 4-AP sensitive Kv channels, but it was resistant to the BKCa blocker TEA and IRK blocker $BaCl_2$. Patch-clamp study showed that $H_2O_2$ induced a dose-dependent increase of Kv currents in rat mesenteric arterial smooth muscle cells (MASMCs), speeding up channel activation and shifting half-point of activation to left. Immunoblot analysis indicated that Kv channel protein is significantly glutathionylated after exposure to $H_2O_2$. Consistent with S-glutathionlyation, pipette application of oxidized glutathione (GSSG) increased Kv currents and occluded $H_2O_2$ effects. Moreover, adding glutathione reductase prevented $H_2O_2$- induced activation of Kv currents, indicating glutathionylation mediated $H_2O_2$ stimulatory effects on Kv currents. Interestingly, *conditions* of increased *oxidative* stress within MASMCs impaired the capacity of $H_2O_2$ to stimulate Kv channels. Not only $H_2O_2$ stimulatory effect was much weaker, but also an inhibitory effect of $H_2O_2$ was unmasked. The present data demonstrate that $H_2O_2$ is a vasodilator in rat mesenteric arteries that activates smooth muscle 4-AP sensitive Kv channels through glutathionylation. Our results further suggest that basal redox status of MASMCs determines the response of Kv currents to $H_2O_2$.

**Key Words:** $H_2O_2$, Kv channel, Artery, Glutathionylation, Oxidative stress

## IC-31

### Blockade by MK801 of Voltage-Gated $K^+$ Currents in Rat Mesenteric Arterial Smooth Muscle Cells

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**Purpose:** A phencyclidine (PCP) derivative MK801 (dizocilpine), is a potent noncompetitive N-Methyl-D-aspartate receptor (NMDAr) antagonist. Recently, another PCP derivative ketamine was reported to block the voltage-gated $K^+$ channel (Kv) in rat mesenteric arterial smooth muscle cells (RMASMCs), which was independent of NMDAr. Kv currents are major regulator of membrane potential (Em) and thus cellular excitability in muscle as well as in neurons. In this study, therefore, we investigated the effect of MK801 on Kv and Em in RMASMCs. **Experimental Approach:** We used whole-cell patch clamp technique for analyzing the effect of MK801 enantiomers on the Kv and Em. **Results:** (+)MK801 concentration dependently inhibited Kv with an IC50 of 89.1 $\mu$M±13.1 and Hill coefficient of 1.05± 0.08. The inhibition was voltage-independent. Time course of the Kv activation was not affected, whereas that of Kv inactivation was accelerated by (+) MK801. The (+)MK-801 inhibition of Kv was slightly use-dependent. However, holing Em at -110 mV without depolarization did not affect

the (+)MK801 inhibition of the Kv. (+)MK801 did not affect the steady-state activation and inactivation of Kv. (+)MK concentration-dependently depolarized Em with concomitant decrease of membrane conductance. (-)MK801 also inhibited the Kv with a similar IC50 (134.0 $\mu$M±17.5) and Hill coefficient (0.87±0.09) as (+)MK801. **Conclusions and Implications:** These results indicate that MK-801 directly inhibits the Kv with state-independent manner in RMASMCs. This MK801 inhibition of Kv should be considered when assessing various pharmacological effects of MK801 such as schizophrenia, neuro-protection, and hypertension.
**Key Words:** MK801, Phencyclidine, Kv, NMDAr

## IC-32

### The Role of Classical Transient Receptor Potential Channel 4 (TRPC4) and 5 (TRPC5) in Polycystic Kidney Disease (PKD)

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Polycystin-1 (PKD1) regulates a number of cellular processes (ex. heterotrimeric G protein, transcription factor etc.) through the formation of complexes with the polycystin-2 (PKD2) ion channel or with other signal transduction proteins. Although $Ca^{2+}$ modulation by polycystins has been reported between transient receptor potential (TRP) channels, the function with TRPC subfamily regulated by G-protein signaling has remained elusive. Here, we recorded the activity of TRPC4/C5 heterologously co-expressed with PKD1 or PKD2 in HEK293 cells. PKD1 activated TRPC4 (40±14 pA/pF) or TRPC5 (104±17 pA/pF) channel by modulating G-protein signaling without change in TRPC4/C5 translocation. Intracellular 0.2 mM GTP$\gamma$S-induced TRPC4/C5 activation was not significantly different in the presence or absence of PKD1. And C-terminal fragment (CTF) of PKD1 did not affect TRPC4/C5 activity due to loss of N-terminus containing G-protein coupled receptor proteolytic site (GPS). We investigated whether TRPC1/C4/C5 assemble to form a heterodimeric channel, even if PKD2 is mainly retained in the endoplasmic reticulum (ER). PKD2 was targeted predominantly to the plasma membrane, especially by TRPC5 but not TRPC1. Our findings indicated an important function between PKD and TRPC4/C5 in modulation of intracellular $Ca^{2+}$ signaling and provided a new potential therapeutic approach targeting TRPC4/C5 channel in polycystic kidney disease.
**Key Words:** PKD, Polycystin, TRPP, TRPC, G-protein

## IC-33

### Remodeling of Caveolae Mediates Stretch-Induced Increase of L-type $Ca^{2+}$ Current in Rat Mesenteric Artery via an Activation of EGFR/JNK Cascade

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**Background:** Voltage-dependent L-type $Ca^{2+}$ channel ($VDCC_L$) current of vascular smooth muscle is facilitated by mechanical stimuli, which is thought to contribute to the myogenic contraction of resistant arteries. However, the molecular basis underlying the $VDCC_L$ activation is controversial. **Aim and Methods:** We investigated the signaling from hypotonic membrane stretch to $VDCC_L$ activation in isolated rat mesenteric arterial smooth muscle cells using patch- clamp, immunocytochemistry, Western blotting, and immunoprecipitation. **Results:** Hypotonic membrane stretch reversibly increased $VDCC_L$ current. Also associated with the hypotonic membrane stretch were increases in EGFR phosphorylation shown by Western analysis. Pretreatment with EGFR inhibitor, AG1478 attenuated the stretch-induced activation of $VDCC_L$ (SIAVL). Furthermore, stretch increased phosphorylations of c-Jun N-terminal protein kinase (JNK), extracellular signal-regulated kinase (ERK), PLCr-1, and caveolin-1. The SIAVL was prevented by inhibiting JNK or PLCr-1 with SP600125 and U73122, respectively, whereas inhibiting ERK pathway with PD98059 had no effect. EGFR was further shown to be upstream of the JNK, with its inhibitor AG1478 preventing JNK activation. Interaction of PLCr-1 with caveolin-1 analyzed by coimmunoprecipitation was increased upon membrane stretch. Both JNK phosphorylation and SIAVL were abolished by U73122. Stretch-induced caveolin-1 phosphorylation was blocked by AG1478 but not by U73122. Cyclodextrin-induced unfolding of caveolae increased EGFR and JNK activation, implying a role of caveolae remodeling in EGFR activation. Furhtermore, cyclodextrin cuased a cytosolic to membrane translocation of Caveolin-1, which was similar to those by stretch. Finally cyclodextrin induced an increase of $VDCC_L$, which was not further enhanced by stretch, indicating that stretch and cyclodextrin increased $VDCC_L$ via common signaling pathway. **Conclusion:** These results indicate that hypotonic stretch activates EGFR tyrosine kinase by caveolae remodeling. EGFR in turn activates $VDCC_L$ via PCLr-1/ PLCr-1/JNK pathway. These results define a novel function for caveolae and EGFR in SIAVL.
**Key Words:** Voltage-Dependent L-type $Ca^{2+}$ channel, Myogenic contraction, Hypotonic stretch, EGFR, Caveolin

## IC-34

### Effect of Stress Hormones on the Cardiac Electrocardiogram, Action Potential duration and hERG $K^+$ Channel

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Cortisone (17-hydroxy-11-dehydrocorticosterone) and hydrocortisone ((11$\beta$)-11,17,21-trihydroxypregn-4-ene-3,20-dione) are steroid hormones having lots of adverse effect on various organ. Human may be exposed to stressful conditions with harmful influences on their physiological and psychological health. To date, the adverse effect of cortisone and hydrocortisone on cardiac functions has not been revealed. Therefore, we studied heart effect to find out the relation between Q-T interval and two stress hormones (cortisone and hydrocortisone) on ECG, action potential duration, PKC phosphorylation status of guinea pig's hearts and hERG $K^+$ channel expressed in Xenopus oocytes. Administration of cortisone and hydrocortisone reduced the Q-T interval of ECG by 15% and 5%, respectively. Also, cortisone and hydrocortisone decreased the action potential duration at 90% of repolarization ($APD_{90}$), $APD_{50}$, and $APD_{20}$ with concentration and time dependences. Western blot analysis demonstrated a time-dependent increase in the phosphorylation of PKC alpa/beta II and PKC gamma. Finally, the tail current of hERG channel are reduced by both cortisone and hydrocortisone. These results suggested that the stresshormones can affect the cardiac electrophysiology either possibly by direct action on ion transport mechanism or via biochemical change of intracellular molecules.

**Key Words:** Cortisone, Hydrocortisone, ECG, APD, PKC

## IC-35

### Functional Expression of Thermo/ Mechanosensitive TRP Channels in Human Periodontal Ligament Fibroblasts

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The periodontal ligament fibroblasts (PLFs) are specialized connective tissue fibers for attachment of the teeth to the alveolar bone and for supporting the tooth to withstand the mechanical stress that occurs during chewing behaviors, continuous orthodontic tooth movement, and temperature changes caused by food intake and endodontic treatment. These thermo-mechanical stresses induce physiological processes such as inflammatory response, periodontal tissue and bone remodeling. However, the mechanism of thermo-mechanical stress-induced cellular response and signal transmission in PLFs remains poorly understood. In this study, we investigated the expression of thermo-mechanosensitive transient receptor potential (TRP) channels and physiological functions under hypo-osmotic stress in primary cultured human PLFs. We found mRNA expressions of TRPV1, TRPV2, TRPV3, TRPV4, TRPM3, TRPM8 and TRPA1. Each specific agonists of TRPV1-4 (heat sensors), TRPM8/TRPA1 (cold sensors) and TRPV4/TRPM3 (mechanical sensors) caused increases in intracellular calcium concentration ($[Ca^{2+}]_i$). Hypo-osmotic stress increased the mRNA expression of receptor activator of nuclear factor-kappaB (NF-$\kappa$B) ligand (RANKL) but not osteoprotegerin (OPG) as well as $[Ca^{2+}]_i$. This increases in $[Ca^{2+}]_i$ was completely inhibited by gadolinium and lanthanum, non-specific plasma membrane $Ca^{2+}$ channel blockers. We confirmed activities of TRPM3 and TRPV4 by a whole-cell patch-clamp technique. Finally, 2-aminoethoxydiphenyl borate and ruthenium red, each blockers of TRPM3 and TRPV4, reduced hypo-osmotic stress-induced increases in $[Ca^{2+}]_i$ and RANKL mRNA expression. These results suggest that the primary cultured human PLFs express various TRP channels and these channels play a crucial role in mediating thermo-mechanical sensation and bone remodeling in teeth.

**Key Words:** Thermo/Mechanosensitive TRP channels, Periodontal ligament fibroblast, Bone remodeling, Calcium signaling

## IC-36

### Shear Stress-Mediated Activation of TRPV5 and TRPV6 Channel Stimulates *Slo1* Channel Causing Membrane Hyperpolarization

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TRPV5 and TRPV6 channels are expressed in distal renal tubules and play important roles in the transcellular $Ca^{2+}$ reabsorption in kidney. They are regulated by multiple intracellular factors including protein kinase A and C, membrane phospholipid $PIP_2$, protons, and divalent ions $Ca^{2+}$ and $Mg^{2+}$. Here, we report that fluid flow that generates shear force within the physiological range of distal tubular fluid flow activated TRPV5 and TRPV6 channels expressed in HEK cells. Flow-induced activation of channel activity was reversible and did not desensitize over 2 minutes. Fluid flow stimulated TRPV5 and 6-mediated $Ca^{2+}$ entry and increased intracellular $Ca^{2+}$ concentration. N-glycosylation-deficient TRPV5 channel was relatively insensitive to fluid flow. In cells coexpressing TRPV5 (or TRPV6) and *Slo1*-encoded maxi-K channels, fluid flow induced membrane hyperpolarization, which could be prevented by the maxi-K blocker iberiotoxin or TRPV5 and 6 blocker $La^{3+}$. In contrast, fluid flow did not cause membrane hyperpolarization in cells coexpressing ROMK1 and TRPV5 or 6 channels. These results reveal a new mechanism for regulation of TRPV5 and TRPV6 channels. Activation of TRPV5 and TRPV6 by fluid flow may play a role in the regulation of flow-stimulated $K^+$ secretion via maxi-K channels in distal renal tubules and in the mechanism of pathogenesis of thiazide-induced hypocalciuria. [This research was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (NRF-2010- 0024789)].

**Key Words:** Flow-mediated $K^+$ secretion, TRPV5, TRPV6, *slo1*, Shear stress

## IC-37

### Ablation of Very Long Acyl Chain Sphingolipids Decreases Gastric Smooth Muscle Contractility in Mice by Upregulating $K_{Ca}1.1$ Channel

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Ion channels act as critical regulators of cell function by modulating membrane potential and $Ca^{2+}$ influx. Sphingolipids are important structural components of cell membranes and, thus, specific sphingolipids, such as ceramide, and glucosylceramide, may affect cell function by modulating expression of ion channels on cell surface and channel activity. We now explore the role of the ceramide acyl chain length in $K_{Ca}1.1$ expression and the channel activity by using a ceramide synthase 2 (CerS2) null mice, which is unable to synthesize very long acyl chain (C22-C24) ceramides. $K_{Ca}1.1$ was upregulated, $K_{Ca}1.1$ current was significantly increased in gastric smooth muscle cells (SMCs) from CerS2 null mice compared to those from age-matched wilt-type (WT) mice. However, no significant difference was found in $Ca^{2+}$ or voltage sensitivity of $K_{Ca}1.1$ channel between WT and CerS2 null SMCs, suggesting that the altered sphingolipid acyl chain length does not affect the channel activity. Phospho-PKC $\zeta$ and phospho-PI3 Kinase p85/p55 expression were markedly increased in CerS2 null gastric SMCs, compared with WT SMCs. The increased $K_{Ca}1.1$ expression in CerS2 null SMCs was reduced by treatment with PKC inhibitor or PKC $\zeta$ -targeted siRNA. Similar changes in $K_{Ca}1.1$, phospho-PKC $\zeta$ and phospho-PI3 Kinase p85/p55 from CerS2 null SMCs were evoked by CerS5 transfection. Agonist increased intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and the increased $[Ca^{2+}]_i$ was sustained in wild-type gastric SMCs, but spontaneously declined in CerS2 null SMCs in spite of the presence of agonist. CerS2 null or $K_{Ca}1.1$- transfected SMCs exhibited phosphorylated myosin light chain down-regulation. Compared with WT mice, the number of gastric mucosal fold and the frequency of spontaneous contraction of gastric smooth muscle was reduced in CerS2 null mice. In addition, the contraction by agonist was sustained in wild-type gastric SMCs, but spontaneously declined in CerS2 null SMCs in spite of the presence of agonist. From these results, we conclude that the sphingolipid acyl chain composition of gastric SMCs regulates $K_{Ca}1.1$ expression on cell surface and thereby contractility of gastric smooth muscle via PKC-mediated pathway.

**Key Words:** $K_{Ca}1.1$ channel, Sphingolipid, Cermide Synthase, Gastric smooth muscle contractiblity

## IC-38

### The $K^+$ Channel Microenvironments in the Brain

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$K^+$ channels, together with the auxiliary regulatory proteins, are transmembrane proteins that regulate the neuronal membrane excitability and the resting membrane potential in the mammalian brain. However, the macromolecular composition of $K^+$ channel microenvironment is still unsolved in brain. Here, we used a proteomic approach combining antibody-based affinity purification with mass spectrometry for the comprehensive and quantitative analyses of the $K^+$ channel's microenvironments in the presence of the diverse detergent. According to stringency of the diverse detergent solubility, our results show that the $K^+$ channels constituents coassemble into the macromolecular complexes of potassium channel with distinct stability and abundance. These results reveal the diversity of the macromolecular architecture of $K^+$ channel complexes and describe the global topological landscape of the $K^+$ channel microenvironment.

**Key Words:** Potassium channel, Mass spectrometry, Microenvironment

## IC-39

### The Effect of Modafinils in Pulmonary Hypertension Rat Models

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Pulmonary arterial hypertension (PAH) is difficult to treat and is characterized by increased pulmonary arterial pressure. PAH causes right ventricular failure and possibly even death due to progressive increase in pulmonary vascular resistance. PAH has been shown to be refractory to most of the conventional pharmacological therapies. Modafinils have been known that the effects include increasing cAMP concentrations in aortic smooth muscle cell (SMC)s and phosphorylated $K_{Ca}3.1$ channels. $K_{Ca}3.1$ channels are related to vessel relaxations and proliferation of SMCs. Six-week-old male Sprague Dawley rats were used. The rats were grouped as follows: control (C) group, subcutaneous injection of saline; Monocrotaline (MCT) group, subcutaneous injection of MCT (60 mg/kg); Modafinil group (MD group), gavage feeding of modafinil (50 mg/kg/day.) 1 day after MCT injection. We sacrificed the rats in weeks 1, 2 and 4. Pulmonary arterial pressure (PAP)s were estimated using catheter introduced to internel jugular vein. The mean right ventricular pressure (RVP) significantly increased in MCT gorup compared to C group and significantly decreased in the MD group compared with the MCT group in weeks 1, 2 and 4. Systemic pressure showed no significant changes in three groups. The ratio of RV/LV+septum significantly increased in MCT group compared to C gorup in weeks 2 and 4 and significantly decreased in MD group compared to MCT group in week 4. After Modafinils treatment, there were improvements of RVH, mean RV pressure. Additional research on

the dose and frequency of Modafinils is needed to determine the optimal parameters for PAH treatment.
**Key Words:** Pulmonary hypertension, Monocrotalines, Modafinils, Potassium channels

## IC-40

### Cdo Regulates Activation of Kir2.1 $K^+$ Channels in Myoblast Differentiation

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Skeletal myoblast differentiation is a well-coordinated process involving cell cycle withdrawal, expression of muscle-specific genes and morphological alterations of myoblasts into multinucleated myotubes by fusion. The activation of Kir2.1 appears to be required for myoblast fusion and it precedes the entry of $Ca^{2+}$ and Stim1 activation. Our previous study suggests that the multifunctional receptor Cdo induces Stim1 phosphorylation by ERK1/2 to promote myoblast differentiation, likely via activation of NFATc3. In this study, we investigate the functional interaction between Cdo and Kir2.1 during myoblast differentiation. The expression of Kir2.1 is induced upon myoblast differentiation and overexpression of Kir2.1 enhances myotube formation. In addition, the expression of Kir2.1 does not alter in Cdo depleted myoblasts however its activity is affected significantly in these cells. Based on our data, we propose that Cdo regulates activation of Kir2.1 upon induction of myoblast differentiation.
**Key Words:** Kir2.1, Cdo, Myoblast

## IC-41

### Closely Spatio-Association of TRPC4 with G$\alpha$i in TRPC4 Activation Process

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Canonical transient receptor potential (TPRC) channels are $Ca^{2+}$-permeable nonselective cation channels that are widely expressed in numerous cell types. Seven different members of TRPC channels are isolated and canonical type of TRP channel family transduces signals of GPCR with various external stimuli. TRPC4 channels are known to be regulated by G$\alpha$i proteins. However, the molecular mechanism how G$\alpha$i proteins activate TRPC4 still remains to be questionable. To investigate the mechanism, we used whole patch clamp and FRET (Föster Resonance Energy transfer). We tagged mTRPC4 and G protein with CFP and YFP, respectively, and transiently transfected HEK293 cells with FRET pair. FRET efficiency between TRPC4 and G$\alpha$ was nearly 8% and was greater than those between TRPC4 and G$\beta\gamma$ (nearly 4%). And QL mutant of G$\alpha$ has nearly 18% of FRET efficiency. At the HEK293 cell transfected with M2 muscarinic receptor, application of carbachol (CCh) increased FRET efficieny from 9.66±4.64 % (n = 7) to 20 % (n = 7). We also found that TRPC4 channel directly interacts with G$\alpha$i2 but not G$\alpha$q, during channel opened. We checked calcium level of HEK293 cells expressed channels and G$\alpha$i2 or G$\alpha$q by using calcium indicator YC6.1(yellow cameleon6.1). And finally we observed that calcium level of cell expressed TRPC4, G$\alpha$i2 and M2.
**Key Words:** TRPC, G protien, Calcium, FRET

## IC-42

### Palmitic Acid Regulation of Calcium Handling in Cardiac Myocytes from Normal and Angiotensin II-Induced Hypertensive Rat

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Free fatty acids (FA) are preferential substrates of cardiac metabolism in healthy heart. However, FA is accumulated in cardiac interstitial and becomes pathogenetic stimuli in the heart under stress. So far, FA regulation of calcium handling processes in mammalian cardiac myocytes remain unclear. Here, we tested palmitic acid (PA) regulation of intracellular $Ca^{2+}$ transients ($[Ca^{2+}]_i$), L-type $Ca^{2+}$ current ($I_{Ca}$) and Na+-$Ca^{2+}$ exchange (NCX) involvement in left ventricular (LV) myocytes from healthy and hypertensive rats. Our results showed that PA (100 $\mu$M) did not increase the amplitude of $Ca^{2+}$ transients in LV myocytes from shams or hypertensive rats (between PA and control: p=0.6 in shams; p=0.6, in hypertension). In contrast, PA prolonged the time constant of $[Ca^{2+}]_i$ decay (tau) in both groups ($p < 0.001$ in shams and p=0.01 in hypertension). Consequently, total $[Ca^{2+}]_i$ during one pulse duration (2Hz) was significantly increased by PA in shams (p=0.04). However, total $[Ca^{2+}]_i$ was not different between control and PA in hypertension (p=0.15). Voltage-clamp experiments showed that the peak amplitude of $I_{Ca}$ (at 0 mV) was significantly reduced in both groups (between control and PA: p=0.004 in shams; p=0.007 in hypertension). In addition, tau of $I_{Ca}$ inactivation was slower by PA (p= 0.008, in shams and p=0.02, in hypertension). Interestingly, the integral of $I_{Ca}$ (total $Ca^{2+}$ influx via $I_{Ca}$) was not different before and after PA supplementation in shams (p=0.5) but was reduced in hypertension (p=0.003). PA reduced NCX activity in shams and was not different from that in hypertension. Taken together, PA increases intracellular $Ca^{2+}$ level by reducing NCX activity or slowing inactivation kinetics of $I_{Ca}$ in shams. In hypertension, PA maintains intracellular $Ca^{2+}$ level despite that $I_{Ca}$ and NCX activity were reduced.
**Key Words:** Palmitic acid, Intracellular $Ca^{2+}$ transient, L-type $Ca^{2+}$ channel, Hypertension, Left ventricular myocyte

## IC-43

### Comparative Effects of Triazolopyridine Antidepressants on L-Type $Ca^{2+}$ Channels Between Rat Isolated and Human Stem Cell-Derived Cardiomyocytes

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a suitable model for studying human cardiac ion channels and for drug safety assessment. The triazolopyridine antidepressants (TAs) trazodone and its analogue nefazodone, a 5-$HT_2$ receptor antagonist with weak serotonin reuptake inhibitor, have been shown to modulate several cardiac ion channels and we previously reported that they inhibited voltage-dependent L-type $Ca^{2+}$ currents (Ica,L) with different sensitivity in rat isolated cardiomyocytes (isolated-CMs). Although TAs' actions on isolated-CMs are well established, little is known about whether the Ica,L characteristics of hiPSC-CMs faithfully recapitulate those of isolated-CMs. Thus, in this study, we investigated the pharmacological effects of TAs on Ica,L of hiPSC-CMs and compared the results obtained by isolated-CMs using whole-cell voltage clamp recordings. All cells were held at −40 mV and depolarized to 0 mV for 300 ms to elicit Ica,L. The specificity of Ica,L was verified using the L-type $Ca^{2+}$ channel antagonist nifedipine, which produced a similar high-affinity block of Ica,L in the isolated-CMs and hiPSC-CMs. The TAs also produced a similar affinity block of Ica,L in the isolated-CMs and hiPSC-CMs, and nefazodone's potency for Ica,L inhibition was higher than that shown by trazodone. To investigate the current-voltage (I-V) relationships, all cells were held at −40 mV and depolarized for 300 ms to potentials ranging from −40 to + 50 mV in 10 mV increments, and peak current amplitudes for each voltage were recorded. The effects of TAs on I-V relationship in both cell lines, however, were different, resulting in a right-shifted I-V curve for the isolated-CMs and a left-shifted I-V curve with increased outward currents for the hiPSC-CMs. This is the first study to report TAs' potent cardiovascular depressant effects in mammalian and human cardiomyocytes by inhibiting cardiac L-type $Ca^{2+}$ channels. Our results suggest that hiPSC-CMs well replicate the effect of TAs on Ica,L compared to the well-established isolated-CMs and it could be useful for drug safety assessment; however, caution should be exercised owing to multiple mechanisms of drug action on each cell line.

**Key Words:** Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), Rat isolated cardiomyocytes (isolated-CMs), Triazolopyridine antidepressants (TAs), L-type Ca²+ channels, Drug safety assessment

## IC-44

### Surface Expression and Trafficking of the voltage-Gated Potassium Channel Kv3.1b through N-Glycosylation

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The potassium ion channel Kv3.1b is a member of the third group of maturely glycosylated voltage-gated ion channel family. The channel allows high frequency firing of neurons when functional and physiologically expressed on the cell surface as a result of controlled modulation of potassium channel outward currents. N-glycosylation has been known to regulate the surface trafficking and function of different ion channels. However, the N-glycosylation dependent mechanisms concerning Kv3 channels remain to be elucidated. Here, we show that N-glycosylation serves a principal role in Kv3.1b surface expression and $K^+$ current regulation in a glycosylation site-dependent manner. We observed that N-glycosylation in N229, in contrast to N220, mediates Kv3.1b intracellular trafficking and surface expression. Our findings suggest that this individual N-glycosylation site is vital for distinct Kv3.1b regulatory functions and provide significant knowledge on the N-glycosylation-dependent molecular mechanisms of Kv3.1b channels.

**Key Words:** N-glycosylation, Trafficking, Kv3.1b

## IC-45

### Molecular Mechanisms of the dual Sensitivity of TREK-2 Channels by Membrane $PIP_2$

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TREK-1 and -2, members of two-pore domain $K^+$ channel (K2P) family, are activated by various chemical and physical stimuli; arachidonic acid (AA), intracellular acidic pH , membrane stretches, etc. Also, TREK channels show dual sensitivity to plasmalemmal phosphatidylinositol 4, 5-bisphosphate ($PIP_2$); inhibition by $PIP_2$ higher than physiological intrinsic level, activation by partial depletion, and complete inhibition by $PIP_2$ scavenging (e.g. poly-L lysine). Consistently, in inside-out patch clamp conditions, application of MgATP (1 mM) inhibits TREK via PI-kinase dependent $PIP_2$ production whilst ATP-free condition activates. After confirming the total inhibition by poly-L lysine, appli-

cation of $PIP_2$ initially activated and then inhibits TREK channels. To elucidate the complicated regulatory sites interacting with $PIP_2$, we made 15 site-directed mutants of TREK-2 in the carboxy-terminal regions close to the trans-membrane segment. Patch clamp study, whole-cell (w-c) and inside-out (i-o) configurations, demonstrated two novel regulatory sites; 1) a cluster of three positive charges (R355, R356, R357), and 2) one negative charge residue (E350). In w-c patch clamp, when all three positive residues were substituted by alanines (R355-7A), TREK-2 channel activity was significantly reduced. Similarly, the deletion of R355-7 also showed suppressed TREK-2 activity. In contrast, TREK-2 E350A mutant was constitutively active, and was not inhibited by MgATP (up to 5mM) under the i-o and w-c configuration. Maximum activation of TREK-2 by 10 $\mu$M AA was not different between wild type and the mutants. The precise modeling and further investigation are requested to understand the complex regulation of TREK-2 by $PIP_2$.
**Key Words:** TREK-2, K2P, $PIP_2$, $K^+$ channel

## MP-1

### Molecular Expression Mechanism of Lipocalin-2 in Pancreatic β-Cells Under Exposure to IL-1β and IFN-γ

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Lipocalin-2 (LCN-2) was known to play a role in obesity and insulin resistance, however, little is known about the expression of LCN-2 in pancreatic islet β-cells. We examined the molecular mechanisms by which proinflammatory cytokines interleukin-1β (IL-1β) and interferon-γ (IFN-γ) induce LCN-2 expression in RINm5F β-cells. IL-1β significantly induced LCN-2 expression while IFN-γ alone did not induce it. IFN-γ significantly potentiated IL-1β-induced LCN-2 protein and mRNA expression. However, promoter study and EMSA showed that IFN-γ failed to potentiate IL-1β-induced LCN-2 promoter activity and binding activity of transcription factors on LCN-2 promoter. Furthermore, LCN-2 mRNA stability and transcription factors NF-κB and STAT-1 were not involved in the stimulatory effect of IFN-γ on IL-1β-induced LCN-2 expression. Meanwhile, Western blot and promoter analyses showed that NF-κB was a key factor in IL-1β-induced LCN-2 expression. Collectively, IL-1β induces LCN-2 expression via NF-κB activation in RINm5F β-cells. IFN-γ potentiates IL-1β-induced LCN-2 expression at mRNA and protein levels, but not at promoter level and the stimulatory effect of IFN-γ is independent of NF-κB and STAT-1 activation. These data suggest that LCN-2 may play a role in β-cell function under an inflammatory condition.

**Key Words:** Lipocalin-2, Interleukin-1β, Interferon-γ, NF-κB, RINm5F cells

## MP-2

### A Protein Arginine Methyltransferase Isoform Controls the HIF-1-Mediated Adaptation to Hypoxia by Reducing De Novo Synthesis of HIF-1 Alpha Protein

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Hypoxia-inducible factor 1α (HIF-1α), which is regulated oxygen-dependently, transactivates numerous genes essential for cellular adaptation to hypoxia. HIF-1α expression is regulated at multiple steps from transcription to protein degradation. Moreover, the stability of HIF-1α protein has been known to be determined by posttranslational modifications such as ubiquitination, sumoylation, neddylation, and acetylation, but the HIF-1α regulation by methylation has not been reported. Protein methylation at arginine residues is an essential process to regulate gene expressions and signal transductions, and is catalyzed by PRMT enzymes. While testing which PRMT isoforms participate in the HIF-1 signaling pathway, we found that one of PRMTs modulates HIF-1α expression under hypoxia. When the PRMT was knocked-down in glioblastoma cells, HIF-1α was expressed even under normoxia and further induced under hypoxia. The transcriptional activity of HIF-1 was evaluated in reporter systems using EPO enhancer-luciferase or VEGF promoter-luciferase vector, and the HIF-1-driven gene expressions were checked by RT-qPCR. These assays demonstrated that functional HIF-1 was induced by PRMT knock-down. We next studied the mechanism of the HIF-1α induction, and found that HIF-1α was induced at the translational level through activated PI3K/Akt/mTOR signaling. Based on these findings, we propose that the PRMT negatively controls de novo synthesis of HIF-1α protein regardless of oxygen level. Given many literatures supporting the cancer promoting action of HIF-1α, the PRMT could be a potential target for cancer therapy.

**Key Words:** HIF-1α, PRMT, de novo synthesis

## MP-3

### G-Protein Regulatory (GPR) Motif of Activator of G-protein Signaling (AGS) 3 Protein Modulates SDF1α-Induced MUC1 Overproduction and Controls Airway Inflammation

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Mucus overproduction and airway obstruction are common features in airway mucosal inflammation. The mechanism by which SDF1α induces MUC1 overproduction, however, has not been fully explored. The aims of this study were two-fold; firstly, to examine the Activator of G-protein signaling (AGS) 3-dependent mechanism by which SDF1α reduces MUC1 gene expression and airway inflammation, and secondly, to identify specific molecules which could suppress SDF1α-induced airway inflammation at a G-protein coupled receptor level. Here, we suggest that SDF1α induces MUC1 gene expression via CXCR4 receptor. Interestingly, SDF1α signaling made an interaction between MUC1 and CXCR4 to regulate physiological phenomena. In addition, we showed that AGS3 plays as a suppressor for SDF1α-induced MUC1 and TNFα gene expressions by regulating with Giα, whereas it could not control MUC1-mediated IL-6 and IL-8 gene expression. More interestingly, G-protein Regulatory (GPR) motif in AGS3 bound to Giα and decreased MUC1 gene expression, whereas increased TNFα gene expression. In addition, GPR mutation (DDQR→DDAR) increased MUC1 and TGFβ gene expression, but decreased TNFα, IL-6, and IL-8 gene expressions. mGPR peptide inhibited significant morphologic changes and inflammatory cell infiltration after SDF1α exposure in mouse lungs. In addition, synthezied mGPR peptide also inhibited inflammatory cytokines in bronchoalveolar lavage (BAL) fluid and lungs.

These results suggest that GPR motif may be essential for regulating MUC1 and cytokines in inflammatory microenvironment and mGPR peptide play as suppressive compound to decrease airway inflammation.
**Key Words:** SDF1/CXCR4, MUC1, AGS3, GPR motif, Inflammatory cytokines

## MP-4(PO-6)

### Dysfunction of PTPRT Contributes to Depressive-Like Behavior Through Imbalance of Inhibitory GABAergic Synapses and Excitatory Glutamatergic Synapse

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Receptor protein tyrosine phosphatase receptor T (PTPRT), a receptor-like Protein tyrosine phosphatase (PTP), has a brain specific expression pattern in central nervous system. The effect of the PTPRT dysfunction in CNS on behavior has not yet been investigated. To investigate the function of PTPRT, we used PTPRT mutant mice which PTPRT in juxta-membrane domain following transmembrane domain of PTPRT was mutated by a substitution. Genotype of PTPRT mice was identified by PCR. We investigated male sexual behavior and maternal behavior, and also evaluated the role of PTPRT against chronic restraint stress. As results, male PTPRT mice showed less sexual behavior which include latency and frequency of copulatory behavior (mount, pelvic thrust and ejaculation) than wild-type (WT) mice ($p<.05$). Although female PTPRT mice could bear pups, many pups of PTPRT died neonatally and survival rate of pups of PTPRT was lower than that of WT ($p<0.05$). Serum corticosterone level was higher and GABA concentration in hypothalamus of male PTPRT mice was lower than that of WT mice ($p<0.05$). In chronic stress (CS) with 6 hours restraint during 14 days, body weight of male PTPRT mice was more decreased from 3 days after CS. Although weight loss of WT mice was stopped at 7 days after CS, weight loss of PTPRT mice was larger occurred than that of WT. Immobility time of PTPRT was longer than that of WT both before and after CS. These results suggest that PTPRT mutant has a depression-like behavior in male and impaired maternal behavior in female. Taken together, dysfunction of PTPRT is contributed to depressive-like behavior due to imbalance of inhibitory GABAergic synapses and excitatory glutamatergic synapse.
**Key Words:** PTPRT, Dysfunctiom, Depression, GABA

## MP-5

### Regulation of Autophagy by TRPM7 Channel Affecting A$\beta$ Production

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Autophagy is a catabolic process for degradation and recycling of cellular components, and the dysfunction of autophagy is related to many neurodegenerative diseases including Alzheimer's disease (AD). Even though intracellular $Ca^{2+}$ level is known to regulate autophagy, the mechanism for the $Ca^{2+}$ regulation of autophagy is unknown. As a ubiquitous $Ca^{2+}$ channel, TRPM7 channel underlies the constitutive $Ca^{2+}$ influx, and related to many neurodegenerative diseases. From these reasons, we hypothesize that $Ca^{2+}$ influx through TRPM7 channel regulates the basal autophagy. When TRPM7 channel expression is increased using tetracycline-inducible expression system, basal autophagy and AMPK phosphorylation (a main regulator for autophagy by $Ca^{2+}$) are increased. In contrast, basal autophagy and AMPK phosphorylation were decreased when TRPM7 channel expression is down-regulated by shRNA. Consistent with this result, basal autophagy, AMPK phosphorylation, are decreased by a specific TRPM7 blocker. Recently, autophagy has been suggested as the A$\beta$ clearance mechanism for AD. We have reported that the activation of TRPM7 channel is chronically suppressed by the presence of familial AD mutants. Consistent with close relationship between TRPM7 channel activity and autophagy, we demonstrated that the basal autophagy is down-regulated via AMPK signaling in FAD mutant cells. Moreover, A$\beta$ secretion was increased by TRPM7- specific shRNA in HeLa-APP cells. Therefore, TRPM7 channel contributes A$\beta$ clearance via basal autophagy.
**Key Words:** TRPM7, Autophagy, Alzheimer's disease

## MP-6

### Role of a Jumonji Histone Demethylase in Osteoclast Differentiation

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Osteoclasts are bone-resorbing multinucleated cells that differentiate from monocyte/macrophage-lineage precursors. Bone destruction and osteoporosis are attributed to increased and activated osteoclasts. Osteoclast differentiation is a complicated process governed by diverse classes of regulators. In particular, nuclear factor?activated T cells c1 (NFATc1) plays a key role in osteoclast differentiation in response to RANKL. Yet, the regulatory mechanism of the NFATc1 gene has not been uncovered so far. A growing body of evidence has demonstrated that histone methylation and demethylation are responsible for epigenetic regulation during stem cell differentiation. Therefore, we tested the possible involvement of a jumonji histone demethylase in the epigenetic regulation of NFATc1 during osteoclast differentiation. We generated transgenic (TG) mice overexpressing the jumonji demethylase and

checked the structures of their long bones using microCT. Consequently, bone volume/tissue volume (BV/TV), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were reduced in 4 month-old TG mice, whereas trabecular thickness (Tb.Th), body weight and tibia/femur length were not. Moreover, we found ex vivo that macrophages from the TG bone marrow have a higher potential for differentiation toward osteoclasts than those of wild type. We also identified that the jumonji histone demethylase associates with NFATc1 in macrophages stimulated by RANKL. These findings suggest that the jumonji histone demethylase potentiates bone resorption by promoting osteoclast differentiation, and imply that the inhibition of this enzyme could be a novel strategy for preventing inflammatory bone destruction or osteoporosis.
**Key Words:** Jumonji histone demethylase, Nuclear factor-activated T cells c1, Osteoclast

## MP-7

### O-GlcNAcylation of β-Amyloid Precursor Protein Reduces β-Amyloid Production by Decreasing Endocytosis

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β-amyloid precursor protein (APP) is transported to the plasma membrane, where it is sequentially cleaved by α-secretase and γ-secretase. This pathway is called non-amyloidogenic pathway, since it precludes the production of hydrophobic β-amyloid peptide (Aβ), the main culprit of Alzheimer's disease (AD). Alternatively, once APP undergoes clathrin-dependent endocytosis, it can be sequentially cleaved by β-secretase and γ-secretase at endosomes, producing Aβ (amyloidogenic pathway). O-GlcNAcylation is a novel type of O-linked glycosylation attaching the monosaccharide β-N-acetylglucosamine (GlcNAc) to serine and threonine residues. Recently, it is shown that O-GlcNAcylation of APP increases the non-amyloidogenic processing of APP and decreases the production of Aβ. In this study, we tested whether O-GlcNAcylation may affect APP processing via regulating its endocytosis. When O-GlcNAcylated APP was increased by using inhibitor of O-GlcNAcase, PUGNAc, the level of APP in the plasma membrane was increased. We also found that PUGNAc selectively attenuated the endocytosis of APP, but not that of transferrin receptor. The level of sAPPα increased, while the level of sAPPβ and Aβ was concomitantly decreased by PUGNAc. Blocking the clathrin-dependent endocytosis by inhibitor prevented the effect of PUGNAc, suggesting that the effect of PUGNAc on Aβ production was mainly mediated through decrease of APP endocytosis. These results strongly indicate that O-GlcNAcylation increases the plasma membrane targeting of APP and selectively decreases endocytosis rate of APP thereby enhancing non-amyloidogenic processing of APP. Thus, O-GlcNAcylation of APP is implied as a potential therapeutic target for AD.
**Key Words:** Alzheimer's disease, APP, Aβ, O-GlcNAcylation

## MP-8

### Pancreatic Beta-Cell Apoptosis under Glucotoxicity is Inhibited By GLP-1-Induced pAKTS473 Activation

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**Aim/hypothesis:** GLP-1 has been shown to protection in hyperglycemia induced β-cell apoptosis in type 2 diabetes. However, the cellular mechanism underlying of GLP-1 at glucotoxicity remain largely unknown. Therefore, the primary objective of this study was to elucidate the molecular mechanism of GLP-1 effect in β-cell. **Mothods:** Experiments were performed on pancreatic acute slice and INS-1 cells. The effects of GLP-1 in mTORC2/pAKT activation and antiapoptosis were investigated using cell viability assay, western blotting, immunocytochemistry, kinase assay and small interfering (si) RNA. **Results:** GLP-1 treatment of pancreatic acute slice and INS-1 suppressed high glucotoxicity-induced apoptosis. The effect of GLP-1 was related to the activation of pAKTSer473 via mTROC2, but not pAKTThr308. GLP-1 induced pAKTSer473 activation and antiapoptosis were abolished by the selective inactivation of mTORC2 using siRNA directed towards rapamysin-insensitive companion of target of rapamysin (RICTOR). Moreover, p-p70S6K (Thr421/Ser424) was also inhibited by siRICTOR. **Conclusion:** This report provides evidence that glucotoxicity-induced β-cell apoptosis is abolished by the mTROC2/pAKT activation via GLP-1. Therefore, GLP-1 induced mTORC2 activation is essential to β-cell function and survival.
**Key Words:** GLP-1, pAKT, mTORC2

## MP-9

### Cytoplasmic Localization and Redox Cysteine Residue of APE1/Ref-1 is Associated with Anti-Inflammatory Activity in Cultured Endothelial Cells

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Apurinic/apyrimidinic endonuclease1/redox factor-1 (APE1/Ref-1) is a multifunctional protein involved in base excision DNA repair and transcriptional regulation of gene expression. APE1/Ref-1 is mainly localized in nucleus, but cytoplasmic localization has also been reported. However, the functional role of cytoplasmic APE1/Ref-1 and its redox cysteine residue is still unknown. We investigated the role of cytoplasmic APE1/Ref-1 on tumor necrosis factor-α (TNF-α)-induced vascular cell adhesion molecule-1 (V-

CAM-1) expression in endothelial cells. Endogenous APE1/Ref-1 was mainly observed in nuclei however, cytoplasmic APE1/Ref-1 was increased by TNF-$\alpha$. Cytoplasmic APE1/Ref-1 expression was not blunted by cycloheximide, a protein synthesis inhibitor, suggesting cytoplasmic translocation of APE1/Ref-1. Transfection of an N-terminus deletion mutant APE1/Ref-1 (29-318) inhibited TNF-$\alpha$-induced VCAM-1 expression, indicating an anti-inflammatory role for APE1/Ref-1 in cytoplasm. In contrast, redox mutant of APE1/Ref-1 (C65A/C93A) transfection led to increased TNF-$\alpha$-induced VCAM-1 expression. Our findings suggest cytoplasmic APE1/Ref-1 localization and redox cysteine residues of APE1/Ref-1 are associated with anti-inflammatory activity in endothelial cells.

**Key Words:** APE1/Ref-1, TNF-$\alpha$, VCAM-1, Endothelial cells

## MP-10

### Silibinin Induces Cell Death Through ROS-Dependent Down-Regulation Notch-1/ERK/Akt Signaling in Human Breast Cancer Cells

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Anticancer activity of silibinin, a flavonoid, has been demonstrated in various cancer cell types. However, the underlying mechanisms were not elucidated in human breast cancer cells. The present study was performed to elucidate underlying mechanism of silibinin-induced cell death of human breast cancer cell line MCF7 and MDA-MB-231. Silibinin suppressed cell viability in a time- and dose- dependent manner in both cell lines. Silibinin-induced cell death was attenuated by antioxidants, N-acetylcysteine (NAC) and Trolox, suggesting that the effect of silibinin was dependent on generation of reactive oxygen species (ROS). Western blot analysis showed that silibinin induced down-regulation of ERK and Akt. When cells were transiently transfected with constitutively active MEK (caMEK) and Akt (caAkt), they showed resistance to silibinin-induced cell death. Silibinin decreased the cleavage of Notch-1 mRNA and protein levels. Notch-1-overexpressed cells were resistant to silibinin-induced cell death. Notch-1 signaling was dependent on ROS generation, and the overexpression of Notch-1 prevented silibinin-induced inhibition of p-ERK and p-Akt. These results indicate that ROS generation and Notch-1 signaling act upstream of the ERK and Akt pathway in the silibinin-induced breast cancer cell death. These data suggest that silibinin may serve as a potential therapeutic agent for human breast cancer cells.

**Key Words:** Breast cacner, Notch1, ROS, Cell death

## MP-11

### Role of Bone Morphogenic Protein-2 on Ostegenic Differentiation of Human Adipose Tissue- and Bone Marrow-Derived Mesenchymal Stem Cells

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The bone morphogenetic proteins (BMPs) belong to a unique group of proteins that includes the growth factor TGF-$\beta$. BMPs play important roles in cell differentiation, cell proliferation, and inhibition of cell growth. BMPs can induce the differentiation of mesenchymal progenitor cells into various cell types, including chondroblasts and osteoblasts. The aim of this study was to analyze the effect of BMP-2 on the adipogenic and osteogenic differentiation of human adipose tissue- (hADSC) and bone marrow (h-BMSC)-derived mesenchymal stem cells. BMP-2 increased osteogenic differentiation in human bone marrow-derived MSCs (hBMSC) without affect their adipogenic differentiation. Unexpectedly, BMP-2 increased adipogenic differentiation of hADSC without affecting osteogenic differentiation at an osteogenic differentiation condition. BMP-2 did not enhance adipogenic differentiation at an adipogenic differentiation condition. Real time PCR analysis showed that hADSC express BMP receptors and SMAD1 and 4, and that BMP-2 increased the expression of BMP2-responsive genes and induced SMAD1 phosphorylation in hADSC. Proteome analysis showed that BMP2 increased adipogenesis-related protein levels. Downregulation of SMAD1 and 4 by the specific siRNAs transfection inhibited BMP2-induced increase of adipogenic differentiation in hADSC. At the condition that adipogenic differentiation was inhibited by the treatment of TNF-$\alpha$, BMP2 stimulated osteogenic differentiation of hADSC. These data indicated that the control of osteogenesis and adipogenesis in MSCs are closely related, and that hADSC have preferential commitment into adipogenic lineages. This works was supported by grant from Ministry of education, science and technology (2012M3A9B402-8558).

**Key Words:** BMP-2, Osteogenic differentiation, hADSC, hBMSC

## MP-12

### Valproic Acid Induces Calu-6 lung Cancer Cell Death via Caspase-Dependent Apoptosis and GSH Depletion

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Valproic acid (VPA) as a histone deacetylase (HDAC) inhibitor has various cellular effects such as differentiation and apoptosis. In the present study, we evaluated the effects of VPA on the growth and death of Calu-6 lung cancer cells. VPA inhibited the growth of Calu-6 cells with an IC50 of approximately 3 mM at 72 hours in a dose-dependent manner. DNA flow cytometric analysis indicated that VPA induced a G2/M phase arrest of the cell cycle. This agent also induced apoptosis, as evidenced by sub-G1 cells and annexin V-FITC staining cells. VPA-induced apoptosis was accompanied by the loss of mitochondrial membrane potential (MMP; $\Delta\psi$m), PARP-1 cleavage, Bax increase, and the activation of caspase-3. All of the tested caspase inhibitors prevented Calu-6 cell death induced by VPA. VPA increased intracellular reactive oxygen species (ROS) levels and induced glutathione (GSH) depletion in Calu-6 cells. Generally, caspase inhibitors did not affect ROS levels in VPA-treated Calu-6 cells, but they significantly prevent GSH depletion in these cells. Furthermore, while the well-known antioxidant, N-acetyl cysteine (NAC) did not affect cell death, ROS level or GSH depletion, L-buthionine sulfoximine (BSO), a GSH synthesis inhibitor, enhanced cell death and GSH depletion in these cells. In conclusion, VPA inhibited the growth of Calu-6 lung cancer cells via caspase-dependent apoptosis, and the inhibition is dependent on GSH level changes. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government through the Diabetes Research Center at Chonbuk National University (2012-0009323) and supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, (2013006279).
**Key Words:** Valproic acid, Apoptosis, Calu-6, Caspase, Glutathione

## MP-13

### $Ca^{2+}$ Response Pattern and Activity to Tastant in Mouse Salivary Gland and Exocrine Glands

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**Background:** Taste gives an appetite and it protects us from poisons. Taste sense is very crucial factor for quality of life and survival. Taste cells utilize G protein-coupled receptors to detect sweet, bitter and umami taste whereas salty and sour taste are detected with ion channels. Recently, some of these taste receptors were identified in non-taste cells. Salivary and other exocrine glands are related to the roles in sensing of taste and transduction of taste. Therefore, we examined whether salivary gland and exocrine glands respond to tastant and analyzed relative difference between several taste stimuli. **Methods:** Submandibular and lacrimal glands and pancreas were digested with collagenase treatment. The acinar cells were loaded with fura-2 and measured $Ca^{2+}$ activity while applying several different tastants. Treated compounds were cycloheximide, denatonium, and phenylthiourea (bitter); saccharine (sweet); glutamate (umami); and carbachol (positive control). **Results:** $Ca^{2+}$ responses elicited by each chemical stimulus showed distinct peak amplitude. Cycloheximide, denatonium and phenylthiourea elicited increase of $Ca^{2+}$ activity with different patterns and dose-dependent manner, depending on each compound and cells. However, saccharine and glutamate did not show change in $Ca^{2+}$ activity. No different $Ca^{2+}$ activity effect showed in $Ca^{2+}$ free medium condition. These results suggest that the $[Ca^{2+}]_i$ increases in response to taste compounds are due to release from intracellular $Ca^{2+}$ stores, and are not derived from $Ca^{2+}$ influx. **Conclusions:** Our study show that murine salivary gland and exocrine glands respond to bitter taste with different range of chemical sensitivities. This suggests that individual cells distinguish between bitter taste and the possibility of other exocrine glands also directly respond to taste. *This work was supported by grant (NO. 2013R1A1A- 2008424) from Ministry of Science, ICT and Future Planning (MSIP), Republic of Korea.
**Key Words:** Taste receptor, Exocrine gland, $Ca^{2+}$ imaging

## MP-14

### Extract from Lycoris Chejuensis Reduces $\beta$-Amyloid Production via Decreasing $\beta$-Amyloid Precursor Protein

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Neurotoxic $\beta$-amyloid (A$\beta$) peptides may play a central role in Alzheimer's disease (AD). A$\beta$ is produced by the proteolysis of amyloid $\beta$ precursor protein (APP). We examined the effect of Lycoris chejuensis (CJ) extract on A$\beta$ and memory impairment in AD experimental models in vitro and in vivo. HeLa cells stably expressing Swedish mutant form of APP were incubated with 50% ethanol extract of CJ. A$\beta$40 and A$\beta$42 levels from the conditioned media were decreased in a dose-dependent manner by CJ. CJ decreased the levels of immature APP as well as mature APP. When double transgenic mice expressing both APPswe and presenilin-1 mutant were treated orally with CJ extract (150 mg/kg) for 4 months, spatial memory was significantly enhanced in Morris water maze test, improving both acquisition and probe phases. The exploration time in novel object recognition test was also increased. Toxic A$\beta$42 level as well as amyloid plaques were significantly decreased in animals treated with CJ extract. Taken together, CJ extract may reduce A$\beta$ by attenuating APP levels. Further research on the constituents of CJ extract and the mechanism of action will be needed.
**Key Words:** $\beta$-amyloid, Alzheimer's disease

## MP-15

### Docosahexaenoic Acid Improves Vascular Function via Up-Regulation of SIRT1 Expression in Endothelial Cells

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n-3-Polyunsaturated fatty acids (PUFAs) protect against myocardial infarction, arteriosclerosis and high blood pressure by stimulating endothelial nitric oxide synthase (eNOS) to increase nitric oxide (NO) production. However, the mechanism remains to be elucidated. This study investigated the role of SIRT1 in the protective effects of docosahexaenoic acid (DHA) in vascular endothelial cells. Exposure of human umbilical vein endothelial cells (HUVECs) to 0.3～30 lM DHA did not affect cell viability, and DHA treatment dose-dependently increased SIRT1 expression. The DHA-mediated increase in SIRT1 expression induced eNOS deacetylation, increasing endothelial NO. However, inhibition of SIRT1 inhibited DHAmediated increases in NO production. This effect was mediated via deacetylation of lysines 496 and 506 in the eNOS calmodulin-binding domain. The effects of DHA were also demonstrated in rat aortic rings, in which DHA treatment increased SIRT1 expression and bioavailable NO. Our results demonstrate that SIRT1 plays an important role in DHA-mediated increases in bioavailable NO via decreased eNOS acetylation.

**Key Words:** eNOS, Nitric oxide, SIRT1, Docosahexaenoic acid

## MP-16(PO-8)

### APE1/Ref-1 Inhibits Protein Kinase C-Induced Mitochondrial Dysfunction in Endothelial Cells

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Phorbol 12-myristate 13-acetate (PMA), an agonist of protein kinase C, induces mitochondrial dysfunction, which is an important pathological factor in cardiovascular diseases. Human apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) is elevated in mitochondria in pathologic condition. In this study, we investigated that the role of APE1/Ref-1 on the PMA-induced mitochondrial dysfunction in mouse endothelial MS-1 cells. PMA-stimulated cells induced mitochondrial hyperpolarization and mitochondrial ROS generation. We observed that elevation of mitochondrial APE1/Ref-1 level is induced following PMA treatment. APE1/Ref-1 overexpression suppressed PMA-induced the disruption of mitochondrial function and this suppression was completely achieved in mitochondria-specific, MTS-APE1/Ref-1-overexpressed cells. These results suggest that PMA-induced mitochondrial translocation of APE1/Ref-1 is closely related in its inhibitory action against the disruption of mitochondrial membrane potential and the increase of mitochondrial ROS in mouse endothelial MS-1 cells.

**Key Words:** APE1/Ref-1, Endothelial cell, Mitochondria, Phorbol 12-myristate 13-acetate

## MP-17(PO-7)

### Interaction of Macrophages with Apoptotic Cells or Gas6 Blocks Epithelial-Mesenchymal Transition

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Apoptotic cell clearance results in the release of growth factors and the action of signaling molecules involved with the maintenance of tissue homeostasis. Here, we investigated the effect of conditioned medium derived from macrophage that had been exposed to apoptotic Jurkat T cell or Gas6 an apoptotic cell bridge molecular in TGF-$\beta$1-induced epithelial-mesenchymal transition (EMT). Exposure of alveolar epithelial cells (LA-4) to TGF-$\beta$1 for 3 days induced a complete conversion of the epithelial cells to myofibroblasts as evidenced by acquisition of spindle-like morphology, loss of E-cadherin, and synthesis of N-cadherin and $\alpha$-smooth muscle actin ($\alpha$-SMA). Conditioned medium derived from macrophages (Raw 264.7 cells) that had been exposed to apoptotic or necrotic Jurkat T cell (Apo CM or Nec CM) were treated in alveolar epithelial cells (LA-4 cells) stimulated TGF-$\beta$1 for 3 days. Treatment with conditioned medium derived from Raw 264.7 cells that had been exposed to apoptotic cells inhibited TGF-$\beta$1-induced loss of E-cadherin and, synthesis of N-cadherin and $\alpha$-SMA. However conditioned medium derived from Raw 294.7 cells that had been exposed to necrotic Jurkat T cells as well as medium derived from cultured apoptotic Jurkat cells alone had no effect on EMT. In addition, Gas6 the bridge protein and a ligand of TAM family receptors. Similarly Gas6, a bridging molecules to link apoptotic cells and their phagocytic receptors, Mer, also blocked TGF-$\beta$1-induced EMT. Therefore, our data suggest that interaction of macrophages with apoptotic cells or Gas6 leads to endogenous potent inhibitors of EMT that represents a new form of epithelial cell homeostasis.

**Key Words:** Conditioned medium, Apoptotic cell, Gas6, TGF-$\beta$1, EMT

## MP-18

### 3, 3'-Diindolylmethane Inhibits Migration and Invasion of Human Colon Cancer Cells through Down Regulation of uPA and SUSD2

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Despite that 3, 3'-diindolylmethane (DIM) inhibits migration and invasion in a variety of cancer cells, the biological invasive function of DIM in colon cancer cell has not been clearly understood. In the present study we investigated the effect of DIM on migration and invasion in colon cancer cells. Human colon cancer cell lines (HCT-116 and DLD-1) were used to test the response to DIM. DIM significantly inhibited the migration ability of colon cancer cells by wound healing assay. The expression of uPA was significantly attenuated by DIM treatment. In addition, DIM significantly decreased mRNA levels of uPA and SUSD2. E-cadherin was significantly enhanced by DIM treatment. Therefore, our results suggest that DIM may decrease invasive capacity of human colon cancer cells through down regulation of uPA and SUSD2.
**Key Words:** DIM, Colon cancer cells, uPA, Metastasis

## MP-19

### Expression Profiling of Ion Channel Genes Predicts Clinical Outcome in Breast Cancer

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**Background:** Ion channels play a critical role in a wide variety of biological processes, including the development of human cancer. However, the overall impact of ion channels on tumorigenicity in breast cancer remains controversial. **Methods:** We conduct microarray meta-analysis on 280 ion channel genes. We identify candidate ion channels that are implicated in breast cancer based on gene expression profiling. We test the relationship between the expression of ion channel genes and p53 mutation status, ER status, and histological tumor grade in the discovery cohort. A molecular signature consisting of ion channel genes (IC30) is identified by Spearman's rank correlation test conducted between tumor grade and gene expression. A risk scoring system is developed based on IC30. We test the prognostic power of IC30 in the discovery and seven validation cohorts by both Cox proportional hazard regression and log-rank test. **Results:** 22, 24, and 30 ion channel genes are found to be differentially expressed with a change in p53 mutation status, ER status, and tumor histological grade in the discovery cohort. We assign the 30 tumor grade associated ion channel genes as the IC30 gene signature. We find that IC30 risk score predicts clinical outcome ($p < 0.05$) in the discovery cohort and 6 out of 7 validation cohorts. Multivariate and univariate tests conducted in two validation cohorts indicate that IC30 is a robust prognostic biomarker, which is independent of standard clinical and pathological prognostic factors including patient age, lymph node status, tumor size, tumor grade, estrogen and progesterone receptor status, and p53 mutation status. **Conclusions:** We identified a molecular gene signature IC30, which represents a promising diagnostic and prognostic biomarker in breast cancer. Our results indicate that information regarding the expression of ion channels in tumor pathology could provide new targets for therapy in human cancers.
**Key Words:** Ion channel, Breast cancer, Gene expression, Molecular signature, Microarray

## MP-20

### Apoptotic Cells/Gas6/Mer Signaling in Macrophages Leads to HGF Secretion Which Promotes Epithelial Cell Proliferation and Wound Repair

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Previously we found that apoptotic cells or Gas6/Mer signaling leads to transcriptional HGF production. The present study investigated the functional relevance of activation of Mer and induction of HGF in macrophages upon exposure to apoptotic cells or Gas6 was evaluated by examining epithelial cell (LA-4 cell) proliferation and wound repair. The conditioned medium derived from RAW 264.7 cells had been exposed to apoptotic cells or Gas6 caused an enhancement of epithelial cell proliferation and wound closure. However, when LA-4 cells were exposed to the conditioned medium with the HGF receptor-blocking antibody or c-Met antagonist PHA, the proliferation of LA-4 cells and wound closure were suppressed. The conditioned medium derived from RAW 264.7 cells had been exposed to apoptotic cells or Gas6 in the presence of neutralizing antibody or siRNA of Mer blocked epithelial cell proliferation and wound closure. Furthermore, we demonstrate that the conditioned medium derived from LA-4 cells had been exposed to apoptotic cells inhibited both spontaneous and $H_2O_2$-induced apoptosis in LA-4 cells. These effects were not observed when phagocytes had been exposed to viable or necrotic Jurkat T cells. Our data provide evidence that apoptotic cells/Gas6/Mer signaling leads to transcriptional HGF secretion by macrophages, that promotes epithelial growth.
**Key Words:** Gas6/Mertk complex, HGF, Epithelial wound repair

## MP-21

### Non-Silent Story in Voltage-Gated Ion Channel

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

**Key Words:** Synonymous, Codon, Silent, Mutation, Voltage-gated ion channel

## MP-22

### Effects of Adenosine and 5'-(N-ethylcarboxamido) Adenosine on Proliferation and Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells

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In this study, we considered possibility that inflammatory-product of tissue microenvironment affects properties of mesenchymal stem cells (MSCs) grafted for tissue regeneration. To determine effect of adenosine, product of tissue-damage or inflammatory-response, for function of MSCs then we confirmed effect of adenosine and 5'-(N-ethylcarboxamido) adenosine (NECA) for proliferation and differentiation of human adipose tissue-derived mesenchymal stem cells (hADSCs) and examined their mechanism. In adenosine receptors, A2A and A2B receptor are highly expressed in hADSC. Adenosine and NECA did not affect the proliferation and ability of adipogenic differentiation, but inhibited osteogenic differentiation in a dose-dependent manner of hADSC. Adenosine and NECA did not increased cAMP concentration of hADSC. However, the pretreatment of H89, a protein kinase A inhibitor, did not affect NECA-induced inhibition of osteogenic differentiation. These results indicate that adenosine and NECA inhibit osteogenic differentiation of hADSC, these effect are independent of cAMP pathway.

**Key Words:** MSC, hADSCs, Adenosine, Adenosine receptors, NECA

## MP-23

### Effect of the Mer inhibtion by Mer/Fc on LXR Activation and Acute Sterile Inflammation

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Mer signal plays the central role in the intrinsic inhibition of the inflammatory response to pathogens by macrophages and dendritic cells. Liver X receptors (LXR$\alpha$ and LXR$\beta$) also have a role for the maintenance of immune tolerance. In the current study, we investigated whether Mer signaling leads to LXR$\alpha/\beta$ induction and suppresses zymosan-induced acute inflammation, using the inhibitor of Gas6 Mer/Fc fusion protein. After zymosan injection, Gas6 expression in peritoneal lavage fluid, spleen and lung increased over the course of inflammation. In addition to increased Gas6 protein, phosphorylation of Mer was also enhanced up to 72 hours after zymosan treatment. Pretreatment with Mer/Fc significantly reduced phosphorylation of Mer at hours 6, 24 after zymosan injection. The levels of pro-inflammatory mediators, TNF-$\alpha$, IL-1$\beta$ and MIP-2, in peritoneal lavage fluid (PLF) were increased at 6 hours and diminished baseline within 24 hours after zymosan treatment. In contrast, treatment with Mer/Fc further increased expression of pro-inflammatory mediators. Anti-inflammatory mediators, TGF-$\beta$1 and HGF, also increased up to 24 hours, but were inhibited by treatment of Mer/Fc. Moreover, Mer/Fc further enhanced neutrophil influx into the peritoneal cavity and the level of proteins in PLF. After zymosan injection, LXR$\alpha/\beta$ protein expression in spleen and lung reduced at 6 hours and recovered the control level at 24 hours. Treatment with Mer/Fc further decreased LXR$\alpha/\beta$ protein levels at 6 h, and the delayed their recovery at 24 h after zymosan treatment. In parallel, ABCA1 and ApoE protein levels were reduced at each time point. These findings suggest that Gas6/Mer signaling contributes to the resolution of acute inflammation through up-regulation of LXR expression and activation.

**Key Words:** Mer receptor tyrosine kinase, Liver X receptor, Zymosan, Mer/Fc, Inflammatory responses

## MP-24

### Identification of Valid Housekeeping Genes for Quantitative RT-PCR Analysis of Human Adipose Tissue-Derived Mesenchymal Stem Cells During Differentiation

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Human adipose tissue-derived mesenchymal stem cells (hADSCs) have therapeutic potential, including the ability to self-renew and differentiate toward multiple lineages. For the accurate determination of differentiation-related gene expression changes during differentiation, quantitative real-time PCR is often the method of choice. The technology is very sensitive, however, without a proper selection of stable housekeeping genes for normalization of real-time PCR, erroneous results may be obtained. In this study, we have compared the gene expression levels of a panel of 6 housekeeping genes, beta-actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), betaglucuronidase (GUSB), TATAA-box binding protein (TBP), ribosomal protein L13a (RPL13A) and tyrosine 3/tryptophan 5-monooxygenase activation protein (YWHAZ) during adipogenic, osteogenic, chondrogenic and hepatogenic differentiation of primary hADSCs. Our study showed that several of the commonly used reference genes including GAPDH and $\beta$-actin were unsuitable for normalization in the conditions we tested, whereas TBP, GUSB were stable across all conditions. Furthermore, we quantified the differential expression of adipogenic, osteogenic, chondrogenic and hepatogenic markers to assess the housekeeping gene variability.

**Key Words:** hADSCs, Differentiation, Housekeeping genes, Normalization

## MP-25

### Isobavachalcone from Psoralea Corylifolia Suppressed LPS-Induced ICAM-1 Expression in Cerebrovascular Endothelial Cells

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Brain inflammation has been implicated in various cerebral diseases. Leukocyte-infiltration into brain parenchyma is critically associated with in the development brain inflammation. Therefore, control of leukocyte infiltration is a very important therapeutic target for the treatment of neurodegenerative diseases accompanied with inflammation such as Alzheimer's disease and stroke. Isobavachalcone (IBC), a flavonoid from Psoralea corylifolia, is known to possess a wide spectrum of biological activities, antibacterial, antifungal, anticancer, anti-reverse transcriptase, antitubercular and antioxidant. Recently, it was reported that IBC suppresses LPS-induced iNOS expression and is expected to be useful for preventing or treating neurodegenerative disease. However, the effect of IBC on leukocyte-endothelial adhesion and expression of intercellular adhesion molecule-1(ICAM-1) in brain endothelial cells remains unexplored. In this study, we examined the effect of IBC on ICAM expression and leukocyte adhesion in bEnd. 3 cells and explored the possible mechanisms therein involved. IBC significantly down-regulated LPS-induced ICAM-1 expression and leukocytes-endothelial adhesion. IBC suppressed LPS-induced sequential events for NF-kB activation, that is, IkB-$\alpha$ phosphorylation, p65 translocation into nucleus and NF-kB transcriptional activity. TLR4 conveys LPS-signal to intracellular compartment via MyD88- and TRIF-dependent pathways, which culminate in the activation of NF-kB. IBC attenuated MALP-2 (a TLR2, 6 specific ligand)-induced ICAM-1 expression and NF-kB transcriptional activity, suggesting inhibition of MyD88-dependent signaling pathway. IBC also down-regulated poly[I:C] (a TLR3 specific ligand)-induced expression of ICAM-1 and IFN-$\beta$, which was mediated by suppression of NF-kB and IFN-$\beta$ transcriptional activity, respectively. These data indicate TRIF-dependent signaling pathway is also blocked by IBC. Taken together, our data suggest that IBC inhibits LPS-induced ICAM-1 expression and leukocyte adhesion in brain endothelial cells and these effects are mediated by blockade of MyD88-dependent and TRIF-dependent signaling pathways and in turn, inhibition of NF-kB activity.

**Key Words:** Isobavachalcone (IBC), Lipopoysaccharide (LPS), Intercellular adhesion molecule (ICAM-1), NF-kB, Cerebrovascular endothelial cells

## MP-26

### Cell Proliferation is Regulated by Extracellular Calcium Sensing Receptor and Caveolin in Cultured Rat Renal Epithelial Cells

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An important role of kidney is to maintain electrolyte and water homeostasis by sensing changes in its extracellular environment. It is well known that chronic hypercalcemia is responsible for a defect in the urine concentration. The cell response by extracellular calcium change is caused through the extracellular calcium sensing receptor (CaSR), which belongs to the type III family of G-protein-coupled receptors. In order to clarify the effect of CaSR in renal epithelial cells, we examined the expression of the CaSR, relationship with cavelin and extracellular calcium-induced cell proliferation and the change of intracellular free calcium in cultured rat renal epithelial cell line, NRK-52E. The intracellular calcium concentration ($[Ca^{2+}]_i$) was increased by an increment of $[Ca^{2+}]_o$. This increment was inhibited by

NPS 2390, an antagonist of CaSR, pretreatment. RT- PCR and Western blot analysis of NRK-52E cells revealed the presence of CaSR, caveolin (Cav)-1 and -2 in both mRNA and protein expressions, but there was no expression of Cav-3 mRNA and protein in the cells. In the isolated caveolae-rich fraction from NRK-52E cells, the CaSR, Cav-1 and Cav-2 proteins were localized in same fractions (fraction number 4 and 5). The immuno-precipitation experiment using the respective antibodies showed complex formation between the CaSR and Cav-1, but no complex formation of CaSR and Cav-2. Confocal microscopy also supported the co-localization of CaSR and Cav-1 at the plasma membrane. Functionally, the $[Ca^{2+}]_o$- induced $[Ca^{2+}]_i$ increment was attenuated by the introduction of Cav-1 antisense oligodeoxynucleotide (ODN). From these results, in NRK-52E cells, the function of CaSR might be regulated by binding with Cav-1. Considering the decrement of CaSR activity by antisense ODN, Cav-1 up-regulates the function of CaSR under normal physiological conditions, and it may play an important role in the diverse pathophysiological processes of CaSR-related renal disorders in the body.

**Key Words:** Calcium sensing receptor, Caveolin, Proliferation

## MP-27

### Upregulation of Mitochondrial Nox4 Mediates TGF-β-Induced Apoptosis in Mouse Podocytes

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Injury to podocyte leads to the onset of chronic renal diseases characterized by proteinuria. Elevated TGF-β in kidney tissue is associated with podocyte damage that ultimately results in apoptosis and detachment. We investigated the proapoptotic mechanism of TGF-β in immortalized mouse podocytes. Exogenous TGF-β1-induced podocyte apoptosis through caspase-3 activation which was related to elevated reactive oxygen species (ROS) levels generated by selective upregulation of Nox4. In mouse podocytes, Nox4 was predominantly localized to mitochondria and Nox4 upregulation by TGF-β1 markedly depolarized the mitochondrial membrane potential. TGF-β1-induced ROS production and caspase activation was mitigated by an antioxidant, the Nox inhibitor DPI, or siRNA for Nox4. A TGF-β receptor I blocker, SB431542, completely reversed the changes triggered by TGF-β1. Knock-down of either Smad2 or Smad3 prevented increase of Nox4 expression, ROS generation, loss of mitochondrial membrane potential, and caspase-3 activation by TGF-β1. These results suggest that TGF-β1-induced mitochondrial Nox4 upregulation via TGF-β receptor-Smad2/3 pathway is responsible for ROS production, mitochondrial dysfunction, and apoptosis, which may at least in part contribute to the development and progression of proteinuric glomerular diseases such as diabetic nephropathy.

**Key Words:** Podocyte, Transforming growth factor-β, NADPH oxidase 4, Mitochondria, Apoptosis

## MP-28

### Auranofin-Induced Lung Cancer Cell Death is Related to Glutathione Level

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Auranofin as an inhibitor of thioredoxin reductase (TrxR) affect many biological processes such as inflammation and proliferation. In this study, we evaluated the effects of auranofin on lung cancer cells such as small cell lung carcinoma Calu-6 cells, and non-small cell lung carcinoma A549, NCI-H460 and NCI-H1299 cells in relation to cell growth, cell death and reactive oxygen species (ROS) and glutathione (GSH) levels. Auranofin decreased the growth of lung cancer cells with an IC50 of approximately 1-4 μM at 24 h in a dose-dependent manner. Auranofin induced G2/M phase arrest in only Calu-6 cells. This agent also induced apoptosis as well as necrosis in lung cancer cells. In relation to ROS and GSH levels, auranofin increased ROS level, especially O2•- in lung cancer cells and induced GSH depletion. While N-acetyl cysteine (NAC; an antioxidant) did not prevent cell death induced by auranofin in lung cancer cells, L-buthionine sulfoximine (BSO; an inhibitor of GSH synthesis) intensified cell death and GSH depletion in these cells. In conclusion, auranofin inhibited the growth of lung cancer cells via apoptosis and necrosis, and the inhibition was closely related to GSH level. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government through the Diabetes Research Center at Chonbuk National University (2012-0009323) and supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, (2013006279).

**Key Words:** Auranofin, Thioredoxin Reductase, Apoptosis, Necrosis, Glutathione

## MP-29

### RhBMP-2 Inhibits Human Gastric Cancer Cells by Inactivation of AURKA and AURKB via c-Myc

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Since rhBMP-2 has been used most commonly as bone graft substitutes, several safety issues including a possible cancer risk have arisen. To investigate the biological ef-

fects of rhBMP on human gastric cancer cells, we performed a microarray experiment to find important gene expression patterns of rhBMP's effects. Using an Illumina bead array platform, we generated gene expression data from human gastric cancer cells (SNU484). The gene expression data showed that the expressions of 111 genes were significantly more than 2 fold up-regulated and 77 genes were down-regulated by rhBMP-2 treatment in SNU 484 cells. Cyclin A and cyclin B were suppressed and c-Myc, AURKA, and AURKB were also down-regulated by rhBMP-2 treatment in SNU 484 cells. We also confirmed the mRNA expressions of c-Myc, AURKA and AURKB were significantly suppressed by rhBMP-2 treatment in SNU 484 cells using qPCR. Protein level of c-Myc was significantly decreased after rhBMP-2 treatment in SNU 484 cells, indicating that rhBMP-2 attenuates the growth of gastric cancer cells via inactivation of AURKA and AURKB via c-Myc.

**Key Words:** Gastric cancer cell, rhBMP-2, Microarray, AurkA/B

## MP-30

### Regulation of Renin Secretion and Blood Pressure in Mice without eNOS and nNOS

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To investigate the role of nitric oxide (NO) in macula densa control of renin secretion, the experiment was performed with eNOS/nNOS-/- mice. We measured the acute response of plasma renin concentration (PRC, ng Ang I/ml hr) to furosemide (40 mg/kg i.p.), L-NAME (50 ng/kg i.p), hydralazine (1 mg/kg i.p), propranolol (10 mg/kg, i.p), candesartan (50 $\mu$g), or quinaprilate (50 $\mu$g) in wild type (WT) and eNOS/nNOS-/- mice. Basal level of PRC (ng Angl/ml hr) was significantly lower in eNOSnNOS-/- mice than in WT mice (1,646 ± 32 versus 912 ± 9; $p<0.05$). Renin content and mRNA were 43 % and 21.2 ± 2.4 % of WT. L-NAME reduced PRC significantly in WT, but had no effect in eNOS/nNOS-/- mice suggesting that other NOS isoforms do not measurably affect renin secretion. Acute stimulation of renin release by furosemide, hydralazine, quinaprilate, or candesartan caused significant increases of PRC in both eNOSnNOS-/- and WT mice, but the absolute changes were great in WT mice. SupermineNONOate and papaNONOate caused marked increases of PRC in wild type mice. Blood pressure and heart rate responses to furosemide, hydralazine, propranolol, candesartan, or quinaprilate were not different between eNOSnNOS gynotypes by radiotelemetry. Maintenance of renin secretory responses to furosemide and other agents in the absence of both eNOS and nNOS indicates that NO is a permissive rather than a mediating factor in the MD control of renin release.

**Key Words:** Renin, Enosnnos-/-mice, Blood Pressure

## MP-31

### Glucosamine-Induced Gluconeogenesis Contributes to Mouse Embryonic Stem Cells Self-Renewal: Involvement of Notch1/FoxO1 Pathway

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Glucosamine (GlcN) increased glucose production and gluconeogenic enzymes (G6Pase and PEPCK) expression. GlcN also increased percentage of the cell population in the S phase and cell cycle regulatory proteins (cyclinE, CDK2, cyclinD1, CDK4), expression, which were blocked by 3-mercaptopicolinic acid (gluconeogenesis inhibitor). Next, GlcN stimulated ROS generation and OGT activation, which were blocked by antioxidant (NAC). Inhibition of OGT (ST045849) decreased GlcN-induced glucose production. GlcN enhanced the OGT-dependent O-GlcNAcylated Notch1 and FoxO1. GlcN increased phosphorylation of JNK, ERK or p38. GlcN also induced expression of cleaved notch1 in nucleus, which was blocked by SP600125 (JNK inhibitor), but not by PD98059 (ERK inhibitor) or SB203580 (P38 inhibitor). In addition, GlcN increased the cleaved Notch1/FoxO1 binding to CSL transcription factor. Blockage of Notch1 (L-685,458; $\gamma$-secretase inhibitor) and FoxO1 (specific siRNA) decreased GlcN-increased G6Pase and PEPCK expression. Additionally, GlcN maintained undifferentiation status while depletion of Notch1 and FoxO1 decreased Oct4, SSEA-1, and alkaline phosphatase activity or increased differentiation markers [GATA4 (endoderm), Tbx5 (mesoderm), Cdx2 (trophectoderm), Fgf5 (ectoderm)] for 3 days. In conclusion, GlcN maintains self-renewal through gluconeogenesis via OGT-dependent regulation of FoxO1 as well as Notch1 in mESCs.

**Key Words:** Stem Cell, Glucosamine, Gluconeogenesis, Self-Renewal, FoxO1, Notch1

## MP-32

### Palmitoylation of TRPML3 Regulates its Surface Expression and Membrane Trafficking in Autophagy

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TRPML3 is a $Ca^{2+}$ permeable cation channel and plays an important role in regulating endocytosis and autophagy. Although TRPML3 shows dynamic subcellular localization during endocytosis and autophagy, the underlying mechanism by which TRPML3 traffics between intracellular compartments is not known. Here we report that TRPML3 un-

dergoes palmitoylation at C-terminal cysteine residues (Cys549-551), and that this palmitoylation is required for membrane trafficking of TRPML3. Surface biotinylation assay revealed that palmitoylation regulates cell surface expression of TRPML3. However, organellar targeting and channel activity of TRPML3 appeared not to be affected by palmitoylation. Inhibition of palmitoylation altered TRPML3 function in endocytosis, leading to increased endocytosis. Palmitoylation had no effect on TRPML3 function in basal autophagy in that both WT and palmitoylation mutant of TRPML3 increase autophagy to a similar extent. Upon induction of autophagy by starvation or cell stressors, however, palmitoylation mutant of TRPML3 could not exacerbate autophagy, indicating that palmitoylation is essential for TRPML3 trafficking to autophagosomes. Importantly, inhibition of TRPML3 palmitoylation markedly reduced autophagic flux during induction of autophagy. Taken together, these findings suggest that palmitoylation is important in cell surface expression of TRPML3 and plays a significant role in TRPML3 functions in endocytosis and autophagy by regulating its trafficking between subcellular compartments.

**Key Words:** TRPML3, Palmitoylation, Membrane trafficking, Autophagy

## MP-33

### Autotaxin Plays an Important Role in hMSC Migration through LPA Receptor 1/3-dependent Adherent Junction Disruption and F-actin Reorganization via G$\alpha$i/G$\alpha$q-mediated $\beta$-Catenin and Rho GTPase Family Activation

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Autotaxin(ATX) is a key enzyme that regulates lysophosphatidic acid (LPA) levels in biological fluids, which exerts a wide range of cellular functions. However, the biological role of ATX in human mesenchymal stem cells (hMSCs) migration and signaling mechanisms remain to be fully elucidated. In the present study, we observed that ATX and LPA treatment increased hMSC migration in a dose-dependent manner. In addition, LPA receptor 1-5 were expressed in hMSC and LPA receptor 1-3 located in lipid raft. In experiments to investigate whether the ATX-induced migration is depended on LPA and its receptors, LPA receptor 1 and 3 siRNA transfection inhibited the ATX-induced cell migration, suggesting that the ATX-induced LPA production stimulates the hMSC migration through LPA receptor 1/3-dependent manner. Furthermore, LPA treatment decreased binding with LPA receptor 1-3 and G$\alpha$ subunits (i, q, or 12). LPA treatment increased the $Ca^{2+}$ influx and PKC phosphorylation, which were blocked by G$\alpha$i and G$\alpha$q siRNA transfection as well as PTX pretreatment, suggesting that the G$\alpha$i and G$\alpha$q have an important role in LPA-induced PKC activation. LPA increased the GSK3$\beta$ phosphorylation and $\beta$-catenin activation in a time-dependent manner. In addition, LPA induced translocation of $\beta$-catenin, snail, and slug from cytosol to nuclear, which was inhibited by PKC inhibitors (staurosporine and bisindolylmaleimide I). LPA stimulates the binding of $\beta$-catenin on E-box located in promoter of CHD1 gene (E-cadherin), but not snail and slug. In addition, ATX and LPA-induced increase in hMSC migration through down-regulation of E-cadherin expression was blocked by $\beta$-catenin specific siRNA transfection. LPA-induced PKC phosphorylation is also involved in Rho GTPase activation and Rac1, CDC42 siRNA transfection abolished LPA-induced F-actin reorganization. In conclusion, ATX stimulates the hMSCs migration through LPA receptor 1/3-dependent decrease of E-cadherin expression and increase of F-actin reorganization via PKC/GSK3B/$\beta$-catenin and PKC/Rho GTPase pathways.

**Key Words:** Autotaxin, Lysophosphatidic acid, Human umbilical cord blood-derived MSC, Migration

## MP-34

### Hydrogen Peroxide Induces Matrix Metalloproteinase-12 Expression to Promote Motility of Human Umbilical Cord Derived Mesenchymal Stem Cells

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Although many studies have examined the role of reactive oxygen species (ROS) in stem cell behavior, their effects on the reciprocal action of matrix metalloproteinase (MMP) regulation and motility in human umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) have not been characterized. Therefore, we examined the involvement of MMP and extracellular matrix (ECM) proteins in $H_2O_2$-induced human UCB-MSC motility and its related signaling pathways. $H_2O_2$ (1 $\mu$M) significantly increased motility after a 24 h incubation, which was inhibited by antioxidant. In experiments to examine related signaling pathways, $H_2O_2$ increased protein kinase C (PKC), extracellular signal-regulated kinases (ERK), and p38 mitogen activated protein kinase (MAPK) phosphorylation, which were attenuated by antioxidant and PKC inhibitors. In addition, $H_2O_2$ increased NF-$\kappa$B, GSK-3$\beta$ phosphorylation, and nuclear localization of $\beta$-catenin. Next, we analyzed 16 MMP genes in human UCB-MSCs but only found eight (MMP-1, -2, -11, -12, -14, -16, -17, and -19 mRNA). Among them, $H_2O_2$ increased MMP-12 and MMP-16 mRNA expression levels. However, $H_2O_2$ only increased MMP-12 protein expression level, but did not change MMP-16 protein expression. Additionally, $H_2O_2$ induced collagen 5 (COL-5) and fibronectin (FN) degradation in medium, but did not affect COL-5 or FN protein expression in cell lysates; these effects were inhibited by the MMP-12 inhibitor MMP408 and MMP-12 siRNA. Subsequently, $H_2O_2$-induced cell motility was inhibited by signal pathway-related siRNA and inhibitors but was not inhibited

by LiCl. These data demonstrate that $H_2O_2$ stimulated human UCB-MSC motility through degradation of COL-5 and FN and by NF-$\kappa$B and GSK-3$\beta$/$\beta$-catenin-dependent MMP-12 expression.

**Key Words:** Umbilical cord blood derived mesenchymal stem cells, Hydrogen peroxide, Matrix metalloproteinase, Extracellular matrix proteins, Motility

## MU-1

### Differences in Somatotype between the Ssireum Athletes and the Non-athletes

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This study was to show somatotype and physical characteristic differences between ssireum athletes and non-athletes. Differences between weight divisions were also examined. In this study, we first observed 32 elite and professional ssireum athletes and 15 non-athletes. The participants were measured with the modified somatotype method of Heath-Carter, resulting in three kinds of somatotype and a balanced type. The non-athletes consisted of two endomorphic, three mesomorphic, five ectomorphic, and five central types. The ssireum athletes consisted of thirty mesomorphs and two endomorphs. Subdividing the athletes' somatotypes resulted in twenty-three endomorphic mesomorphs, six mesomorph-endomorphs, two mesomorphic endomorphs, and one balanced mesomorph, respectively. Ssireum athletes had higher weights, body mass index, and endomorphic and mesomorphic component values than did the non-athletes. However, the ectomorphic component in the athletes was lower than in the non-athletes. Furthermore, a higher weight division was positively correlated with a higher body mass index and endomorphic and mesomorphic components, but negatively correlated with the ectomorphic component. Our study provides in part physical characteristics of ssireum athletes to establish a reference for sports rehabilitation.
**Key Words:** Somatotype, Ssireum athletes, Sports rehabilitation

## MU-2

### Role of TRPC3 in Endothelium-Dependent Relaxation

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Canonical transient receptor potential (TRPC) channels are $Ca^{2+}$- permeable nonselective cation channels that regulate ion homeostasis and intracellular $Ca^{2+}$ signaling in numerous cell types. Important physiological functions such as vasoregulation and neuronal growth have been assigned to this class of ion channels. We investigated the role of TRPC3 channel in the modulation of vascular contractility. Myogenic tones, contractile responses, changes in $[Ca^{2+}]_i$, releases of nitric oxide (NO), and nitrite/nitrate levels were measured. Transmural pressure-induced myogenic contraction was not different between wild type (WT) and TRPC3 knock-out (KO) mice. Phenylephrine-induced contraction was slightly weaker in TRPC3 KO than WT mice in the 40 mmHg. Acetylcholine-induced vasorelaxation and changes in $[Ca^{2+}]_i$ was inhibited in TRPC3 KO mice. Pre-treatment with the selective TRPC3 inhibitor Pyr3 significantly decreased ACh-induced vasorelaxation of WT mice. In endothelial cell experiments, ACh-induced increase in $[Ca^{2+}]$ was attenuated in TRPC3 inhibitor, Pyr3,-treated cells. ACh-induced NO release was smaller in arteries from TRPC3 KO than those in WT mouse. Also treatment with acetylcholine increased nitrite/nitrate levels in WT mouse, but not TRPC3 KO mouse. These results suggest that TRPC3 contributes to endothelial NO-mediated vasorelaxation in mouse mesenteric arteries. (This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NO. NRF-2011-0029459).)
**Key Words:** Transient receptor potential channel (TRPC), TRPC3 KO mouse, Vascular contractility, Nitric oxide, Vasorelaxation

## MU-3

### Effect of Skin Regeneration and Composition of the Essential Oil from Artemisia Montana Pampan

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Artemisia Montana Pampan, a herbaceous perennial, belongs to the compositae family and consists of various composition such as essential oil, pyrethrin, choline, adenine, inulin, and carotene. Artemisia Montana pampan is an oriental medicine that has been extensively used for deworming, digestion, treatment of chronic gastroenterocolotis and antipyretic. However, the biological activity of essential oil from Artemisia Montana Pampan on skin has not been investigated. In the present study, we tested the effect of the essential oil from Artemisia Montana Pampan on cellular events (migration and proliferation) relative to skin regeneration using normal human keratinocytes (HaCaT) and human dermal fibroblast (CCD-986sk). The essential oil was extracted from Artemisia Montana Pampan by steam distillation and its components were analyzed using gas chromatography-mass spectrometry. Total 49 components were identified from the essential oil. The essential oil did not show cytotoxic effects on HaCaT and CCD-986sk at the concentration of 0.0001 to 1 $\mu$g/ml, respectively. In proliferation assay, the essential oil increased the proliferation in HaCat in dose dependent manner, which reached a maximum at a concentration of 0.1 $\mu$g/ml (153.1±2.7% of control), but did not affect that in CCD-986sk. In migration assay, the essential oil induced

migration in CCD986 at the concentration of 1 μg/ml (150.2±1.7% of control), but not in HaCaT. Moreover, the essential oil dose-dependently induced the phosphorylations of Akt and ERK 1/2 in HaCat. Finally, in ELISA assay, the essential oil induced synthesis of type Ⅳ collagen in HaCaT, but not in CCD-986sk. On the other hand, the essential oil did not affect synthesis of type Ⅰ collagen in both HaCaT and CCD-986sk. These results indicate that essential oil from Artemisia Montana Pampan by steam distillation induces the proliferation and synthesis of type Ⅳ collagen in human skin keratinocytes and migration in dermal fibroblasts and may affect the events of skin regeneration and wound healing in human skin. Therefore, this study may provide new information for development of a new compound exerting bioactivity in skin.

**Key Words:** Artemisia Montana Pampan, Essential oil, Keratinocyte, Fibroblast, Type IV collagen

## MU-4

### Methanol Extract of Poncirus Fructus Modulates Pacemaker Activity in Interstitial Cells of Cajal from the Murine Small Intestine

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**Ethnopharmacological relevance:** Poncirus fructus (PF) has been widely used as a traditional medicine in Eastern Asia, especially to ameliorate the symptoms of gastrointestinal (GI) disorders related to abnormal GI motility. Aim of the study: Poncirus fructus (PF), also known as Poncirus trifoliata (L.) Raf. (Rutaceae), is widely used as a traditional medicine in Eastern Asia mainly to ameliorate the symptoms of gastrointestinal (GI) disorders related to abnormal GI motility. In a previous study, a methanol extract of PF was found to have particularly potent gastroprokinetic effects. Interstitial cells of Cajal (ICCs) are pacemaker cells in the gastrointestinal tract, but the action mechanisms of PF extract in mouse small intestinal ICCs have not been investigated. Therefore, in the present study, we investigated the effects of a methanol extract of PF (MPF) in mouse small intestinal ICCs. In addition, we sought to identify the receptors involved. **Materials and Methods:** Enzymatic digestions were used to dissociate ICCs from small intestines. The whole-cell patch-clamp configuration was used to record potentials (current clamp) from cultured ICCs. In addition, we analyzed intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_i$). **Results:** MPF decreased the amplitudes of pacemaker potentials in ICCs, and depolarized resting membrane potentials in a concentration dependent manner. Y25130 (a 5-HT3 receptor antagonist) and RS39604 (a 5-HT4 receptor antagonist) blocked MPF-induced membrane depolarizations, whereas SB269970 (a 5-HT7 receptor antagonist) did not. Pretreatment with $Na^+$ or $Ca^{2+}$-free solution or thapsigargin (a $Ca^{2+}$-ATPase inhibitor in endoplasmic reticulum) abolished the generation of pacemaker potentials and suppressed MPF-induced activity. $[Ca^{2+}]_i$ analysis showed that MPF increased $[Ca^{2+}]_i$. Furthermore, treatments with PD 98059, SB203580, or JNK II inhibitor blocked MPF-induced membrane depolarizations in ICCs. **Conclusion:** These results suggest that MPF modulates pacemaker potentials through 5-HT3 and 5-HT4 receptor-mediated pathways via external $Na^+$ and $Ca^{2+}$ influx, and via $Ca^{2+}$ release from internal stores in a mitogen-activated protein kinase dependent manner. The study shows MPF is a good candidate for the development of a gastroprokinetic agent. In view of the effects of MPF on ICCs, further research is required, particularly to identify the active compound(s) involved and to determine their action mechanisms.

**Key Words:** Poncirus fructus, Poncirus trifoliata (L.) Raf., Interstitial Cells of Cajal, 5-HT, Gastrointestinal Motility

## MU-5(PO-1)

### Stromal Interaction Molecule 1 (STIM1) Regulates Sarcoplasmic/Endoplasmic Reticulum Calcium-ATPase 1a (SERCA1a) in Skeletal Muscle

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Stromal interaction molecule 1 (STIM1) mediates $Ca^{2+}$ movements from the extracellular space to the cytosol through a store-operated $Ca^{2+}$ entry (SOCE) mechanism in various cells including skeletal muscle cells. In the present study, to reveal the unidentified functional role of the STIM1 C-terminus from 449 to 671 amino acids in skeletal muscle, binding assays and quadrupole time-of-flight mass spectrometry were used to identify proteins binding in this region along with proteins that mediate skeletal muscle contraction and relaxation. STIM1 binds to sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase 1a (SERCA1a) via this region (called STIM1-SBR). The binding was confirmed in endogenous full-length STIM1 in rabbit skeletal muscle and mouse primary skeletal myotubes via co-immunoprecipitation assay and immunocytochemistry. STIM1-knockdown in mouse primary skeletal myotubes decreased $Ca^{2+}$-uptake from the cytosol to the sarcoplasmic reticulum (SR) through SERCA1a only at micromolar cytosolic $Ca^{2+}$ concentrations, suggesting that STIM1 could be required for the full activity of SERCA1a possibly during the relaxation of skeletal muscle. Various $Ca^{2+}$ imaging experiments using myotubes expressing STIM1-SBR suggest that STIM1 is involved in intracellular $Ca^{2+}$ distributions between the SR and the cytosol via regulating SERCA1a ac-

tivity without affecting SOCE. Therefore, in skeletal muscle, STIM1 could play an important role in regulating $Ca^{2+}$ movements between the SR and the cytosol.
**Key Words:** Stromal interaction molecule 1 (STIM1), Sarcoplasmic/endoplasmic reticulum $Ca^{2+}$-ATPase 1a (SERCA1a), Skeletal excitation-contraction coupling (skeletal ECC), Dihydropyridine receptor (DHPR), store-operated $Ca^{2+}$ entry (SOCE)

## MU-6

### Mechanism of the 5-HT Mediated Vasoconstriction in Rat Mesenteric Artery: Receptor-Specific Roles of Caveolae, Src Tyrosine Kinase, and $K_v$ Channels

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**Background:** Recent studies suggest differential roles of caveolae, cSrc tyrosine kinase, and voltage-gated $K^+$ channel (Kv) among G-protein-coupled receptors including 5-$HT_{2A}$R and $\alpha$-adrenoceptor. The purpose of this study was examined the receptor specific role of 5-HT and $\alpha$-adrenoceptor in rat mesenteric artery smooth muscle cells. **Methods and aim:** Isometric arterial tension measurement, nystatin-perforated patch-clamp technique, electron microscopy, and Western blotting analysis were used for examining roles of caveolae, Src tyrosine kinase, PKC, and $K_v$ in the 5-$HT_{2A}$R and $\alpha$-adrenoceptor-mediated vasoconstriction in rat mesenteric artery. **Results:** Pretreatment of cSrc tyrosine kinase inhibitor $PP_2$ almost completely prevented the 5-$HT_{2A}$R-mediated $K_v$ inhibition and vasoconstriction. On the contrary, vasoconstriction by $\alpha_1$- adrenoceptor was relatively less inhibited by $PP_2$, but was markedly inhibited by PKC inhibitior chelerythrine. Inhibition of PKC did not affect the 5-$HT_{2A}$R-mediated responses. The $K_v$ inhibitor 4-aminopyridine failed to evoke additional vasoconstriction after 5-HT-induced vasoconstriction, verifying that $K_v$ inhibition mediates the 5-HT effect. Disruption of caveolae by methyl-$\beta$-cyclodextrin not only inhibited 5-HT-induced vasoconstriction but also rescued 4-aminopyridyne-induced vasoconstriction after vasoconstriction by 5-HT, indicating that integrity of caveolae is required for the 5-HT-induced $K_v$ inhibition. Accordingly, methyl-$\beta$-cyclodextrin treatment prevented the 5-$HT_{2A}$R-mediated $K_v$ inhibition. However, vasoconstriction by $\alpha_1$-adrenoceptor was not inhibited by methyl-$\beta$-cyclodextrin treatment. Western blot analysis revealed that cSrc tyrosine kinase is phosphorylated by 5-HT, but not by norepinephrine. The 5-HT-induced cSrc tyrosine kinase phosphorylation was also inhibited by methyl-$\beta$-cyclodextrin treatment. **Conclusion:** From these results, we conclude that caveolae-dependent cSrc tyrosine kinase activation and the subsequent $K_v$ inhibition is the main signaling of the 5-$HT_{2A}$R-mediated vasoconstrciton, whereas caveolae-independent PKC activation largely contributes to the $\alpha$-adrenoceptor-mediated vasoconstriction in rat mesenteric artery.
**Key Words:** Serotonin, $\alpha$-adrenoceptor, Src tyrosine kinase, Caveolae, Voltage-gated $K^+$ channels

## MU-7

### Angiopoietin 1 Enhances the Proliferation and Differentiation of Skeletal Myoblasts

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Angiopoietin 1 (Ang1) plays an important role in various endothelial functions, such as vascular integrity and angiogenesis; however, less is known about its function outside of the endothelium. In this study, we examined whether Ang1 has direct effects on skeletal muscle cells. We found that Ang1 exhibited myogenic potential, as it promoted the proliferation, migration, and differentiation of mouse primary skeletal myoblasts. The positive effect of Ang1 on myoblast proliferation could have been mediated by the a7 and b1 integrins. We also found that Ang1 potentiated cellular $Ca^{2+}$ movements in differentiated myotubes in response to stimuli, possibly through the increased expression of two $Ca^{2+}$-related proteins, namely, Orai1 and calmodulin. Ang1 also increased Orai1 and calmodulin expression in mouse hearts in vivo. These results provide an insight into the molecular mechanisms by which Ang1 directly affects the myogenesis of striated muscle.
**Key Words:** Ang1, Skeletal muscle, Calcium, Proliferation, Differentiation

## MU-8

### Role of Myogenic eNOS in the Thromboxane A2-Pretreated Pulmonary Arteries Combined with Increased Wall Tension

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Nitric oxide (NO) is a potent vasodilator, which is conventionally thought to be released from endothelial cells via eNOS activation. We recently suggested that phenylephrine-pretreated skeletal arteries show robust hypoxic

contraction (HVC), which is mediated by a hypoxic inhibition of eNOS expressed in smooth muscle layer (Han et al., 2012). Such role of myogenic eNOS might be also critical in pulmonary artery (PA) where hypoxic pulmonary vasoconstriction (HPV) is physiological important. Isometric contraction of rat PA was measured using Myograph, and the muscular expression of eNOS was confirmed by using RT-PCR and immunohistochemistry. Unlike DFA, with partial contraction by applying 3 nM U46619 (TXA2 agonist) in endothelium denuded PAs, additional contraction by non-specific NOS inhibitor [nitro-L-arginine methyl ester (L-NAME)] was feeble. However, with combined increase in wall tension equivalent to a luminal pressure elevation from 10 to 30 mmHg, L-NAME induced robust contraction of PAs (more than 100% of 80 mM KCl contraction). A partial depolarizing condition (20 mM KCl) combined with 3 nM U46619 induced similar sensitivity to L-NAME. Interestingly, PGF2a (1 $\mu$M) pretreatment alone could induce the robust contractile response to L-NAME whereas angiotensin II or 20 mM KCl alone did not. Neither neuronal NOS (nNOS) inhibitor [S-methyl-L-thiocitrulline (SMTC)] nor the inducible NOS (iNOS) inhibitor (1400W) induced the additional contraction in the PAs with the pre-stretch combined with U46619. Taken together, the above findings suggest that PA smooth muscle cells express eNOS that is activated a specific class of agonists and combined conditions such as mechanical stretch. The supposed activation of eNOS by TXA2 and increased wall tension might contribute to the relatively low peripheral resistance of pulmonary circulation and pulmonary arterial pressure.

**Key Words:** eNOS, Pulmonary artery smooth muscle

## MU-9

### Substrate Dependent Regulation Mitochondrial NADH and Membrane Potential in Permeabilized Single Ventricular Myocytes of Rat

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The mitochondrial respiration generates the proton motive force (PMF) for the production of ATP. For studying mitochondrial functional study, we need to know the consequences of the experimental conditions affecting mitochondrial status. In previous reported studies, they used variety combinations of mitochondrial substrates to energize mitochondria. However, it was not clear what the consequences in mitochondria with those substrates were. In this report, we tested the change of NADH and mitochondrial membrane potential with the combinations of the typical mitochondrial substrates such as malate, pyruvate, or glutamate. We used the isolated permeabilized cardiac myocytes of rat. And also, we measured the oxygen consumption rate by those substrates. Pyruvate is the final product from the glycolysis as a mitochondrial substrate, however, surprisingly, pyruvate alone could not maintain mitochondrial NADH or membrane potential. Each substrate alone could not maintain NADH level. Only malate and pyruvate combination could maintain NAHD level and mitochondrial membrane potential. Interestingly, glutamate could maintain the mitochondrial membrane potential, even though its mechanism is not clear. We think it may be related to complex II oxidation. FCCP was used to invoke oxygen consumption. With pyruvate or malate alone, FCCP could not induce oxygen consumption, however, it could induce the oxygen consumption in the conditions of both substrates. From the above results, we suggest when the mitochondria related experiments were done, it is necessary to select the mitochondrial substrates carefully and to check the the mitochondrial status by those conditions. We conclude that both pyruvate and malate, at least, were necessary to energize mitochondria. We think both citric acid cycle and malate/aspartate shuttle system were required to optimally energize the mitochondria (This work was supported by the grant from NRF (No. 2013023166).

**Key Words:** Mitochondria, Pyruvate, Malate, Glutamate, FCCP

## MU-10

### Protective Effect of Essential Fatty Acids against Palmitic Acid-Induced Impairment of Glucose Uptake Involves both AKT and AMPK in C2C12 Myotubes

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The most common saturated fatty acid, palmitic acid (PA) has been shown to induce insulin resistance and impaired glucose uptake in skeletal muscle. This study was to examine the protective effect of essential fatty acids (EFAs), linoleic acid (LA) and $\alpha$-linolenic acid (ALA) against PA-induced impairment of glucose uptake using C2C12 myotubes. PA significantly decreased glucose uptake of myotubes in the absence and presence of insulin. However, co-supplementation of different unsaturated fatty acids prevented PA-induced decrease in glucose uptake. Among them, polyunsaturated LA (C18:2) and ALA (C18:3) showed stronger effect than that of corresponding monounsaturated fatty acid, oleic acid (OA, C18:1) in terms of enhancing glucose uptake activity. To determine molecular mechanisms underlying the action of EFA, we have investigated the effects of fatty acid on the activation of protein kinase B (Akt) and 5'-adenosine monophosphate-activated protein kinase (AMPK) signaling pathways which are important mediators in regulation of cellular glucose uptake. The results showed that EFAs restored suppressed insulin signaling in PA-treated myotubes, which is characterized by the increased phosphorylation of Akt and dephosphorylation of serine kinases (PKC$\theta$ and JNK). Furthermore, EFAs activated AMPK and acetyl-CoA carboxylase (ACC), which can be prevented using an AMPK inhibitor, adenine 9-$\beta$-D-arabinofuranoside (araA). Preincubation of araA significantly decreased EFA-stimulated glucose uptake, indicating that AMPK pathway is also involved in action of

EFA. Thus, we concluded that protective effect of EFAs against PA-induced impairment of glucose uptake associated with its ability to enhance glucose uptake via Akt and AMPK activation in C2C12 myotubes.

**Key Words:** Essential fatty acid, Glucose uptake, Akt, AMPK, C2C12 myotubes

## NC-1

### The Effect of Functional Electrical Stimulation of Stroke Patients on Balance and Activities of Daily Living

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The present study investigated the effect of functional electrical stimulation of stroke patients in a sitting position on balance and activities of daily living. Functional electrical stimulation was applied to stroke patients (6 male, 3 female) while in a sitting and supine position. Functional electrical stimulation was applied six times for 30 minutes each for a total of six weeks. The timed up and go values at weeks 2, 4, and 6 after functional electrical stimulation in a sitting position were noticeably decreased in a time-dependent manner, compared with controls. In the sitting, the functional reach test values were significantly increased in a time-dependent manner. The same values in the supine position weakly showed a similar pattern to those in the sitting position. Furthermore, the functional independent measurement values in the sitting position were markedly increased in a time-dependent manner. In the sitting position, the intensity of functional electrical stimulation was markedly decreased in a time-dependent manner. The same values in the supine position weakly showed a similar pattern to those in the sitting position. These results suggest that the conditions of stroke patients in both the sitting and supine positions after electrotherapy were improved and that functional electrical stimulation had a greater effect in the sitting position.
**Key Words:** Stroke patients, Functional electrical stimulation, Balance

## NC-2

### Respiratory Analysis of the Children after Cerebral Palsy

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Respiratory dysfunction generally considered the main cause of death in cerebral palsy. However, the change of respiratory function after cerebral palsy with or without ambulatory has not been fully understood. Therefore, the present study is designed to evaluate divided rest breathing and forced breathing in school-aged children with cerebral palsy compared with those normal children. We studied fourteen children with cerebral palsy aged six to ten years and fourteen children with normal development aged six to eleven years. The ratio of the transverse diameters of upper chest and lower chest measured on a radiograph. Spirometry was performed using a spirometry. Chest ratio of cerebral palsy was smaller than normal groups in upper rib cage. This tendency was greater in non-ambulatory cerebral palsy than ambulatory cerebral palsy. In Circumference analysis, all measurement was statistically no significant results. In EVC during rest breathing, significant differences between non-ambulatory cerebral palsy and normal subjects. MV in rest breathing was significant differences between non-ambulatory cerebral palsy and normal groups. Tv in rest breathing was significant differences between ambulatory cerebral palsy, non-ambulatory cerebral palsy, and normal subjects. However, in EVC of forced breathing, no significant differences between ambulatory, ambulatory cerebral palsy, and normal groups. In IVC of forced breathing, significant differences between ambulatory cerebral palsy and normal subjects. These results suggest that respiratory dysfunction in cerebral palsy may be critical problems in area of rehabilitation.
**Key Words:** Respiratory dysfunction, Cerebral palsy, Rehabilitation

## NC-3

### Zinc Chelation Reduces Traumatic Brain Injury-Induced Neurogenesis in the Subgranular Zone of the Hippocampal Dentate Gyrus

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Several studies have shown that traumatic brain injury (TBI) increase hippocampal neurogenesis in the rodent brain. However, the mechanism underlying increased neurogenesis after TBI remains unknown. Continuous neurogenesis occurs in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) in the adult brain. The mechanism why neurogenesis actively occurs in this hippocampal area is not clear. A high level of vesicular zinc is localized in the presynaptic terminals of the SGZ (mossy fiber). The mossy fiber of dentate granule cells contains high levels of chelatable zinc in their terminal vesicles, which can be released into the extracellular space during neuronal activity. Previously, our lab presented that a possible correlation may exist between synaptic zinc localization and high rates of neurogenesis in this area after hypoglycemia or epilepsy. Using a weight drop animal model to mimic human traumatic brain injury, we tested our hypothesis that zinc plays a key role in modulating hippocampal neurogenesis after TBI. Thus, we injected a zinc

chelator, clioquinol (CQ, 30 mg/kg), into the intraperitoneal space to reduce brain zinc availability twice per day for 4 days. Neuronal death was evaluated with Fluoro Jade-B and NeuN staining to determine whether CQ has neuroprotective effects after TBI. The number of degenerating neurons (FJB (+)) and live neurons (NeuN (+)) was similar in vehicle and in CQ treated rats at 1 week after TBI. Neurogenesis was evaluated using BrdU, Ki67 and doublecortin (DCX) immunostaining 1 week after TBI. The number of BrdU, Ki67 and DCX positive cell was increased after TBI. However, the number of BrdU, Ki67 and DCX positive cells was significantly decreased by CQ treatment. The present study shows that zinc chelation did not prevent neurodegeneration but did reduce TBI-induced progenitor cell proliferation and neurogenesis. Therefore, this study suggests that zinc has an essential role for modulating hippocampal neurogenesis after TBI.

**Key Words:** Traumatic brain injury, Zinc, Hippocampus, Neurogenesis, Clioquinol, Subgranular zone

## NC-4

### Ghrelin Attenuate GABAergic Inhibitory Synaptic Transmission on Dorsal Raphe Neurons

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Ghrelin, an orexigenic peptide, is released from upper intestine and provoke hunger. Ghrelin has multiple biophysiological activities and modulate metabolism, acting like growth-hormone, and increase awaking level. In recent experiments, it is known that some of the appetite regulating hormones also acting as sleep regulator by modulating neuronal activity in arousal system. Although generally known by experience that hunger make difficult to sleep but whether ghrelin's involve in awakening is not known. Axons of the ventrolateral preoptic nucleus neuron project multiple arousal area including DR and inhibit neuronal activity by releasing GABA. The activity of the dorsal raphe nucleus increase in the proportion of the level awaking. The hypotonic benzodiazepine agonists suppress neuronal activity of the dorsal raphe by potentiating $GABA_A$ receptor responses. Therefore, we test ghrelin effects on GABAergic inhibitory postsynaptic currents (IPSCs) of dorsal raphe using patch clamp method. Throughout the experiments, the spontaneous IPSCs was isolated in a continuous presence of the NBQX (20 $\mu$M). Ghrelin (100 nM) significantly decreased frequency of spontaneous IPSCs without change amplitude and decay time. This result suggests that ghrelin reduce GABA release on GABAergic presynaptic terminal without change property of postsynaptic $GABA_A$ receptors. Thus, our result implies that ghrelin may exert sleep-wake action by attenuating spontaneous IPSCs by presynaptic mechanism.

**Key Words:** Ghrelin, Dorsal raphe, IPSCs

## NC-5

### Activated Microglia Contribute to the Generation of Spasticity after Spinal Cord Injury

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Spasticity is a common syndrome and a major cause of disability in patients with spinal cord injury (SCI). Spasticity is usually defined as a velocity-dependent increase in the tonic stretch reflex, resulting from the increased excitability of spinal neurons. But, the pathogenesis of spasticity following SCI in patients remains uncertain. Several experimental evidences have been shown that activated microglia involve in hyper-excitability of spinal neurons by evoking inflammatory process. Enhanced excitability of spinal neurons by activated microglia may play a role in generating spasticity. The purpose of this study was to test the role of activated microglia in the generation of spasticity in SCI animal model. SCI was made at T12 in adult male Sprague Dawley rats by NYU impactor. To assess motor function, BBB (Basso, Beattie, and Bresnahan) locomotor rating scale and Combined Behavioral Score (CBS) was measured before and after SCI. To assess the degree of spasticity, modified Ashworth scale (MAS) was measured before and after SCI, or minocycline administration, respectively. Immnohistochemistry was performed to assess the expression of microglia at lumbar spinal segments (L5-6) in normal, SCI, saline and minocycline groups. Spasticity was maintained above 3 grades of MAS from 28 days after SCI. Locomotor function was not recovered from 35 days after injury and combined motor function was no longer improved from 28 days after injury. After SCI, expression of activated microglia increased. Proportion of activated microglia and spasticity was decreased after minocycline administration. The study showed that rats with 50 mm contusive SCI reproduce sustained spasticity and expression of activated microglia in the spinal cord increase after SCI. Minocycline decreased degree of behavioral sign of spasticity with inhibition of activated microglia in SCI rats. Therefore, these results suggest that activated microglia can involve in spasticity of rats with contusive SCI.

**Key Words:** Microglia, Spasticity, Spinal cord injury

## NC-6

### The Mechanical and Chemical Stimulation into the Intervertebral Discs (IVDs) of Rats Can Evoke the Neuronal Excitation in Afferent Fibers Innervating IVDs

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Low back pain (LBP) can be originated from the excessive mechanical stress or inflammation with facet joints, intervertebral discs (IVDs), nerve roots, ligaments and muscles which constitute the low back structure. To understand the underlying mechanism of discogenic LBP, it should be considered whether/how mechanical or chemical stimulus can affect mechanoreceptor and/or chemoreceptor in peripheral nerve terminal innervating IVDs. According to some results from human and animal studies, it has been known that nerve fibers reach the IVDs through the sinuvertebral nerves or from branches of the paravertebral sympathetic trunks, however, to date there has been no study to explore the properties of afferent fibers in IVDs by using in vivo electrophysiology. Male SD rats (300-350 g; Orient Bio, Korea) were used for in vivo single nerve recording. Neuronal activities of paravertebral sympathetic trucks in normal rats were measured and analyzed by counting spikes per second while mechanical (vonfrey filament, 1-2-4-6-10 g) and chemical stimulation (KCl 0.2 M) were being applied to IVDs. Neuronal excitation was displayed in slowly or rapidly adaptive pattern. The intra-discal injection of 0.2 M KCl into IVDs evoked the neuronal excitation. The present study implicate that the sensory information from IVDs can be transmitted to spinal cord and furthermore excessive mechanical or chemical stimulation produce discogenic low back pain.
**Key Words:** Intervertebral discs (IVDs), Paravertebral sympathetic trucks, in vivo single nerve recording

## NC-7

### Pulse Shape Effect on Evoking Retinal Ganglion Cell (RGC) Responses in rd1 Retina

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Extracting optimal electrical stimulation parameters is one of the most important elements for the development of retinal prosthesis. Previously, we compared polarity effect of pulse on evoking RGC responses in retinal degenerated (*rd1*) mice. Not many studies have been performed with different pulse shapes. Therefore, here, we used 3 different pulse shapes and investigated pulse shape effect on evoking RGC responses. The well-known animal model for RP, rd1 ($Pde6b^{rd1}$) mice at postnatal 8~9 weeks were used. From the *ex-vivo* retinal preparation (n=19), retinal patches were placed ganglion cell layer down onto 8×8 MEA and RGC responses were recorded while applying electrical stimuli. All pulses were charge balanced, biphasic, cathodic phase-1st current pulses with same charge of rectangle pulse; 1) biphasic rectangle pulse (I: intensity, D: duration), 2) biphasic triangle pulse with double intensity (2xI, D) or double duration (I, 2xD) 3) ramp pulse (2xI). For intensity (or duration) modulation, duration (or amplitude) of the pulse was fixed to 500 $\mu$s (30 $\mu$A), changing the intensities (or duration) from 2 to 60 $\mu$A, 60 to 1000 $\mu$s. With applying 50 pulses with 1 Hz frequency, averaged response of RGC spikes was defined as positive response when the number of RGC spikes for 400 ms after stimulus was 30 % increase than spontaneous RGC spikes. RGC responses were well modulated with rectangle, triangle, and ramp pulse regardless of amplitude or duration modulation. In amplitude modulation, triangle pulse with double duration (I, 2xD) is more efficient than rectangle and triangle pulse with double intensity at I=5 $\mu$A, 10 $\mu$A. In duration modulation, triangle pulse with double duration (I, 2xD) is the most efficient pulse shape at D=60 $\mu$s, 100 $\mu$s. In comparison with rectangle pulse and ramp pulse, linear increase ramp pulse (2xI) is the most efficient pulse shape at I=5 $\mu$A, 10 $\mu$A while at 40 $\mu$A and 50 $\mu$A, rectangle pulse is the most efficient.
**Key Words:** Retinal ganglion cell, rd1 mice, Biphasic current pulse, Amplitude modulation, Duration modulation

## NC-8

### Two Compartment Model of Spontaneous Firing in Midbrain Dopamine Neurons: Proximal Dendrite as an Initiator and Soma as a Counteract Balancer

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In spontaneously firing midbrain dopamine neurons, action potential generated at the proximal dendrite from which axon develops, propagates to the soma. Therefore, the rate of spontaneous firing is believed to be determined by electrical coupling between the soma and dendrites. However, this model, based on homogeneous electrical properties of all compartments, does not explain various dynamic aspects of glutamate-evoked firing diversity. Here we propose the initiator-conteract blancer model in which proximal dendrites act as an action potential initiator and the soma plays as a counteract balancer. Glutamate can generate high frequency firing and pause in vivo and in vitro. When we stimulated series of small areas along a dendrite by caged-glutamate photolysis, glutamate excites dopamine neurons in which high frequency firing was generated similarly within proximal dendritic region, but post-firing pause was rapidly decayed with distance from the soma. Local dendritic $Ca^{2+}$-uncaging experiments reveal that $Ca^{2+}$-induced suppression of spontaneous firing purely depended on the amplitude of the $Ca^{2+}$ spikes or closeness to the soma. All these data suggest that high frequency firing generated at proximal dendrites propagates to the soma in that $[Ca^{2+}]_c$ accumulates tonically according to the firing frequency, thereby leading to the frequency-dependent suppression of spontaneous firing. In contrast to the firing generation, the tonic rises in $[Ca^{2+}]_c$ in the slow

and large soma compartment leads to post-firing pause, thereby acting as a controller to the fast-responding proximal dendrites.
**Key Words:** Dopamine neuron, Midbrain, Spontaneous firing

## NC-9

### Changes in Oxidative Stress and Neuronal Activity of Ventrolateral Preoptic Nucleus during Sleep-Wake Cycle

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Sleep is as an important mechanism that recovers the cell function to the normal state by decreasing reactive oxygen species (ROS) and oxidative stress developed during wakefulness. Sleep-wake cycle is regulated by a balance between sleep-inducing neurons and wake-inducing neurons. The ventrolateral preoptic nucleus (VLPO) of the hypothalamus is known as a sleep-inducing area in sleep-wake cycle. The VLPO is composed of GABAergic and galaninergic neurons which inhibit various wake-promoting regions of the brain. ROS are produced as a normal product of cellular metabolism and induce oxidative stress and endoplasmic reticulum (ER) stress. Reactive nitrogen species including nitric oxide (NO) is also produced by oxidative metabolism of the cells and NO is well known as a biological messenger. In this study, we examined the relation between changes in ROS and NO during sleep-wake cycle and the cellular activity of the sleep-inducing VLPO neurons. The results indicate that the sleep-inducing function of the VLPO may be regulated by ROS and NO during sleep- wake cycle.
**Key Words:** Sleep, Ventrolateral preoptic nucleus, GABA, Oxidative stress

## NC-10

### The Inhibitory Role of Kappa Opioid Receptors on Acute Knee Joint Pain in Rats

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The opioidreceptors (ORs)in both central and peripheral nervous systemare well known to play an important role in alleviating the pain. Ors which are divided into three subtypes ( $\mu$OR, $\delta$OR and $\kappa$OR) decrease neuronal excitability by coupling to G$\alpha$i/o protein, inhibiting adenylatecyclase and attenuating the release of excitatory neurotransmitter. However, it is less known whether $\kappa$ORin knee joint have inhibitory role on arthritic pain. To answer this question, we examined whether the activation of $\kappa$OR in knee joint can reverse the reduction in weight load and inhibit neuronal discharge in response to mechanical stimulation in carrageenan-induced arthritic pain . 1) Behavior test Carrageenan (1%, 50 $\mu$l/200 g) was injected into the knee joint space to induce arthritis in SD rats (male, 250~300 g). On 4hr after carrageenan injection, we administered U50488 (kappaopioid agonist; 0.1 nM, 10 nM, 1 mM/70 $\mu$l) or saline (70 $\mu$l/200 g) into knee joint cavityand measured consecutively the peak value of weight load in hindlimbs at pre, 4, 6, 8hr and 1 day after carrageenan injection. 2) In-vivo single nerve recording On 3hr after carrageenan injection, rat was deeply anaesthetized by 25% urethane. With end tidal $CO_2$ and body temperature maintained with physiological level, the saphenous nerve was transected, teased with sharpened watchmaker forceps and then placed over platinum recording electrode. Identifying nerve fibers innervating the knee joint, we recorded the neuronal activities evoked by mechanical stimulation. We administered U50488 (kappa opioid agonist; 10 nM, 1 mM /70 $\mu$l) or saline (70 $\mu$l / 200 g) to knee joint cavity through catheter. We measured conduction velocity and counted neuronal firing per second while Von-frey filaments (6 and 26 g) were applied to knee joint at base,10, 20, 30, 40 and 60 min We found that U50488 at dose of 0.1 nM, 1 $\mu$M reversed significantly the reduction of weight load on 6 and 8 hr after carraageenan injection and decreased significantly neuronal excitation 10 min later after U50488 injection. In this study our results indicate that peripheral $\kappa$OR in knee joint have inhibitory role on carrageenan-induced arthritic pain.
**Key Words:** Peripheral kappa opioid, Knee joint, Pain, In-vivo recording

## NC-11

### Functional Neural Differentiation of Human Bone Marrow Mesenchymal Stem Cell Lines Transfected with Neurogenin-1

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Bone marrow contains two types of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). The studies using human MSCs have difficulty in obtaining sufficient number and homogenous population of human MSCs to investigate detailed cellular and molecular properties. To circumvent these problems, we have previously generated an immortal human MSCs cell line (BM3.B10) via retroviral vector encoding v-myc. B10 human MSCs carry normal human karyotype of 46,XX and express MSC specific markers and differentiate into adipocytes, osteocytes, chondrocytes or neurons when grown in differentiation media. In the present study, we generated an enriched population of neurons from MSCs via trasfection of B10 MSCs with Neurogenin-1(Ngn1) gene, a mem-

ber of proneural basic helix-loop-helix transcription factors, and investigated the functional neural differentiation by RT-PCR, Real-time PCR, western blot and. whole-cell mode patch clamp techniques.
**Key Words:** B10, Neurogenin-1, Mesenchymal stem cell

## NC-12

### Calbindin Dysfunction Underlies Hyperexcitability of Dentate Granule Cells in Alzheimer Model Mice

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Calbindin is implicated in the pathogenesis of Alzheimer's disease (AD); however, the underlying mechanisms are not well understood. Here, we discovered that the endogenous $Ca^{2+}$ buffer capacity in dentate granule cells (GCs) is reduced in 1-2 month-old AD model mice (Tg2576) to a level comparable to calbindin-$D_{28K}$ knock-out (CBKO) mice. The reduction of $Ca^{2+}$ buffering in Tg2576 GCs was mimicked by the exogenous application of amyloid $\beta$ protein (A$\beta$) and restored by the antioxidant Trolox, suggesting that calbindin dysfunction is caused by A$\beta$-induced oxidative changes. Functional and molecular analyses of Tg2576 and CBKO GCs revealed that hyperexcitability, reduced A-type $K^+$ currents, and reduced Kv4.1 mRNA expression were commonly observed in both mice. Furthermore, spontaneous seizure activity was observed in CBKO mice. Our data provide the first evidence for a causal link between A$\beta$-induced oxidative changes, calbindin dysfunction, and the hyperexcitability of dentate GCs, which may underlie aberrant network activity in AD.
**Key Words:** Tg2576, Kv4.1, Antioxidant, Amyloid $\beta$, Seizure

## NC-13

### Neuroprotective Effects of Mesenchymal Stem Cells on Transient Global Cerebral Ischemia-Induced Neuron Death

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Global cerebral ischemia is the most important cause of poor prognosis after successful resuscitation from cardiac arrest. Therapeutic induction of hypothermia (maintenance of core body temperature between 32°C and 34°C for 12 to 24 hours, TH (Therapeutic hypothermia)) in reduction of the neurologic damage from global cerebral ischemia has been reported from various laboratory and clinical researches, and it is recommended as one of the standard post-resuscitation managements. However, alternatives or complements for TH are necessary due to its technical difficulty in induction of recommended temperature and keeping recommended rate of rewarming, which limits the application of TH. We aimed to show the effect of stem cell on the neurologic recovery after transient global cerebral ischemia including the comparison with that of current standard therapy, TH. Rats were subjected to 7 minutes of transient global cerebral ischemia and randomized into 4 intervention groups: placebo control, TH, human mesenchymal stem cell (MSC), and combined TH and MSC. Hippocampal neuronal death was evaluated at 7 days after ischemia by Fluoro Jade B. Analysis of variance (ANOVA) showed the differences between control and TH and between control and TH/MSC in CA1. Activated microglia and infiltrated macrophages were evaluated at 7 days after ischemia by immunostaining for CD11b. ANOVA showed the differences between sham groups and control, sham groups and TH, control and TH, control and MSC, TH and MSC, and control and TH/MSC. IgG immunostaining was performed to detect blood brain barrier (BBB) disruption, ANOVA showed the differences between sham groups and control, control and TH, and control and TH/MSC. Myeloperoxidase (MPO) immunostaining was done to detect neutrophil infiltration in the hippocampus. ANOVA failed to show a significant difference in MPO(+) cell count among 8 sham operation and ischemia groups. 4HNE immunostaining was performed to detect oxidative injury. ANOVA showed the differences between sham groups and control, sham groups and TH, control and TH, control and MSC, and TH and TH/MSC. Administration of MSC after transient global cerebral ischemia has a large protective effect on hippocampal neuron death comparing with current standard treatment option, TH. The present results suggest combined treatment of MSC and hypothermia warrants a potential therapeutic strategy for intervention of global cerebral ischemia after cardiac arrest.
**Key Words:** Ischemia, Stem Cell, Hippocampus, Neuron Death, Hypothermia

## NC-14

### Increased Inhibitory Influence on the Periaqueductal Gray Disrupts Endogenous Analgesic System during Chronic Neuropathic Pain State

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Neuropathic pain is a pathological pain caused by damage to the peripheral or central nervous system. Neuropathic pain patients are suffering from spontaneous continuing pain and allodynia that are often unbearable and intractable. Peripheral and central mechanisms of neuropathic pain include maladaptive change of the processes related to sensory transduction, nociceptive signal transmission and cortical pain perception. Above these, we speculated that dysfunction of the endogenous analgesic system might be occurred during neuropathic pain state concurrently. To observe the possible change of the endogenous analgesic function, brain glucose metabolisms of the neuropathic pain animals and control animals were measured with 18F-Fluorodeoxyglucose (FDG) - Positron Emission Tomography (PET) scan and compared. Analysis of brain images revealed that metabolic relationship between the periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) was altered in neuropathic pain animals, showing negative correlation between two regions. We could reverse this negative correlation into positive correlation by activating endogenous analgesic effect sufficient to cancel out the mechanical allodynia using chemical manipulation of the ventrolateral PAG (VL-PAG). Based on this observation, we hypothesized that the negative correlation appeared from neuropathic pain animals might be reflecting the increased inhibitory activity to the RVM-projecting VL-PAG neurons (PAG-RVM neuron). To investigate this possibility, we injected retrograde tracer into the RVM to identify PAG-RVM neurons and recorded the spontaneous inhibitory postsynaptic currents (sIPSCs) coming to the labeled PAG-RVM neurons. We could observe that the sIPSC inputs coming to the PAG-RVM neurons of the neuropathic pain animals were significantly increased compared to sIPSCs of the sham controls. This result shows that influences of the inhibitory neurons on PAG-RVM neurons are increased during chronic neuropathic pain state, which disrupts activation of the endogenous analgesic system.

**Key Words:** Neuropathic pain, Brain image, Periaqueductal gray, Neuron, Analgesia

## NC-15

### Exercise Training Normalizes Imbalanced Sympathetic Nerve Activity in Rats with Heart Failure

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Exercise training (ExT) has been known to be beneficial to patients with heart failure (HF). Previous studies reported that ExT decreases renal sympathetic nerve activity (RSNA) in anesthetized HF rats. However, underlying mechanisms are not well understood. Here, we examined the heart rate variability in rats in vivo and firing rate, EPSCs and IPSCs in the paraventricular neurons projecting to the rostral ventrolateral medulla (PVN-RVLM) in ExT or sedentary (Sed) rats. HF rats were induced by ligation of left descending coronary artery. 3-4 weeks after HF ligation surgery or sham surgery, the rats were exercised on a motor-driven treadmill for 4 week period. Electrocardiogram data were collected for 24 h at week 8 following the surgery using DSI Dataquest A.R.T.TM system and analyzed by Kubios HRV software. Electro-physiological parameters were recorded by slice patch clamp combined with the retrograde labeling technique. The results indicated that ExT (1) decreased the differences of heart rate between light and dark phase; (2) increased the value of LF/HF ratio at the dark phase, which represents sympathovagal balance; (3) decreased the firing rate, which is associated with an increase in IPSC frequency. In conclusion, the present study reveals that ExT normalizes the blunted diurnal variation of sympathovagal balance in HF rats in vivo and the enhanced neuronal excitability from presympathetic PVN-RVLM neurons in HF rats.

**Key Words:** Heart rate variability, LF/HF ratio, Slice patch clamp, Firing rate, IPSC

## NC-16

### Regulation of CpG Methylation of Neurogenin 2 Promoter Region during Neuronal Differentiation of Neural Precursor Cells

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Neural stem cells perform self-renewal and differentiate into the mouse committed progenitor cells. These common progenitors give rise to glial and neuronal precursors, which can terminally differentiate into neurons and glial cells. Proneural basic helix loop helix (bHLH) transcription factors, Neurogenin (Ngn) 1 and 2 are a critical transcription factor during neuronal differentiation. Glial differentiation of neural precursor cells (NPCs) is regulated by methylation pattern of GFAP promoter region. However, methylation pattern of Ngn gene promoter region in neuronal differentiation have never been elucidated. In this study, we examined whether regulation of methylation in Ngn2 promoter region influences Ngn2 expression and also whether demethylation promotes neuronal differentiaton through Ngn2 expression. First, we examined the expression of neuronal marker, MAP2 and marker of NPC, Nestin to show whether NPCs can differentiate into neuron. During neuronal differentiation, expression of MAP2 increased whereas expression of Nestin decreased. Next, we investigated methylation pattern of Ngn2 promoter region NPCs with methylation-specific PCR (MSP). Methylation of Ngn2 promoter region reduced and unmethylation of Ngn2 promoter region increased during neuronal differentiation, and also expression of Ngn2 increased. Treatment of demethylation agent, 5-Aza-2-deoxycytine into

NPCs decreased methylation of Ngn2 promoter and also increased unmethylation. These data demonstrated that regulation of methylation pattern in Ngn2 promoter region is closely correlated with neuronal differentiation of NPCs. This work was supported NRF funded by the MEST (2013-029298) and NRF through Chronic Inflammatory Disease Research Center (2012R1A5A2051428).
**Key Words:** Neural precursor cells, Neurogenin2, Neuronal differentiation, Promoter methylation

## NC-17

### Seizure Susceptibility in Immature Brain due to Lack of Cyclooxygenase-2-Induced Prostaglandin $F_{2\alpha}$

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The immature brain is prone to seizure; however, the mechanism underlying this vulnerability has not been clarified. Febrile seizure is common in young children, and the use of non-steroidal anti-inflammatory drugs for febrile seizure is not recommended. In previous studies, we established that prostaglandin (PG)$F_{2\alpha}$, a product of cyclooxygenase (COX), acts as an endogenous anticonvulsant in the adult mouse. Therefore, we assumed that COX-2 activity was involved with seizure susceptibility in early life. In the present study, immature mice (postnatal day 9) were far more prone to kainic acid (KA)-induced seizures than mature mice (after postnatal day 35). Seizure activity began later in immature mice, but was more severe and was unaffected by a potent COX inhibitor, indomethacin; in contrast, indomethacin aggravated seizure activity in mature mice. Immature mouse brains exhibited little basal COX-2 expression and little KA-induced COX-2 induction, while KA-induced COX-2 expression and $PGF_{2\alpha}$ release were prominent in mature brains. During brain development, COX expression was increased and glycosylated in an age-dependent manner, which are necessary for COX enzyme activity. Intracisternal $PGF_{2\alpha}$ administration also reduced KA-induced seizure activity and mortality. Taken together, low COX activity and the resulting deficiency of $PGF_{2\alpha}$ may be an essential cause of increased seizure susceptibility in immature. **Acknowledgement:** This study was supported by the National Research Foundation (NRF) grant funded by the Korea government (MEST; 2013-029298), the Korea Science and Engineering Foundation through the Chronic Inflammatory Disease Research Center (NRF-2012R1A5A2051428) and a grant of the Korean Health Technology.
**Key Words:** Cyclooxygenase, Febrile seizure, Kainic acid, Prstaglandin $F_{2\alpha}$

## NC-18

### Muscarinic Activation Induces Long-Term Synaptic Depression via Endocannabinoid Signaling in Layer 2/3 Pyramidal Neurons of Rat Visual Cortex

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Activation of cholinergic receptors controls basal synaptic transmission and long-term synaptic plasticity involved in learning, memory, and adaptive sensory processing. Endocannabinoid system is also known to be related with synaptic plasticity in both excitatory and inhibitory synapses in various brain areas. Endocannabinoid signaling is involved in cholinergic induction of long-term depression (LTD) in synaptic transmission in the hippocampus and the dorsal cochlear nucleus. Although, in the visual cortex, the two neuromodulatory systems are abundantly expressed, their mechanistic interaction underlying short- and long-term synaptic plasticity remains unknown. In this study, we investigated the interactions between the muscarinic and the endocannabinoid systems in the regulation of synaptic transmission in layer 2/3 pyramidal neurons of rat visual cortex using the whole-cell patch-clamp technique in slices. Synaptic responses were evoked by bipolar tungsten electrode located in underlying layer 4. Bath application of cholinergic agonists showed no effect on the amplitude of the postsynaptic current elicited by pressure-applied glutamate. However, the muscarinic cholinergic agonist muscarine (10 $\mu$M) decreased the basal amplitude of excitatory postsynaptic potentials (EPSPs) and induced LTD, while it increased the paired-pulse ratios of EPSPs, suggesting that its action was mediated by presynaptic mechanisms. In addition, the endocannabinoid receptor agonist WIN55212-2 (3 $\mu$M) decreased the basal amplitude of EPSPs and induced LTD. Muscarine-induced LTD was blocked by the endocannabinoid CB1 receptor antagonist AM251 (2 $\mu$M), while acute depression of basal synaptic transmission was not affected. In our previous study, muscarinic LTD was blocked by the intracellular application of calcium chelator BAPTA (10 mM). These results suggest that muscarinic presynaptic LTD might be mediated indirectly by the endocannabinoid signaling in layer 2/3 pyramidal neurons of the rat visual cortex. Supported by the Basic Science Research Program through the NRF (2013-056534).
**Key Words:** Muscarine, Long-Term depression (LTD), Endocannabinoid, Visual cortex

## NC-19(PO-13)

### Mechanisms of Electroacupuncture-Induced Analgesia on Neuropathic Pain in Animal Model

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**Background:** Neuropathic Pain (NP) “Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system” -NeuPSIG (Special Interest Group on Neuropathic Pain) Electro-acupuncture (EA) Acupuncture is reported to be efficacious in various fields of pain, such as, lower back pain, chronic knee pain and chronic headache. **Objective:** To guide future efforts in the advancement of neuropathic pain treatment and to expand on clarification of the effect of EA on neuropathic pain and quantify its mechanism. **Methods:** 1. Opioidergic recepetors Selective $\mu$ ($\beta$-FNA), $\delta$ (naltrindole), $\kappa$ (nor-BNI) antagonists were administered intrathecally with EA. The effects are blocked by $\mu$ and $\delta$ selective opioid antagonists but not by $\kappa$ selective opioid antagonists 2. Adrenoreceptors $\alpha$1-and $\alpha$2-adrenoreceptors antagonists ( prazosin or yohimbine) were administered intrathecally with EA. The relieving effects were blocked by the $\alpha$2-adrenoreceptors antagonist yohimbine but not by the $\alpha$1-adrenoreceptors antagonist prazosin 3. Serotonergic recpetors Serotonin receptor antagonists of 5-HT1A (NAN-190), 5-HT2A (ketanserin), and 5-HT3 (MDL-72222) were injected intrathecally with EA. The relieving effects were blocked by the 5-HT1A antagonist and by the 5-HT3 antagonist significantly, but not by the 5-HT2A antagonist 4. Cholinergic receptors Pirenzepine (M1 muscarinic receptor antagonist), methoctramine (M2 muscarinic antagonist), 4-DAMP (M3 muscarinic antagonist) were injected intrathecally with EA. Only M1 muscarinic receptor antagonist completely blocked the relieving effect of EA 5.GABAergic recepetors GABAergic recpetors gabazine (GABAA receptor antagonist) or saclofen (GABAB receptor antagonist) were intrathecally injected. The relieving effects were blocked by both gabazine and Saclofen. **Results:** Data from our experiments show that spinal $\mu$ and $\delta$ opioid receptors, $\alpha$2-adrenoreceptors, 5-HT1A and 5-HT3 serotonergic receptors, M1 muscarinic receptors, GABAA and GABAB GABAergic receptors are involved in the analgesic effect of EA on neuropathic pain in the CNS. **Discussion:** Well-designed, rigorous and large randomized clinical trials are necessary to assess the effect of EA in clinic.
**Key Words:** Electroacupuncture, Neuropathic pain, Neurotransmitter, Analgesia

## NC-20(PO-14)

### Balance between the Proximal Dendritic Compartment and the Soma Determines Spontaneous Firing Rate in Midbrain Dopamine Neurons

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Midbrain dopamine (DA) neurons are slow intrinsic pacemakers that require elaborate composition of many ion channels in the somatodendritic compartments. Understanding the major determinants of spontaneous firing rate (SFR) of the midbrain DA neurons is important, because it determines basal DA levels in target areas including the striatum. Since spontaneous firing occurs synchronously at the soma and dendrites, the electrical coupling between the soma and dendritic compartments has been regarded as a key determinant for SFR. However, it is not known whether this somatodendritic coupling is served by the whole dendritic compartments or parts of them. By taking advantage of dissociation of morphologically well-preserved DA neurons from the rat substantia nigra pars compacta (SNc), here we describe that the balance between the proximal dendritic compartment and the soma determines SFR. Isolated DA neurons from the SNc showed a wide range of the soma size and a variable number of primary dendrites, but preserved quite a consistent SFR. The SFR was correlated neither with the soma size nor with the number of primary dendrites, but strongly with the area ratios of the proximal dendritic compartments to the somatic compartment. Elimination of excitability from the distal dendrites by tetrodotoxin puff had no effect on SFR. Local glutamate uncaging experiments revealed a much higher excitability in the proximal dendritic compartments than in the distal dendrites. These data indicate that the proximal dendritic compartments, not the whole dendritic compartments, play a key role in the somatodendritic balance determining SFR in the midbrain DA neurons.
**Key Words:** Neuronal morphology, Spontaneous firing rate, Dendritic excitability, Dopamine neuron, Somatodendritic balance

## NC-21(PO-15)

### Toll-Like Receptor 3 Contributes to Inflammatory Schwann Cell Activation and Wallerian Degeneration after Peripheral Nerve Injury

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It is well known that Schwann cells play an important role in Wallerian degeneration after peripheral nerve injury. Recently, it was reported that toll-like receptor (TLR) signaling contributes to Wallerian degeneration. Also, we have previously reported that TLR ligands-induced inflammatory Schwann cell activation via TLR2 and 3. However, the role of TLR3 in Wallerian degeneration after peripheral nerve injury is poorly understood. In this study, we investigated the role of TLR3 in Wallerian degeneration after peripheral nerve injury. First, sciatic nerve crush injury reduced the number of degenerating myelin axons in TLR3 KO mice. After 7 days, TLR3 KO mice showed delayed sciatic nerve degeneration compared with WT mice. We also examined the expression of proinflammatory or nerve regeneration-associated genes such as monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-a), and iNOS in Schwann cells in vitro and in vivo. The TLR ligands-induced proinflammatory gene ex-

pression was reduced in Schwann cells derived from TLR3 KO mice. Similarly, the sciatic nerve injury-induced proinflammatory gene expression was also reduced in TLR3 KO mice. Taken together, our data show that TLR3 is required for the inflammatory Schwann cell activation and contribute to wallerian degeneration after peripheral nerve injury.

**Key Words:** Toll-like receptor 3, Schwann cells, Wallerian degeneration, Sciatic nerve injury

## NC-22

### The Beneficial Effect of Pharmacological Manipulation of Mitochondrial Membrane Potential ($\triangle \Psi$m) on Mitochondrial Calcium Overload in Primary Cortical Neurons and Isolated Mitochondria

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It has been demonstrated that even a small mitochondrial depolarization is sufficient to prevent neuronal cell death by suppressing mitochondrial calcium overload since mitochondrial membrane potential ($\triangle \Psi$m) contributes to determining a driving force for calcium to enter the mitochondria. Therefore, mitochondrial depolarization has been recently evaluated as a novel mechanism of neuroprotection via inhibiting neurotoxic mitochondrial calcium overload during neuronal insults. We previously demonstrated that ethanolic peel extract of Citrus sunki Hort. ex Tanaka and its active compounds are capable of inducing a neuroprotective mitochondrial depolarization. In this study we investigated the neuroprotective effect of a non-steroidal anti-inflammatory drug (NSAID) indomethacin-induced partial mitochondrial membrane depolarization in glutamate-induced excitotoxicity model and clarified the underlying mechanism of action in both primary cortical neurons and isolated mitochondria. The results demonstrated that neuronal viability was significantly increased by indomethacin treatment in glutamate-exposed primary cortical neurons as a glutamate-induced neurotoxicity model. This neuroprotective effect was abolished by 5-hydorxydecanoate (5HD), a ATP-sensitive $K^+$ channels (mitoKATP) channel blocker. This blockade of mitoKATP channels by 5HD treatment significantly inhibited indomethacin-induced mitochondrial depolarization and also abolished indomethacin-induced inhibitory effect on mitochondrial calcium overload. It also suppressed mitochondrial dysfunction-associated parameters such as reactive oxygen species (ROS) generation. Taken together, these results suggest that blockade of mitochondrial $Ca^{2+}$ overload via depolarization of mitochondrial membrane plays a critical role in the mechanism underlying neuroprotective effects of indomethacin. In addition, the molecular target of indomethacin is considered as ATP-sensitive $K^+$ channels (mitoKATP). These findings raise the issue of a novel beneficial role of pharmacologically-induced mitochondria depolarization in neuroprotection.

**Key Words:** Mitochondria, Mitochondrial calcium, Mitochondrial membrane potential, Cell death, Glutamate, Indomethacin

## NC-23

### Beta-lapachone Improved the Intracellular Energy Status against Metabolic Insult in Astrocyte

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Energy homeostasis in the brain, a highly cooperative tissue between neurons and astrocytes, is mainly maintained with oxidative phosphorylation of glucose, a principle substrate. However, when glucose supplementation is restricted, other alternative energy substrates (e.g., monocarboxylic acids, ketone bodies, amino acids, and fatty acids) can be utilized as metabolic fuels. We have studied energetic rescue effect of beta-lapachone ($\beta$LA) in focal cerebral ischemia-reperfusion model and iodoacetate-induced neuronal death model. $\beta$LA stimulated utilization of amino acids, glutamate and glutamine, via activation of phosphate-activated glutaminase (PAG) and glutamate dehydrogenase (GDH) for ATP restoration, finally leading to neuroprotection. Here, we examined whether $\beta$LA also improves the intracellular energy status in astrocyte, via the way neurons protected. First, $\beta$LA demonstrated significant protection via ATP generation when iodoacetate induced severe energy depletion and death. In astrocyte, ATP generation by $\beta$LA may not be involved in the activation of GDH as a GDH activator, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid did not show the protection in metabolic stress. Indeed, $\beta$LA induced the oxidation of lactate to pyruvate via activation of lactate dehydrogenase by regulation of NAD+/NADH ratio. Supplementation of pyruvate, a main mitochondrial substrate overcame ATP depletion, leading to functionally active mitochondria. Our findings suggest that $\beta$LA stimulated the utilization of monocarboxylic acids for improvement of the intracellular bioenergetics against metabolic stress in astrocytes. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2009-0076242), (2013-029298), (NRF-2012R1A-5A2051428).

**Key Words:** Beta-lapachone, ATP, Protection

## NC-24

### The Plausible Neuroprotective Mechanism How Dieckol might Attenuate Vicious Cycle of Positive Feedback Loop in TLR4 Signaling through NADPH-ROS Pathway

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Early blockade of microglial activation could be a very effective treatment of brain stroke since it inhibits delayed neuronal cell death in the penumbra region of brain ischemia. Therefore, much attention has been paid to therapeutic strategies aimed at suppressing microglial overactivation. In the present study, the neuroprotective mechanism of dieckol, one of the phlorotannins, was investigated in microglia-mediated neurotoxicity models. The results demonstrated that dieckol exerts reactive oxygen species (ROS)-scavenging activity and intracellular ROS were markedly reduced by dieckol (50 $\mu$M) treatment in lipopolysaccharide (LPS, 1 $\mu$g/ml)-stimulated BV-2 microglial cells. Moreover, dieckol markedly suppressed LPS-induced expression of Nox2 (also known as gp91$^{phox}$) responsible for superoxide production. Dieckol treatment potently suppressed LPS-stimulated nitric oxide (NO) production in a dose-dependent manner in both primary microglia and BV-2 cell line. Moreover, dieckol significantly inhibited secretion of interleukin 6 (IL-6) and tumor necrosis factor $\alpha$ (TNF-$\alpha$), phosphorylation of mitogen- activated protein kinases (MAPKs) and Akt, and nuclear translocation of nuclear factor $\kappa$B (NF-$\kappa$B) in LPS-stimulated BV-2 microglial cells. In addition, dieckol significantly attenuated neuronal cell death induced by treatment of the conditioned media containing neurotoxic secretary molecules from LPS-stimulated microglia. These neuroprotective effects of dieckol were also confirmed in a neuron--microglia co-culture system. Taken together, these results suggest that a strong anti-oxidant dieckol suppresses excess microglial activation and microglia-mediated neuronal cell death via downregulation of NADPH oxidase-ROS signaling as a positive feedback pathway of LPS-induced Toll-like receptor 4 (TLR4) signaling.

**Key Words:** Microglia, NADPH oxidase, ROS, TLR4, Neuroinflammation, Dieckol

## NC-25

### Mechanism of Long-Term Synaptic Depression of Type I Metabotropic Glutamate Receptor Signaling in Cerebellar Parallel Fiber-Purkinje Cell Synapse

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Metabotropic glutamate receptors (mGluRs) are well-known for their contribution to various forms of synaptic plasticity. They not only induce long-term potentiation and depression of ionotropic glutamate receptors, but also raise internal calcium concentration and regulate gene expressions. Recently, it has been found that in cerebellar tissues, mGluR itself can undergo long-term depression, which can be described as metaplasticity. This LTD of mGluR is known to be highly dependent on intracellular $Ca^{2+}$ level; however, its precise mechanism is still unidentified. Thus here in this study, the underlying mechanism of LTD of mGluR1 is investigated. Phosphorylation and/or internalization of the receptor and dendritic glutamate secretion was examined as desensitization of G-protein coupled receptors (GPCRs) can be induced by these factors. Drugs which inhibit phosphorylation and internalization of the receptor were treated and brief depolarizations were used to induce dendritic glutamate release. Our data suggest that this form of LTD is irrelevant to phosphorylation of the receptor by second messenger-dependent protein kinases, but internalization of the receptor may take place during LTD of mGluR1 and also dendritic glutamate release may be linked to this LTD.

**Key Words:** Metabotropic glutamate receptors (mGluRs), Cerebellar purkinje cells, Synaptic plasticity, Phosphorylation, Internalization, Dendritic glutamate release

## NC-26

### Cyanidin-3-Glucoside Inhibits Glutamate-Induced $[Zn^{2+}]_i$ Increase by Inhibition of $[Ca^{2+}]_i$, Reactive Oxygen Species, and Mitochondrial Depolarization in Cultured Rat Hippocampal Neurons

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Glutamate has been reported to induce an increase in intracellular free $Zn^{2+}$ concentration ($[Zn^{2+}]_i$) in neurons through $Ca^{2+}$ signaling, oxidative stress, and mitochondrial dysfunction. Cyanidin-3-glucoside (C3G), a member of the anthocyanin family, is a potent natural antioxidant. However, effects of C3G on glutamate-induced $[Zn^{2+}]_i$ homeostasis remain unknown. We studied how C3G affects glutamate-induced $[Zn^{2+}]_i$ increase in cultured rat hippocampal neurons from embryonic day 17 maternal Sprague-Dawley rats using digital imaging methods for $Zn^{2+}$, $Ca^{2+}$, reactive oxygen species (ROS), and mitochondrial membrane potential. Reproducible $[Zn^{2+}]_i$ increases were elicited by applying glutamate (100 $\mu$M) for 7 min at 30 min intervals (relative to peak 1=101.03±2.10 % n=38). Pretreatment with C3G (100 ng/ml to 1 mg/ml) for 30 min inhibited the glutamate-induced $[Zn^{2+}]_i$ response in a concentration-dependent manner ($IC_{50}$=14.26 $\mu$g/ml). Pretreatment with C3G (15 $\mu$g/ml) for 30 min significantly inhibited glutamate-induced both $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ responses (50.3±4.50% and 56.4±3.50%, respectively). Pretreatment with the reductant DTT (50 $\mu$M) for 5 min significantly blocked glutamate-induced $[Zn^{2+}]_i$ increase, whereas it did not affect glutamate-induced $[Ca^{2+}]_i$ increase. Each pretreatment with C3G (15 $\mu$g/ml) for 30 min or DTT (50 $\mu$M) for 5 min blocked glutamate (100 $\mu$M), $H_2O_2$ (100 $\mu$M) and DTDP (30 $\mu$M)-induced generation of ROS. C3G also blocked glutamate-induced mitochondrial depolarization. In addition, treatment with C3G attenuated

glutamate-induced release of lactate dehydrogenase in cultured rat hippocampal neurons. All these results suggest that cyanidin-3-glucoside inhibits glutamate-induced $[Zn^{2+}]_i$ increase in cultured rat hippocampal neurons by inhibition of calcium signaling, ROS formation, and mitochondrial depolarization, which is involved in neuroprotection. This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022013)" Rural Development Administration, Republic of Korea.
**Key Words:** Flavonoid, Glutamate, Mitochondrial membrane potential, Reactive oxygen species, $Zn^{2+}$

## NC-27

### The Difference of Neuronal Differentiation between B10 Cells and B10 Transfected with Neurogenin-1 in Bone Marrow Mesenchymal Stem Cell Lines

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Bone marrow contains two types of stem cells, hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). The studies using human MSCs have difficulty in obtaining sufficient number and homogenous population of human MSCs to investigate detailed cellular and molecular properties. To circumvent these problems, we have previously generated an immortal human MSCs cell line (BM3.B10) via retroviral vector encoding v-myc. B10 human MSCs carry normal human karyotype of 46,XX and express MSC specific markers and differentiate into adipocytes, osteocytes, chondrocytes or neurons when grown in differentiation media. In the present study, we generated an enriched population of neurons from MSCs via trasfection of B10 MSCs with Neurogenin-1(Ngn1) gene, a member of proneural basic helix-loop-helix transcription factors, and investigated the functional neural differentiation by RT-PCR, Real-time PCR, western blot and. whole-cell mode patch clamp techniques.
**Key Words:** Mesenchymal stem celll, B10, Neurogenin-1

## NC-28

### The Effect of JAK3 Inhibition on Growth Factor Withdrawal-induced Cell Death

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Apoptosis is evolutionally conserved, and plays central roles during normal development of the nervous system. The number of embryonic neuroepithelial cells (NECs) is regulated by programmed cell death when NECs are substantially proliferated by growth factors (GFs). The extrinsic factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), as well as intrinsic protein regulate growth, proliferation and differentiation. Therefore, in culture system, addition of both bFGF and EGF is critical to maintain and expand NECs. The withdrawal of these GFs induced apoptosis through intrinsic mitochondrial pathway in NECs. However, the mechanisms underlying the fate of NECs to either survive or die are incompletely understood. We previously reported that JAK3 inhibition induced neuronal differentiation accompanied by neurite outgrowth in enriched condition of GFs such as EGF, bFGF. In the present study, effect of JAK3 inhibition on GF-withdrawal death of cultured NECs from embryonic mice brain (P13.5 day) was examined with or without treatment of JAK3 inhibitors. The GF-withdrawal decreased cell viability in time-dependent manner, however treatment of JAK3 inhibitors increased cell viability. The trypan blue stained cells which were dead, were increased in GF-withdrawal condition, in contrast those were decreased by JAK3 inhibitors. For further confirmation, we also performed propidium iodide (PI) and calcein-AM double staining. The number of calcein-AM stained cells, which were alive, was increased strikingly by JAK3 inhibitor. However, the number of PI-positive cells was similar between JAK3 inhibitor treated and non-treated condition. Jak3 inhibitor increased the ability of proliferation and differentiation of NECs. In conclusion, we identified that inhibition of JAK3 drives NPCs toward cell survival in GF-withdrawal condition. Therefore, we propose that JAK3 may have a critical role on growth factor withdrawal-induced apoptotic cell death.
**Key Words:** Neuroepithelial cells, Growth Factor, Cell death, JAK3

## NC-29

### Analysis of Spatio-Temporal Network Dynamics in the Brain: in vivo Two-Photon Imaging

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Neuronal systems are complicated dynamical systems. Neurons influence one another, forming complex patterns of the networks, and change their patterns dynamically over time. Therefore, in order to elucidate how the neuronal systems operate, the spatio-temporal network of the neurons should be understood. In vivo two-photon calcium imaging provides an opportunity of investigating the dynamics of the spatio-temporal network at a single cell resolution by allowing measuring the activity of multiple cells simultaneously. Here, we performed two-photon calcium

imaging in the primary somatosensory cortex and the cerebellum using anesthetized mice. Cells were imaged either with Oregon BAPTA-1 (OGB-1) or by inducing the expression of G-CaMP3. Preprocessed calcium signals were constructed into the spatio-temporal networks by employing sliding-window analysis. Network dynamics of the spontaneous activity were explored with regard to the physical location of the cells using network dynamics movie. In order to explore the dynamics of the neural networks in low dimensional space, we applied principle component analysis (PCA) to connectivity matrix of the spatio-temporal network, projecting the population activity onto a new state space.
**Key Words:** Spatio-temporal network, in vivo Two-photon imaging, Dynamics

## NC-30

### Regulation of Spontaneous Firing by GABA (A) Receptor and GABA (B) Receptors in the Midbrain Dopamine Neurons

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The primary inhibitory neurotransmitter, GABA, activates two distinct GABA (A) and GABA (B) receptors in the brain. Dopamine neurons in the midbrain express both GABA (A) and GABA (B) receptors in the soma and dendrites. At least 70 % of the afferents to substantia nigra dopamine neurons are GABAergic and excitatory and inhibitory afferent inputs harmonically regulate firing activities. However, it is not clear how differently each GABA receptor in the soma and dendrites participates in regulation of spontaneous firing in the dopamine neurons. Therefore, using a patch-clamp recording and GABA uncaging techniques, we studied regional actions of GABA receptors on the spontaneous firing in the acutely isolated rat dopamine neurons. Application of either a GABA (A) receptor agonist, isoguvacine hydrochloride or a GABA (B) receptor agonist, baclofen completely suppressed spontaneous firing in the dopamine neurons. Also GABA was able to completely inhibit spontaneous firing under the presence of either GABA (A) receptor antagonist or GABA (B) receptor antagonist, suggesting that spontaneous firing can be inhibited by activation of only one type of GABA receptors. When we stimulated a various part of a dopamine neuron with caged GABA, activation of GABA receptors in any part of a neuron, such as the soma, proximal dendritic, and distal dendritic regions, inhibited spontaneous firing. However, in the soma, activation of GABA (A) or GABA (B) receptors equally suppressed spontaneous firing, but in the proximal and distal dendrites, GABA (B) receptors more strongly inhibited spontaneous firing than GABA (A) receptors. From these data, we conclude that the spontaneous firing of dopamine neurons could be arrested by the inhibitory action of both GABA (A) and GABA (B) receptors, equally in the soma and in a GABA (B)-dominant way in the dendrites.
**Key Words:** Dopaminergic neuron, Inhibition, GABA (A)/(B) receptors, Spontaneous firing, Cell-attached recording

## NC-31

### JAK/STAT3 Pathway in Microglia was Involved in Learning and Memory

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Microglia are recently emerged as key players for synaptic plasticity in normal brain development and adult physiology. In particular, they actively regulate synaptic pruning and remodeling. To test how microglia have influence on neuronal activity, we used microglia specific STAT3 knockout mice via Cre-Lox recombination system. First of all, we performed a variety of behavior tests. Morris water maze (MWM) test showed that STAT3 KO group was improved in learning and memory (WT=8; KO=9). MWM probe test also verified our results. This implicates that one of the signal pathways in microglia might be included in neuronal function. To concrete this behavior results, we used electrophysiological approach in order to investigate synaptic activity changes. TTX-induced miniature excitatory postsynaptic current (mEPSC) was measured through whole-cell patch recording for determining whether it came from presynaptic or postsynaptic. Statistically cumulative probability of inter-events interval (IEI) showed that synaptic events were more frequently occurred in STAT3-defient mice. However, amplitude of events (distribution and average) had no difference between control and knock-out groups. This implicates microglia related to STAT3 pathway regulate presynaptic effects not postsynaptic effects. In conclusion, synapse-microglia interaction from microglia STAT3-deficient mice model makes possible to expand our understanding of learning and memory.
**Key Words:** Synapse, Microglia, STAT3, mEPSC

## NC-32(PO-16)

### Liver Cirrhosis Attenuates Excitability of Aortic Baroreceptor Neurons: Involvement of Voltage-Gated Sodium Channels

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Arterial baroreflex is a central mechanism for maintenance of cardiovascular homeostasis. Several evidence has shown that liver cirrhosis in humans and experimental animal models is associated to impaired baroreflex sensitivity (BRS). To date, however, cellular mechanisms underlying the cirrhosis-induced baroreflex dysfunction remain unknown. In the present study, we hypothesized that liver cir-

rhosis would attenuate excitability of aortic beroreceptor neurons located in the nodose ganglion. To address the hypothesis, cirrhotic rats were produced by bile duct ligation (BDL). Three-week after BDL, expression of the cirrhosis markers such as a-smooth muscle actin, collagen, and transforming growth factor-$\beta$ was significantly increased in liver. These findings were consistent with histological examination of the liver tissues from BDL rats. At the same period of time, BRS was significantly impaired in BDL rats. Under the current-clamp mode of patch-clamp technique, action potentials (AP) were recorded in Di-I-labeled aortic baroreceptor neurons from sham control and BDL rats. BDL attenuated the excitability of tonic (A-type) and phasic (C-type) neurons by increasing rheobase and reducing AP amplitude. Consistent with the findings, TTX-sensitive and TTX-insensitive sodium currents were significantly decreased in two types of aortic baroreceptor neurons. RT-PCR analysis revealed that BDL down-regulated Nav1.7, Nav1.8, and Nav1.9 in aortic baroreceptor neurons. Taken together, these data suggest that BDL-induced liver cirrhosis impairs BRS through attenuating excitability of aortic baroreceptor neurons. The ionic mechanisms underlying the hypoexciatability may include down-regulation of voltage-gated sodium currents. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2013424).
**Key Words:** Baroreflex sensitivity, Baroreceptor neuron, Liver cirrhosis, Bile-duct ligation

## NC-33

### Lower Level of Inhibitory Synaptic Transmission and Higher Hyperpolarization-activated current in the Vestibulo-Cerebellum

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In accordance with functional classification, the cerebellum is categorized as three subdivisions, the cerebro-cerebellum, the spino-cerebellum, and the vestibulo-cerebellum (VC). It has been well known that the VC, the flocculonodular lobe comprised of flocculus, paraflocculus, and lobule IX and X of vermis, receives substantial vestibular input directly from vestibular nuclei, and then its extensive output projects to vestibular nuclei. Among several types of neurons in the cerebellar cortex, Purkinje cells (PCs) on which external inputs via parallel fibers and climbing fibers converge are the exclusive output neurons and so regarded as the principal neurons of the cerebellar circuits. Therefore, understanding properties of PCs associated with input-output is necessary for comprehending the operating principles of the cerebellar circuitries. With distinguishable characteristics of input and output functions in the vestibulo-cerebellum, previous studies have shown that PCs in the VC have distinct electro-physiological properties. Besides excitatory inputs by parallel fibers and climbing fibers, PCs receive inhibitory inputs from molecular layer interneurons which form feed-forward inhibition influencing the fidelity of PCs output. However, there has been no research regarding inhibitory synaptic transmission of the VC. In this study, we investigated the nature of inhibitory synaptic transmission in the VC and their functional implication, using whole cell patch clamp techniques. Since cerebellar vermis, which is subdivided into ten lobules, has both the VC (lobule IX and X) and the spino-cerebellum (the other lobules), we compared PCs between lobules III~VI and lobule X of vermis. We found that inhibitory inputs into PCs of the VC have much lower amplitude and frequency than those in other cerebellar regions. In this aspect of the relationship between amplitude and rise time, it is plausible that this difference is due to basket cell-PC synapse rather than stellate cell-PC synapse. A recent study showed that hyperpolarization-activated current (Ih) of PC plays a crucial role in neuronal computation, constraining synaptic inhibition. We also recorded hyperpolarization-induced inward current and membrane sag % of PCs, both of which represent a degree of Ih expression. Our results manifested that Ih of PC in the VC is much higher than one in the other lobules. We expect that PCs of the VC not only receive different level of inhibitory input but also process the inhibition distinguishingly from the other cerebellar regions in a manner of adopting higher Ih.
**Key Words:** Cerebellum, Inhibitory Interneuron, Hyperpolarization-activated current, Vestibulo-cerebellum, Purkinje cell

## NC-34

### Chronic Network Activity Blockade causes Decrease of Input Resistance via Upregulation of Ih and Ik Conductance

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Cerebellar Purkinje cells (PC) are the major output of the cerebellar cortex and integrate afferent inputs which are composed of excitatory and inhibitory neurons. Alteration of intrinsic or synaptic properties affects neuronal activity, and has been proposed as a mechanism of learning and memory. One of the factors which influence on neuronal activity, input resistance contributes to the level of membrane potential in response to current coming in or out of the cells. It has been reported that neuronal activity can be homeostatically modulated to provide stability against the disturbance of neuronal activity as well as imbalance of synaptic transmission. It has been widely accepted that prolonged activity blockade causes alteration of synaptic strength or intrinsic excitability. In terms of intrinsic properties, however, how cerebellar PCs response to chronic activity blockade is yet to be investigated. In this work, we hypothesized that prolonged network inhibition affects the input resistance of cerebellar PCs and the alteration is

mediated by some of ion channels. To test this hypothesis, we used organotypic cerebellar slice cultures and electrophysiological techniques. Network activity was totally blocked by application of 1 $\mu$M of TTX for 2 days. First, we observed that the input resistance was decreased in chronic activity blocked group. Based on studies postulating the hyperpolarization-activated current (Ih) contributes to input resistance, we measured sag voltage and Ih conductance. Both sag voltage and Ih were increased in blockade group, and this result was parallel to previous studies. Since it is well known that voltage gated potassium current (Ik) also contributes to determining input resistance, Ik was measured to verify whether the voltage gated potassium channel (Kv) conductance is altered by blockade of network activity. Our data showed that the Ik was increased in activity deprived group at supra-threshold voltage and this alteration was abolished with 4 $\mu$l TEA, a potassium channel blocker. In addition, effect of inhibition of network activity on input resistance was rescued by application of each blockers. Together with other studies, these data suggest that intrinsic properties of cerebellar PCs respond to prolonged inhibition of network activity by decreasing the input resistance with upregulated conductance in both Kv and HCN channels.

**Key Words:** Purkinje cells, Intrinsic, Homeostatic regulation, Slice culture

## NC-35

## Maturation of GABAergic Inhibition is a Critical Regulator of the Induction of Long-Term Potentiation in the Rat Visual Cortex

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Visual cortex is unique in the study of synaptic plasticity since the plasticity is well induced only during a specific period of early developmental stage called critical period. Although many factors could be involved in the regulation of the critical period, GABAergic inhibition has been proven to have a paramount importance in the opening and closing of the critical period. To evaluate the importance of GABAergic inhibition more systematically, we investigated the effects of pharmacologic modulation of GABAergic inhibition on the induction of long-term potentiation (LTP) before, during, and after the critical period. Time table of maturation of inhibition was measured as an excitation-inhibition ratio using whole-cell patch clamp technique in layer 2/3 pyramidal neurons of rat visual cortical slices at 2, 3, 5, 8, and 12 postnatal weeks. NMDAR-dependent LTP of layer 2/3 field potential was evoked by theta burst stimulation of underlying layer 4. GABAergic inhibition was either 20% increased or decreased with adequate concentration of GABAA receptor agonist and antagonist, respectively. GABAergic inhibition matured until 8 weeks of age. LTP could not be induced in 2-week-old rats. After 2 weeks of age, LTP induction abruptly reached peak in 3-week-old rats and then declined with aging. The decline of the induction of LTP was well correlated with the maturation of GABAergic inhibition. In all age groups except 2 weeks of age, LTP induction was suppressed by GABAA receptor agonist and enhanced by GABAA receptor antagonist. These results suggest that GABAergic inhibition is an important regulator of the induction of LTP if only the machinery for LTP induction is equipped during development. Threshold level of GABAergic inhibition is known to be necessary for the opening of the critical period. Thus, our results further suggest that threshold level of GABAergic inhibition should be maintained for some duration to open the critical period since acute modulation of inhibition was not sufficient to induce LTP in 2-week-old rats. Supported by the NRF (2013-056534).

**Key Words:** Visual cortex, GABAA receptor, LTP, Inhibition

## SC-1

### Role of Lysophosphatidic Acid in Salivary Epithelial Cells

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**Purpose:** Lysophosphatidic acid (LPA) is a naturally occurring bioactive lysophospholipid involved in numerous physiological responses. LPA plays a critical role in autoimmune disease, but the intracellular signaling pathways regulating these processes remain elusive. **Materials and methods:** Human submandibular(SMG) gland cells were used in immunoblotting, calcium imaging and patch clamp recording. LPA, U73211, 2-APB were purchased from Sigma (St. Louis, MO, USA) and Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). **Results:** LPA increased levels of the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). Application of plasma membrane calcium ATPases (PMCA) inhibitor, o-vanadate delays the reduction of $[Ca^{2+}]_i$ after LPA application and sustains $[Ca^{2+}]_i$ at a higher level than the resting state. Treatment with LPA also induced a outward current (calcium-activated $K^+$ ($BK_{ca}$) activation), which was attenuated by the LPA receptor antagonist ALLM, PLC inhibitor U-73122 and IP3 inhibitor 2-APB. Interestingly, high concentration of LPA (100 $\mu$M) induced calcium-mediated proteolysis (calpain) activation and reduced PMCA protein levels. **Conclusion:** Our results suggest that LPA trigger $Ca^{2+}$ signaling and calpain activation in human salivary epithelial cells.
**Key Words:** LPA, SMG, PMCA, Calpain

## SC-2

### Alteration of Regulated Exocytosis in Parotid Gland Acinar Cells from TRPML1$^{-/-}$ Mice

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Deletion or loss-of-function mutations in transient receptor potential mucolipidosis (TRPML) 1 causes mucolipidosis type IV characterized by a psychomotor retardation, corneal opacity, retinal degeneration, and achlorhydria. TRPML1 is expressed in late endosomes and lysosomes and controls lysosomal pH. *In vitro* studies of constitutive membrane trafficking suggest that TRPML1 plays a role in delivery or fusion of late endosomes and lysosomes, resulting in accumulation of material in the lysosomes and induction of autophagy. However, the role of TRPML1 in regulated exocytosis is not known well. In our previous work, TRPML1$^{-/-}$ mice recapitulate many features of the human disease, including neuronal degeneration and achlorhydria, suggesting that TRPML1 may have a role in regulated exocytosis, perhaps in membrane retrieval. To further explore the role of TRPML1 in regulated exocytosis, we investigated salivary gland and pancreatic exocytosis in vivo/in vitro. We found the right shift of the dose response to CCK8-mediated $Ca^{2+}$ increase in pancreatic acini from TRPML1$^{-/-}$ mice and that deletion of TRPML1 induced right shift the dose response to agonist-stimulated amylase release, and enhanced secretion in response to high agonist stimulation. However, in TRPML1$^{-/-}$ mice salivary glands, there was no apparent effect on fluid secretion with treatment of pilocarpine, an agonist of muscarinic receptor, or mixture of pilocarpine and isoproterenol, an agonist of adrenergic receptor. In contrast, in TRPML1$^{-/-}$ mice, amylase secretion was increased in both parotid glands *in vivo/in vitro*. In parotid acini from TRPML1$^{-/-}$ mice, the agonist-related amylase secretion was increased in a time-dependent and a dose-dependent manner, respectively. These results suggest that TRPML1 deletion may be related with exocytosis of the secreting vesicles or related with retrieval of membrane in mouse salivary gland acinar cells.
**Key Words:** TRPML1, Salivary gland acinar cells, Amylase secretion, Exocytosis

## SC-3

### Alteration of RANKL-Induced Osteoclastogenesis in Primary Cultured Osteoclasts from Homer2/3$^{-/-}$ Mice

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Calcineurin-dependent nuclear factor of activated T cells, cytoplasmic 1 (NFATc1) pathway plays an important role in receptor activator of nuclear factor-$\kappa$B lignad (RANKL)-induced osteoclast differentiation. RANKL-induced $Ca^{2+}$ oscillations lead NFATc1 activation and osteoclast differentiation. The Homer proteins, cytoplasmic scaffolding proteins, not only interact with $Ca^{2+}$ signaling proteins such as G protein-coupled receptors, inositol 1,4,5-triphosphate receptors ($IP_3Rs$), and transient receptor potential channels in plasma membrane microdomains, but also compete with calcineurin for NFAT binding in T cells. However, the role of Homer proteins during osteoclastogenesis is not known. This study examined the effect of loss of Homer2/3 protein on osteoclast differentiation in Homer2/3 deficient mice (Homer2/3$^{-/-}$). The bone density of Homer 2/3$^{-/-}$ mice decreased compared with wild-type mice (WT). However, we did not found a difference of RANKL-induced $Ca^{2+}$ oscillations between WT and Homer2/3$^{-/-}$ mice. After RANKL treatment, there was markedly increased NFATc1 protein expression and translocation of NFATc1 into the nucleus during osteoclastogenesis of the Homer2/3$^{-/-}$ bone marrow-derived macrophages (BMMs). In addition, RANKL treatment of Homer2/3$^{-/-}$ BMMs increased formation of multinucleated cells. These results suggest that Homer2/3 play a critical role as a negative regulator for osteocla-

stogenesis.
**Key Words:** Homer2/3, Calcium signaling, NFATc1, Osteoclast differenciation

## SC-4

### $Ca^{2+}$ Signaling and Cytokine Release by Asthma Related Allergens in Human Gingival Epithelial Cells

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Asthma is a chronic inflammatory disease of the airway characterized by variable airflow obstruction and bronchial hyper-responsiveness. In addition, patients with asthma have been reported that high prevalence of dental caries and gingivitis, which are induced by low salivation due to the medication for asthma. However, it is not known that asthma related allergens such as house dust mite (HDM) and German cockroach extract (GCE) have direct effects on the generation of gingival inflammation. In the present study, we investigated the level of the NLR family pyridine domain-containing 3 (NLRP3) inflammasome, interlukin-8 (IL-8), IL-6 and IL-1$\beta$ mRNA expression and the characteristics of $Ca^{2+}$ signal by GCE and HDM in the human gingival epithelial cells. HDM and GCE induced increases in NLRP3 and proinflammatory cytokines mRNA expression level and intracellular concentration of $Ca^{2+}$ ($[Ca^{2+}]_i$), respectively. Endotoxin-free GCE activated protease-activated receptor (PAR) 2, but endotoxin-free HDM did not activate any PARs. In human gingival epithelial cells, whereas lipopolysaccharide, a Gram-negative endotoxin, did not induce $Ca^{2+}$ signal, HDM and GCE containing endotoxins induced $Ca^{2+}$ signal from thapsigargin (Tg), an inhibitor of sarco/endoplasmic reticulum $Ca^{2+}$ ATPase, -sensitive $Ca^{2+}$ stores via phospholipase C (PLC)/inositol 1,4,5-trisphosphate ($IP_3$) pathway. These results suggest that asthma related allergens induce $Ca^{2+}$ signaling and cytokine release in human gingival epithelial cells.
**Key Words:** Gingival epithelial cells, Calcium signaling, Asthma, Allergen

## SC-5(PO-9)

### Cytosolic and Mitochondrial Matrix Alkalinisation is Involved in Superoxide Generation and Cytotoxicity by High Phosphate in Insulin Secreting Cells

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Phosphorus is an essential element as a constituent of body and plays a critical role in energy metabolism and cell signaling. However, high phosphate level in plasma elicits serious detrimental consequences to the body including vascular calcification. Impaired insulin secretion has been reported in chronic renal failure with hyperphosphatemia, the mechanism of which remains to be clarified. We investigated the pathophysiologic role of pH changes and ROS increase in high phosphate-induced cytotoxicity of insulin secreting cells. Extracellular high phosphate (2~5 mM) dose-dependently increased superoxide generation, depolarized mitochondrial membrane potential, induced caspase activation and cell death in rat insulinoma cell line INS-1. Intriguingly, high phosphate (3 mM) significantly alkalized both cytosol (from 7.08±0.03 to 7.81±0.04, $p<0.0001$) and mitochondrial matrix pH (from 7.17±0.02 to 7.35±0.05, $p<0.05$). Extracellular alkaline condition further enhanced this alkalinisation by high phosphate, which accelerated mitochondrial superoxide generation and cell death. Conversely, acidic condition rescued from all the changes by phosphate described above. In permeabilized cells, alkaline extramitochondrial pH facilitated more phosphate uptake, whereas acidic pH prevented it. Pathogenic actions of high phosphate were prevented by blockers of mitochondrial phosphate transporter (mersalyl, butylmalonate) and mitochondrial ROS scavengers (mitoTEMPO, MnTBAP). We suggest that phosphate accumulation with cytosolic and matrix alkalinisation closely participates in mitochondrial ROS generation and cell death by high phosphate, which might be involved in the secretory defects of pancreatic $\beta$-cell and/or other pathogenic conditions in hyperphosphatemia.
**Key Words:** Phosphate, Mitochondrial superoxide, Mitochondrial pH, Cytotoxicity

## SY-1

### Effects of Essential Oil Extracted from Chrysanthemum boreale Makino on Skin Regeneration and Wound

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Chrysanthemum (chrysanthemum boreale Makino), which belongs to the compositae family, is widely distributed in Korea, China, Japan and Eastern countries. Its essential oil has also been reported to have a variety of biological activities such as anti-cancer and anti-radical. However, effects of the essential oil on regeneration of skin and wound healing have not been reported. In the present study, we investigated whether essential oil from chrysanthemum affects cell proliferation, the phosphorylation of Akt and ERK1/2, and type IV collagen synthesis in normal human keratinocytes (HaCat) and human dermal fibroblasts (CCD-986sk). Essential oil was extracted from chrysanthemum by steam distillation and analyzed using gas chromatography-mass spectrometry. The essential oil contained total 42 compounds including camphor, $\beta$-cubebene and piperitone. To test cellular experiments, we solubilized essential oil from chrysanthemum using Transcutol-CG, which is widely used as a non-cytotoxic solubilization agent in cosmetics. Treatment with the essential oil did not show cytotoxicity on HaCat and CCD-986sk at the concentration of 0.0001 to 1 $\mu$g/ml, respectively. The essential oil dose-dependently induced the proliferation of HaCat, which showed maximal response at a concentration of 0.1 $\mu$g/ml (176.33±7.73% of control), but dose-dependently inhibited the proliferation of CCD-986sk, which reach a maximum at a concentration of 1 $\mu$g/ml (71.92±2.46% of control). The essential oil also induced the phosphorylations of Akt and ERK 1/2 in HaCat in a dose-dependent manner. Moreover, the essential oil enhanced the type IV collagen synthesis in keratinocyte compared with intact control. This study demonstrates that essential oil from chrysanthemum by steam distillation stimulates the growth of human skin keratinocytes, probably through Akt and ERK1/2 pathway, but suppresses the growth of human dermal fibroblast, and that the essential oil promote the type IV collagen synthesis on kerainocytes. Therefore, essential oil from chrysanthemum may help skin regeneration and wound healing in human skin, and also be a possible cosmetic material for skin beauty.

**Key Words:** Chrysanthemum boreale Makino, Essential oil, Keratinocyte, Fibroblast, Proliferation

## SY-2

### Diluted Bee Venom Injection Reduces Ipsilateral Mechanical Allodynia in Oxaliplatin-Induced Neuropathic Mice

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Oxaliplatin, which is used as one of anti-cancer drugs, commonly induces peripheral neuropathic pain. We have previously reported that an injection of diluted bee venom (DBV) produced a significant anti-nociceptive effect in several pain models of mice or rats. In this study, we evaluated time- and dose-dependent development of oxaliplatin-induced mechanical allodynia in bilateral hind paws of mice, and investigated the effect of DBV injection on this mechanical allodynia. DBV (0.1 mg/kg) was subcutaneously injected into the Zusanli acupoint 2 weeks after oxaliplatin (10 mg/kg) injection. One hour after DBV injection, we observed a significant reduction of mechanical allodynia in the ipsilateral hind paw, but not in the contralateral hind paw to DBV injection site. We subsequently examined whether this effect of DBV was related to the activation of peripheral nerves in DBV injected site, and then whether it was mediated by the activation of spinal cord alpha-2 adrenoceptors or opioid receptors. Subcutaneous pre-injection of 2% lidocaine (40 mg/kg) into the Zusanli acupoint completely blocked the anti-allodynic effect of DBV. Intrathecal pretreatment with yohimbine (25 $\mu$g/mouse), an alpha-2 adrenoceptor antagonist, also prevented the anti-allodynic effect of DBV, whereas pretreatment with naloxone (20 $\mu$g/mouse), an opioid receptor antagonist, did not block the effect of DBV. Taken together, these findings demonstrate that DBV injection into the Zusanli acupoint significantly reduces ipsilateral mechanical allodynia generated by oxaliplatin in mice, and also suggest that this anti-allodynic effect is dependent on the peripheral nerve activation in injected site and spinal cord alpha-2 adrenoceptors.

**Key Words:** Bee venom, Oxaliplatin, Mechanical allodynia, Alpha-2 adrenoceptor

## SY-3

### Angiotensin III Protects Ischemic Injury of Hearts through Mitochondrial KATP Channel and Antioxidant Pathway

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Angiotensin (Ang) III is metabolized from Ang II by aminopeptidase (AP) A and in turn, Ang III is metabolized to Ang IV by APN. Ang III exerts its protective effect via angiotensin II type 2 receptor (AT2R), but the effect of Ang III on ischemic reperfusion (I/R) injury is still controversial. The aim of this study was to investigate whether Ang III protects heart function against I/R injury. After sacrifice of

Sprague-Dawley rats, global ischemia was performed using isolated perfused Langendorff hearts. Hearts were perfused with Krebs-Henseleit (K-H) buffer for 20min pre-ischemic period with and without Ang III followed by a 20 min global ischemia and 50 min reperfusion. Pretreatment with Ang III (1 $\mu$M) for 10 min before ischemia increased recovery rate of the post-ischemic left ventricular developed pressure (LVDP) and±dp/dt, and decreased post-ischemic left ventricular end-diastolic pressure (LVEDP) as compared with untreated I/R group. Ang III markedly decreased the infracted size and attenuated the increased lactate dehydrogenase level in effluent during reperfusion. Pretreatment with AT2R antagonist or mitochondrial KATP channel blocker for 15min before ischemia attenuated the improvement of LVEDP, LVDP, and±dp/dt induced by Ang III. Post-ischemia increased the concentration of atrial natriuretic peptide in coronary effluent, which attenuated by Ang III treatment. Decreases on Mn-superoxide dismutase (SOD), catalase, and heme oxygenase-1 levels by I/R were increased by Ang III treatment. Increases in Bax, caspase-3 and caspase-9 levels, and a decrease in Bcl-2 level by I/R were attenuated by Ang III treatment. These results suggest that the cardioprotective effect of Ang III against I/R injury may be partly mediated through mitochondrial KATP channels and antioxidant pathway. This work was supported by the National Research Foundation of Korea grant funded by the Korea government (2012-0009322) and by a grant from the ministry of Science & Technology (MoST)/Korea Science & Engineering Foundation (KOSEF) (2010-0021808).
**Key words:** Angiotensin III, AT2 receptor, Ischemia, Apoptosis, Mitochondrial KATP channels

## SY-4

### Angiotensin-(1-9) Stimulates ANP Secretion via AT2 Receptor and PI3K/NO/sGC/PKG/ Pathway

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Renin-angiotensin system (RAS) plays an essential role in cardiovascular homeostasis. The peptide hormone angiotensin II (Ang II), generated by angiotensin converting enzyme (ACE), mainly regulates cardiovascular system via its receptors. More recently, we have reported that Ang-(1-7) stimulates ANP secretion via Mas receptor. However, Ang-(1-9), converted from Ang I by ACE 2, is still unknown. The aim of the present study is to determine whether Ang-(1-9) stimulates ANP secretion using isolated perfused beating atria and to find out its signaling pathway. Ang-(1-9) augmented ANP secretion and concentration. Ang-(1-9) induced-ANP secretion was increased from 5% to 60% by 3 uM at low-stretch atrial condition. This stimulatory effect of Ang-(1-9) on ANP secretion was attenuated by the pretreatment with Ang II type 2 receptor (AT2R) antagonist but not by AT1R or Mas receptor antagonist. In addition, pretreatment with inhibitors of phosphatidylinositol 3 kinase (PI3K), protein kinase B (Akt), endothelial nitric oxide synthase (eNOS) or guanylyl cyclase (GC) blocked the attenuation of ANP secretion by Ang-(1-9). However, Ang-(1-9) did not influence atrial contractility and ECF translocation. In high-stretch atrial condition, Ang-(1-9)-induced ANP secretion was accentuated more than in low-stretch atrial condition by 3 uM (from 137% to 219%). In vivo study, acute infusion of Ang-(1-9) increased plasma ANP level without blood pressure change and this stimulatory effect is also attenuated by AT2R antagonist, not by Mas receptor antagonist. These results suggest that Ang-(1-9) stimulates ANP secretion via AT2R, PI3K, Akt, eNOs, GC pathway. We try to do further study to find out the signaling mechanism involved these responses. This work was supported by the National Research Foundation (2012-0009322) and the ministry of Science & technology (MoST)/Korea Science & Engineering foundation (KOSEF) (2010-0021808).
**Key Words:** Renin-angiotensin system, Atrial natriuretic peptide, Angiotensin-(1-9), AT2 receptor

## SY-5

### Sustained Hypertension causes Pancreatic-Cell Dysfunction Partly due to Oxidative Stress

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It is well-known that cardiovascular disorders and diabetes mellitus are closely related. However, there are a few reports showing direct evidence that hypertension can induce diabetes or related disorders. The purpose of this study is to evaluate whether sustained hypertensive rats may have diabetes mellitus or related disorders and to define its mechanisms. Two-kidney, one-clip hypertensive (2K1C) rats were used 6 month after surgery. Oral glucose tolerance test (OGTT) was impaired in 2K1C rats compared to age-matched sham rats, but fasting blood glucose level was not different between both groups. Number of pancreatic islets and size of pancreatic $\beta$-cells compared with cells were markedly decreased in 2K1C rats and basal insulin level in plasma was decreased. The response of insulin secretion to high glucose in isolated islect cells from 2K1C rats was decreased compared to sham rats. Advanced glycation end products (AGE) and 8-hydroxydeoxyguanosine (8-OHdG) increased, and oxygen radical antioxidant capacity (ORAC) tended to decrease in pancreatic tissue of 2K1C rats. At 3 months after surgery, water, captopril (100 mg/kg) or $\alpha$-lipoic acid (100 mg/kg) was feed orally for 3 months. Treatment with captopril or $\alpha$-lipoic acid improved OGTT with an increased plasma insulin level. Immunohistochemistry showed that decreased number of pancreatic islets and size of $\beta$-cells were improved in 2K1C rat fed with captopril or $\alpha$-lipoic

acid, as compared to sham rats. In addition, the levels of AGE, 8-OHdG, and ORAC in pancreatic tissue of 2K1C rats fed with captopril or $\alpha$-lipoic acid returned to control sham rats. Therefore, we suggest that sustained hypertension may cause $\beta$-cell dysfunction due to oxidative stress followed by diabetic condition. This work was supported by This work was supported by the National Research Foundation (2012-0009322) and the ministry of Science & technology (MoST)/Korea Science & Engineering foundation (KOSEF) (2010-0021808).
**Key Words:** Hypertension, Diabetes, Oxidative stress, $\beta$-cell, Advanced glycation end products

## SY-6

### Effects of FK506 on Long-Term Potentiation Observed by Optical Imaging in Organotypic Hippocampal Slice

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Electrophysiological and biochemical assays were used to observe long-term changes in synaptic efficacy following electrical and/or pharmacological manipulation of synaptic function. We investigated the effects of FK506 on synaptic plasticity in organotypic hippocampal slice culture (OHSC) using optical imaging system. Hippocampal slices were prepared from 6-8 day rats using a tissue chopper and placed on a membrane insert. 18 hr after KA (kainic acid) treatment, significant delayed neuronal death which was quantified by PI staining was detected in CA3 and CA1 regions at 24 hr after KA treatment. The neuronal death was significantly prevented at 24 hr after 0.1 $\mu$M FK506 treatment. In electrophysiology study, optical signals were observed by long-term potentiation (LTP) stimulation of the Schaffer collateral pathway. The improvement in amount of LTP was found in FK506-treated group. These results suggest that FK506 may have a beneficial role in recovery of synaptic efficacy following KA-induced neuronal cell death.
**Key Words:** Long-Term potentiation, Organotypic hippocampal slice culture, FK506, Optical imaging, Synaptic plasticity

## SY-7

### Beneficial Effect of Gastrodia Elata Ethanol Extract on High-Fructose Diet-Induced Metabolic Syndrome

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Metabolic syndrome including insulin resistance, dyslipidemia and hypertension is a cluster of risk factor of cardiovascular disease. Gastrodia elata Blume, a widely used traditional herbal medicine, was reported with anti-inflammatory and anti-diabetes activity. Aim in the present study was to investigate that ethanol extract of Gastrodia elata Blume (EGB) ameliorate metabolic signs of high-fructose (HF) diet induced metabolic syndrome. Rats were fed either the 65% HF diet with/without EGB 100 mg/kg/day for 8 weeks. Treatment with EGB was significantly suppressed the increments of epididymal fat weight, blood pressure, plasma triglyceride, leptin and total cholesterol levels, respectively. EGB treatment also elevated high-density lipoprotein (HDL) cholesterol and oral glucose tolerance. Staining with hematoxlin-eosin and oil-red-o showed that marked increase of adipocyte size and hepatic accumulation of triglycerides, and these increases were prevented by EGB. EGB ameliorated endothelial dysfunction by down-regulation of endothelin-1 (ET-1) and adhension molecules in the aorta. In addition, EGB suppressed mRNA expression of the cytokines (TNF-$\alpha$, IL-6 and MCP-1) related with hepatic proinflammation. Moreover, EGB induced markedly up-regulation of phosphorylation AMPK$\alpha$ in the liver, muscle and fat. These results indicate that EGB ameliorates obesity, insulin resistance, dyslipidemia, hypertension and fatty liver in HF diet rats. Take together, Gastrodia elata Blume may be a beneficial therapeutic approach for metabolic syndrome.
**Key Words:** Gastrodia elata Blume, Metabolic syndrome, Hypertension, AMPK, High-fructose

## SY-8

### Effect of Poria Cocos on Puromycin Aminoucleoside-Induced Nephrotic Syndrome in Rats

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Nephrotic syndrome is associated with altered renal handling of water and sodium along with changes of aquaporins (AQP) and epithelial Na channel (ENaC). The dried sclerotia of Poria cocos Wolf (WPC) has been used for the treatment of chronic edema and nephrosis. This study was conducted to evaluate the effects of WPC on puromycin aminonucleoside (PAN)-induced renal functional derangement and the change of renal AQP2 and ENaC expression. The nephrotic syndrome rat models were constructed by PAN 75 mg/kg injection and then were treated with losartan (30 mg/kg/day) or WPC (200 mg/kg/day) for 7 days. WPC group was significantly improved the proteinuria and ascites. Plasma levels of triglyceride, total cholesterol, and low density lipoprotein (LDL)-cholesterol were significantly decreased in WPC group. In addition, WPC group attenuated the PAN-induced increase in protein and mRNA levels of AQP2 and ENaC subunit. WPC significantly suppressed PAN-induced organic osmolytes regulator such as serum- and glucocorticoid-inducible protein

kinase (Sgk1), and sodium-myo-inositol cotransporter (SMIT) mRNA expression. Taken together, WPC improves nephrotic syndrome including proteinuria and ascites through inhibition of AQP2 and ENaC expression. Thus, WPC is involved in the body-fluid regulation via inhibition of water and sodium channels against renal disorder such as edema or nephrosis.
**Key Words:** WPC, PAN, Ascites, ENaC, AQP2

## SY-9

### Building Statistical Criteria for Eye Movement Behaviour

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Vestibule-ocular reflex (VOR) and optokinetic response (OKR) is well-known eye movement for stabilizing image on retina. Since vestibular and visual stimulation can trigger VOR and OKR respectively, we built up the system with turn table for vestibular input and drum for visual input to record these two reflective behaviours. In former studies, three different protocols are applied to measure basal eye movement performance of mice. Considering each protocols are showing the velocity dependency, we took the data with four different velocities. The stimulation is given by sinusoidal curve and output is also produced with sinusoidal curve. This data can be analyzed two big categories. One is 'Gain' that dividing amplitude of reflex by amplitude of given stimulation and the other one is 'Phase' that phase difference between reflex curve and stimulation curve. We recorded more than fifty wild type mice (C57-BL/6) four times of same protocols in each mouse model in different days. From this big data set, we set up two statistical criteria to detect outlier mouse. One is mean of each model's gain and phase. It is possible that some mice can have extraordinary performance, but considering that protocols after measuring baseline, those mice should be sorted out. The other one we have concerned was standard deviation (SD). Since each model has different basal performance, it is hard to analyze with raw gain and phase data. Therefore, we calculated SD of each protocol from each model's daily record because SD represents the data's reliability. We set the criteria with z-scores. For the first 'Mean Filter', we applied quite strict threshold. As z-score that below 1.96 which represents 97.5% of probability, we made the threshold based on this value. Each z-score of protocol which over 1.96 z-score and between 1.96 and 1.5 were set as 1 point and 0.5 point, respectively and then we separated the model that has total point more than 2. After this selection, we sorted rest of models with 'SD Filter'. Usually, each model has none or one over 1.96 of z-score and some of them has more than two. We decided to take models with none or one over 1.96 z-score and therefore, thirty six out of fifty one wild type mice were selected. The 'Mean Filter' and 'SD Filter' were regenerated based on the selected mice and we applied this filter to another mouse line (pcp2-cre, C57BL/6 based). As we expected, the filters separated some abnormal mice and we firmly assure that these filter work properly.

*These authors contributed equally to this work
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**Key Words:** Behaviour, VOR, Eye movement

## SY-10

### Endogenous ACh Tonically Stimulates ANP Secretion in Rat Atria

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Exogenous ACh is known to stimulate ANP secretion concomitantly with a decrease in atrial dynamics. However, the role of intrinsic acetylcholine in the regulation of ANP secretion remains unknown. Recently, it was shown that non-neuronal as well as neuronal ACh is present in the cardiac atria. Machinery for the ACh synthesis, store, and release were identified in the rat atrial cardiomyocytes. From these findings we hypothesize that endogenously released ACh is involved in the regulation of ANP secretion in an autocrine or paracrine manner in the atria. Experiments were performed in isolated beating rat atria. ANP was measured using radioimmunoassay. To increase the availability of the ACh in the extracellular space of the atrium, its degradation was inhibited with an inhibitor of acetylcholinesterase. Acetylcholinesterase inhibition with physostigmine increased ANP secretion concomitantly with a decrease in atrial dynamics in a concentration- dependent manner. Inhibitors of $M_2$ muscarinic cholinergic receptor, methoctramine, and $K^+_{ACh}$ channels, tertiapin-Q, abolished the physostigmine-induced changes. The effects were not observed in the atria from rats treated with pertussis toxin. Furthermore, the physostigmine-induced effects were attenuated by an inhibitor of high-affinity choline transporter, hemicholinium-3, which is a rate-limiting step of ACh synthesis. Inhibitors of mAChR signaling pathway and ACh synthesis also attenuated the basal levels of ANP secretion and accentuated atrial dynamics. These findings suggest that endogenously released ACh tonically stimulates ANP secretion from atrial cardiomyocytes via activation of $M_2$ mAChR-Gi/o-$K^+_{ACh}$channel signaling. It is also suggested that the ACh-ANP signaling is implicatedin cardiac physiology and pathophysiology.
**Key Words:** Atrial natriuretic peptide muscarinic acetylcholine receptor hemicholinium-3 $K^+_{ACh}$ channel pertussis toxin

## SY-11

### Oryeongsan Suppressed Diabetes-Associated Renal Fibrosis in Cultured Rat Mesangial Cells

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The pathological change of diabetic nephropathy is represented kidney hypertrophy, inflammation, and renal fibrosis. Oryeongsan, the traditional oriental herbal formula, is widely used for the treatment of nephrosis, dropsy, and uremia. Thus, this study examined whether Oryeongsan (1-50 $\mu$g/ml) attenuate high-glucose (HG)-promoted rat mesangial cell proliferation and matrix accumulation, major features of diabetic glomerulosclerosis. As results, thymidine incorporation under HG was significantly accelerated, which was inhibited by Oryeongsan in a dose dependent manner. Pre-treatment of Oryeongsan induced down-regulation of cyclins/CDKs and up-regulation of CDK inhibitor, p21waf1/cip1 and p27kip1 expression. In addition, HG enhanced expression of fibrosis biomarkers such as collagen IV and connective tissue growth factor (CTGF), which was markedly attenuated by Oryeongsan. Oryeongsan increased HG-inhibited membrane type-1 matrix metalloproteinase expression (MT1-MMP) and MMP-2 promotor activity, whereas suppressed HG-induced tissue inhibitor of matrix metalloproteinase-2 (TIMP-2) expression. Moreover, oryeongsan promoted extracellular matrix degradation through disturbing transforming growth factor $\beta$ (TGF-$\beta$)-Smad signaling. This study further revealed that Oryeongsan ameliorated HG-induced mesangial inflammation accompanying induction of intracellular cell adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1). Moreover, pretreatment of Oryeongsan inhibited NF-$\kappa$B translocation in HG-exposed mesangial cells. Taken together, these results demonstrate that Oryeongsan has protective effect against renal proliferation, fibrosis, and inflammation. Therefore Oryeongsan may be specific therapies targeting renal dysfunction leading to diabetic nephropathy.

**Key Words:** Oryeongsan, Mesangial cell, High glucose (HG), Collagen IV, TGF-$\beta$1/Smad

## SY-12

### Effect of Methanol Extract of Berberis Amurensis Rupr on Penile Erectile Function

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The aim of the present study is to investigate whether a methanol extract of *Berberis amurensis* Rupr (BAR) augments penile erection. Preconstracted with phenylephrine (PE) in isolated endothelium-intact rabbit corpus cavernosum, BAR relaxed penile smooth muscle in a dose-dependent manner, which was inhibited by pretreatment with $N^G$-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, and 1H-[1,2,4]-oxadiazole- [4,3-$\alpha$]-quinoxalin-1-one (ODQ), a soluble guanylyl cyclase (sGC) inhibitor. BAR-induced relaxation was significantly attenuated by pretreatment with tetraethylammonium (TEA), a nonselective $K^+$ channel blocker for $BK_{Ca}$, $IK_{Ca}$ and $SK_{Ca}$, and charybdotoxin, iberiotoxin, apamin, a selective $Ca^{2+}$ sensitive $K^+$ channel inhibitor, respectively. BAR increased cGMP levels of the corpus cavernosum in a concentration-dependent manner. In addition, BAR caused increase of peak intracavernous pressure (ICP), ICP/MAP ratio and area under the carve (AUC) in a dose dependent manner in SD rats. Taken together, these results suggest that BAR augments penile erection via NO/cGMP system and $Ca^{2+}$ sensitive-$K^+$ Channel in corpus cavernosum.

**Key Words:** *Berberis amurensis* Rupr, NO, cGMP, Corpus cavernosum

## SY-13

### Sigma-1 Receptor Mediates Intracellular Calcium Level of Cultured Astrocyte in Rats and Contributes to Mechanical and Thermal Hypersensitivity in Mice

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Recent studies have indicated that the spinal sigma-1 receptor (Sig-1 R) plays a critical role in the acute and chronic pain. However, the distinct role of Sig-1 R in astrocyte, another critical pain modulator, has not been clearly elucidated. We designed this study to determine the role of Sig-1 R in astrocyte function. Primary astrocyte cultures were prepared from 1 day old newborn Sprague-Dawley rat cerebral cortices. Double immunocytochemistry revealed that the Sig-1 R was co-localized with glial fibrillary acidic protein (GFAP, an astrocyte marker)-positive cells. Treatment with a Sig-1 R agonist, PRE-084 dose dependently (0.1, 1, 10, and 20 $\mu$M) increased intracellular calcium concentration. This increased intracellular calcium level was blocked by the pretreatment of selective Sig-1 R antagonist, BD-1047 (10 $\mu$M). We have treated ethylene glycol tetraacetic acid (EGTA, 1.5 mM), extracellular calcium chelator, and antagonized Sig-1 R agonist-induced effect. Also, we have treated neurosteroid known to endogenous ligand of Sig-1 R. Blockade of Sig-1 R using the progesterone (1 $\mu$M) abolishes DHEA (1 $\mu$M, Sig-1 R agonist)-induced effect. Activation of spinal Sig-1 R increased the co-localized expression of GFAP with Sig-1 R. Collectively, our results suggested that the Sig-1 R of astrocyte may have a potential to modulate cell signaling pathway via the mediation of intracellular calcium level and mechanical and thermal hypersensitivity.

**Key Words:** Sigma-1 receptor, Astrocyte, Calcium, Intrathecal injection

## SY-14

### An Increase of $PIP_3$ by ROS is Involved in the Induction of Mechanical Hyperalgesia in a Rat Model of Peripheral Neuropathy

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Reactive oxygen species (ROS) in the spinal cord, which plays a crucial role in central sensitization, has been implicated in neuropathic pain. Phosphatidylinositol (3,4,5)-triphosphate ($PIP_3$), which functions as downstream effector for cell survival and proliferation, also plays a vital role in strengthening of synaptic efficacy and perhaps in central sensitization. Although ROS has known to be linked to cell survival through PI3-kinase pathway, it is uncertain whether such a linkage plays a pivot role in neuropathic states. In the present study, we investigate whether $PIP_3$ expression was regulated by ROS in the neuropathic state. Mechanical hyperalgesia of hind paw, evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was induced using naive rats either by L5 spinal nerve ligation (SNL) or by intrathecal (i.t.) injection of ROS donor tertiary-butyl hydroperoxide (t-BOOH) at L5 spinal cord. $PIP_3$ expression was assayed by ELISA and visualized by immunohistochemistry following nerve ligation or t-BOOH injection. The activity of phosphoinositide 3'-phosphatase known as PTEN (phosphatase and tensin homolog) that regulates $PIP_3$ level was assayed by IP-western blotting. Either L5 SNL or i.t. t-BOOH in naive rats induced mechanical hyperalgesia. Pretreatment with ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN) prevented the early development of L5 SNL-induced mechanical hyperalgesia. In both SNL and t-BOOH injection groups, $PIP_3$ expression in the lumbar spinal cord was upregulated in the early phase following nerve ligation and t-BOOH injection. The level of oxidized inactive form of PTEN was elevated, which may lead to $PIP_3$ accumulation, in both SNL and t-BOOH injection groups. The results indicate that upregulation of spinal $PIP_3$ expression is involved in L5 SNL-induced mechanical hyperalgesia that depends on inactivation of PTEN through the action of ROS. The research was supported by a grant from Stem Cell Research Center (SC-4140).

**Key Words:** Reactive oxygen species (ROS), $PIP_3$, Neuropathic pain

## SY-15

### Effect of Opioid and Adrenergic Antagonists on Electroacupuncture-Induced Antinociception in Mice with Paclitaxel-Evoked Neuropathic Pain

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This study was designed to determine the antinociceptive effect and related neuronal mechanism of electroacupuncture (EA) on the paclitaxel-induced neuropathic pain in mice. Paclitaxel (4 mg/kg, i.p.) was administered once a day for 5 consecutive days. For EA stimulation, mice were anesthetized by Zoletil® (1.2 mg/kg) and Rompun® (0.24 mg/kg) to prevent restraint stress and all behavioral measurements were performed after the recovery from anesthesia. Repeated EA stimulation (1 mA, 2 Hz) performed into Zusanli (ST36) acupoint bilaterally for 30 min significantly attenuated paclitaxel-induced mechanical allodynia and thermal hyperalgesia. In a separate set of experiment, intrathecal administration of naloxone, prazosin, idazoxan or propranolol was used to determine the EA's antinociceptive effect on paclitaxel neuropathic pain. Results of this study revealed that opioidergic, $\alpha 2$ and $\beta$ adrenoceptors are important for EA's effect. Moreover, EA stimulation remarkably suppressed the paclitaxel-enhanced NR2B phosphorylation of spinal dorsal horn. In conclusion, EA stimulation into Zusanli acupoint significantly diminished paclitaxel-induced neuropathic pain in mice via the activation of spinal opioid, $\alpha 2$ and $\beta$ adrenoceptors.

**Key Words:** Electroacupuncture, Opioid, Adrenergic, Paclitaxel, NR2B

## SY-16

### Sigma-1 Receptor Contributes to Induction of Below-Level Mechanical Allodynia after Spinal Cord Injury via iNOS Activation

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Previous studies have demonstrated that activation of the spinal sigma-1 receptors (Sig-1Rs) induce pain hypersensitivity via nitric oxide (NO) signaling pathway and contribute to peripheral nerve injury-induced chronic neuropathic pain. The present study determined whether spinal Sig-1Rs modulate astrocyte activation and inducible nitric oxide synthase (iNOS)/NO signaling, leading to the spinal cord injury (SCI)-induced below-level chronic neuropathic pain. SCI was performed by transverse hemisection of the right thoracic spinal cord between T11-12 vertebral segments in mice. SCI-induced mechanical allodynia and thermal hyperalgesia were evaluated to examine the effect of treatment with the Sig-1R antagonist, BD1047, during the induction phase. Immunohistochemistry and western blotting were performed to determine potential SCI-induced changes in Sig-1R expression, astrocyte activation, and iNOS expression in lumbar spinal cord dorsal horn.

Sig-1R expression was increased bilaterally with specific localization in astrocyte following SCI, which peaked after 1 week and then declined. SCI induced robust and persistent below-level mechanical allodynia and thermal hyperalgesia in both hindpaws, which developed after 1 week and was maintained after 4 weeks. Intraperitoneal administration with BD1047 dose-dependently attenuated mechanical allodynia, but not thermal hyperalgesia, and significantly reduced the bilateral SCI-induced increase in GFAP and iNOS expression. Furthermore, intrathecal administration with the iNOS inhibitor, L-NIL, dose-dependently reversed SCI-induced below-level mechanical allodynia in both hindpaws, but not thermal hyperalgesia. These findings demonstrate that spinal Sig-1Rs are increasingly expressed in astrocyte during the induction phase following SCI and modulate not only astrocyte activation but also iNOS expression in lumbar spinal cord dorsal horn, and ultimately contribute to the SCI-induced induction of below-level chronic mechanical allodynia.
**Key Words:** Astrocyte activation, Below-level neuropathic pain, iNOS, Sigma-1 receptor, Spinal cord injury

## SY-17

### Enhanced Responses of DRG Neurons to Serotonin Induce Chronic Pruritus in a Rat Model of Atopic Dermatitis

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Atopic dermatitis (AD) is a chronic inflammatory skin disease accompanied with serious chronic pruritus, a major diagnostic criterion and a hallmark of the disease. Pruritus-induced scratching deteriorates the disease and thus relieving pruritus has been accepted as an optimal management of AD. Nevertheless, pathophysiological mechanisms underlying chronic pruritus of AD remain unclear. In the present study, we tried to identify pruritus-evoking mediators and their pathogenesis, using a novel rat model of AD which was produced by neonatal capsaicin treatment (J Dermatol Sci, 2012;67(2):111-9). First, we tried to examine which substances are able to evoke pruritus in naïve rats. Some candidate substances (histamine, chloroquine, serotonin, SLIGRL-NH2, compound 48/80) were injected intradermally into the rostral back skin. Histamine and chloroquine did not cause any responses at all, whereas serotonin induced excessive scratching behavior that was prevented by pretreatment of 5-HT2 receptor antagonists, such as ketanserin and SB206553. In addition, rats showed considerable responses to SLIGRL-NH2, a PAR2 agonist, and compound 48/80, a substance leading to degranulation of mast cells. In the AD rats whose dermal mast cells were observed to be highly activated, the DRG cells responsive to serotonin were remarkably reduced, but these remaining cells exhibited significantly larger response to serotonin, but not histamine. Consistently, serotonin evoked enhanced scratching behaviors in the adult AD rats, unlike histamine. Further, spontaneous scratching in the AD rats was markedly relieved by a single injection of 5-HT2 receptor antagonists and chronic treatment of ketotifen, a mast cell stabilizer. These results indicate that enhanced response of DRG neurons to serotonin released from activated dermal mast cells mediates persistent pruritus through 5-HT2 receptor in the AD rats.
**Key Words:** Itch, Serotonin, Mast cell, Atopic dermatitis, 5-HT2R

## SY-18

### Liquiritigenin, a Licorice Flavonoid, Ameliorates TNBS-Induced Colitis in Mice

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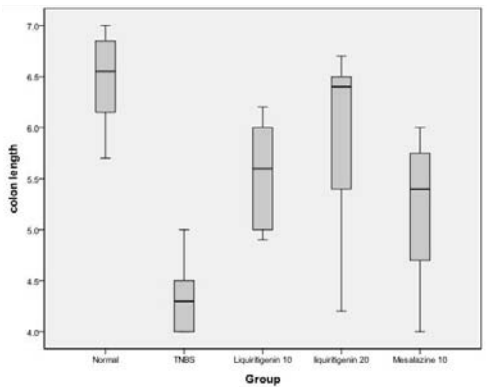
**Objective:** Glycyrrhizae radix (G. radix, licorice, liquorice), the root of the glycyrrhiza plant species, is popularly used for life-enhancing properties and the treatment of injury or swelling as well as for detoxification in traditional oriental herbal medicine. These extracts of G. radix contain flavonoids and the pentacyclic triterpene saponin as major constituents. A major flavonoid isolated from G. radix is liquiritin, which is glycosidic form of liquritigenin. liquritigenin is a metabolite of liquiritin and is known to be actually absorbed from colon. In terms of wide applications of licorice and its therapeutic potential, result of many study demonstrate the important pharmacology of liquiritigenin, one of major active components in extracts of G. radix, and offer the possibility of its therapeutic application for inflammatory bowel diseases. **Material and method:** Male ICR mice were randomly divided into five groups: Normal and TNBS-induced colitic groups, colitis treated with Liquiritigenin 10 mg/kg and 20 mg/kg respectively or sulfasalazine 10 mg/kg. TNBS colitis induction was performed except normal group and treated with Liquirutugenin, & sulfasalazine except active control group (TNBS-induced colitic groups). After treatment for 3days, treatment effect is measured by body weight, colon length, Macroscopic score, pro-inflammatory cytokines (IL1-$\beta$,IL-6, TNF-$\alpha$, IL-10) and histological score. **Results:** As compared to active control group (TNBS-induced colitic groups), Liquiritigenin treatment group showed inhibition of colon shortening and myeloperoxidase activity. Inhibition of the expression of proinflammatory cytokines,TNF-$\alpha$, IL-1$\beta$, and IL-6 were also seen in Liquiritigenin treatment group.
**Key Words:** Liquiritigenin, TNBS colitis, Licorice, Liquorice




## SY-19(PO-10)

### Reduced Synaptic Nitric Oxide Function Contributes to Neuronal Excitation of Medullary-projecting PVN Neurons in Rats with Myocardial Infarction

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

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

## SY-20

### Repetitive Treatment with Diluted Bee Venom Relieves Mechanical Allodynia and Restores Intraepidermal Nerve Fiber Loss in Oxaliplatin-Induced Neuropathic Mice

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The chemotherapeutic agent, oxaliplatin produces a robust painful neuropathy similar to various other neuropathic conditions, which result in loss of intraepidermal nerve fibers (IENFs). Our previous study reported that a single injection of diluted bee venom (DBV) produced an anti-allodynic effect in oxaliplatin-induced neuropathic mice. In this study, we were to investigate more potent effects of repetitive DBV treatment on mechanical allodynia and its potential effect in oxaliplatin-induced loss of IENFs. DBV (0.1 mg/kg, s.c.) was administered once a day for 18 days beginning on day 15 after oxaliplatin injection. The time-course change of mechanical paw withdrawal threshold was examined, and immunohistochemistry using the pan-neuronal marker protein gene product 9.5 was performed on glabrous skin of the hind-paw foot pad to stain for IENFs. We observed a significant increase of mechanical threshold at 60 min after single DBV injection, and this maximal effect was gradually enhanced by repetitive DBV treatments. Interestingly, the basal mechanical threshold prior to daily DBV injection was also increased steadily, and then peaked at day 14 after DBV injection. In addition, the oxaliplatin-induced decrease of IENFs was significantly restored in repetitive DBV-treated mice as compared to that in saline-treated group. We subsequently examined whether these long-term effects of DBV were associated with the activation of alpha-2 adrenoceptors. Repetitive pretreatment with yohimbine (5 mg/kg, s.c.), an alpha-2 adrenoceptor antagonist, completely prevented both the anti-allodynic effects and the increase of IENFs shown in repetitive DBV-treated mice. Collectively, these findings demonstrate that repetitive DBV treatment potently relieves mechanical allodynia and restores the loss of IENFs in oxaliplatin-induced neuropathic mice, and that these effects of DBV are closely linked to the activation of alpha-2 adrenoceptors.

**Key Words:** Bee venom, Oxaliplatin, Mechanical allodynia, Intra-epidermal nerve fibers, Alpha-2 adrenoceptor

## SY-21

### The Role of TLR2 in Alcohol-Related Behaviors

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Alcoholism is one of the most prevalent substance abuse disorders and represents major health problems. Toll-like receptors(TLRs) play an important role in the innate immune response. Recent research suggests that TLR may play a role in brain injury and neurodegeneration. In additions, alcohol activates brain NF-κB transcription of proinflammatory cytokines, TLR and other genes amplifying NF-κB transcription and innate immune response. Therefore, alcohol consumption could induces brain damage and lead to neurodegeneration. Our study was designed to evaluate whether TLR2 cause alcohol-induced neurodegeneration and alcohol-related behavior. We studied the role of TLR2 in alcohol-related behaviors using TLR2 knockout (KO) mice. TLR2 KO mice consumed more alcohol than wild-type (WT/C57BL6) mice in a two-bottle free-choice test. After two-bottle test, TLR2 KO mice showed less anxiety-related behaviors as determined by testing on the light-dark box and open-field. Impairment in reward mechanisms in TLR2 KO mice was confirmed by the lack of alcohol-evoked conditioned place preference. In conclusion, our results suggest that TLR2 could be a potential target to modulate alcohol-related behavior and alcohol-induced neurodegeneration.

**Key Words:** Alcoholism, Toll-like receptor, Neurodegeneration, Behavior

## SY-22

### CaMKII Contributes to NR2B Ser1303 Phosphorylation in the Early Phase of Neuropathic Pain after Nerve Injury

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NR2B, one of the NMDAR subunit, plays a key role in neuroplasticity. Previous our data clearly proved that NR2B Ser1303 is phosphorylated for a long time after nerve injury. Thus, this experiment is to investigate which kinases phosphorylate NR2B ser1303 after peripheral nerve injury. Therefore, we evaluated that expression of CaMKII and PKC, interaction between ser1303 and CaMKII or PKC, and whether these kinases are involved in paw withdrawal threshold to mechanical stimuli using inhibitors after nerve injury or not. We used L5 SNL model in Sprague-Dawley rats. Ipsilateral side of L5 spinal cord dorsal horn was obtained time after injury to analyze protein expression. PE10 was inserted into subarachnoid space to administrate inhibitors. CaMKII inhibitor (AIP; 10 and 50 nmol) or PKC inhibitor (CHE; 10 and 50 nmol) were intrathecally injected divided into early phase (1-4 days) and late phase (7-21 days) after injury. Paw withdrawal thresholds were assessed with series of von Frey filaments using up-down method. Co-immunoprecipitation (co-ip) was performed to analyze the interaction between ser1303 and CaMKII or PKC. Ser1303 phosphorylation was increased from early phase to late phase after injury. Auto-phosphorylated site of CaMKII (Thr286) was phorphorylated only within 24 hours after injury, and total CaMKII expression was not changed. On the other hand, PKC expression was little increased 7 days to 14 days. The interaction between Ser1303 and CaMKII was greatly increased 6 hours to 4 days, and the interaction between Ser1303 and PKC was little appeared 7 days to 14 days after injury. Interestingly, increasing range of paw withdrawal threshold was larger when 10 and 50 nmol of AIP was intrathecally injected in early phase than in late phase at same rat. Also, paw withdrawal threshold was increased by 50 and 100 nmol of CHE in late phase rather than in early phase at same rat, however, little side effect was appeared. Ro 25-6981 (NR2B antagonist) and AIP did reduce the interaction between ser1303 and CaMKII, but CHE did not change the interaction between ser1303 and CaMKII. Thus, CaMKII may contribute to early phase of Ser1303 phosphorylation and PKC may partially involve to late phase of Ser1303 phosphorylation after nerve injury that contribute to central sensitization.

**Key Words:** NR2B, Neuropathic pain, Ser1303, CaMKII, Spinal nerve ligation

## SY-23

### Blockage of Microglial Interleukin-1β Lead to Development of Carrageenan Induced Mirror-Image Pain via Astrocyte Activation in the Rats

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Mirror-image pain (MIP) is an abnormal phenomenon in which damage on one side of the body results in pain from contralateral unaffected area. However, it is not clear which mechanism is involved in development of MIP. Interaction between astrocyte and microglia play an important role for regulating nociceptive processing in the spinal cord. Interleukin-1β (IL-1β) in spinal cord, which is well known to be increased in peripheral inflammation, is mainly released from activated microglia. Also, IL-1β is known to modulate activity of neighboring astrocytes. The present study was designed to examine whether microglial IL-1β regulates astrocyte activation and development of contralateral mechanical allodynia (CMA). After 2% carrageenan was injected into hind-paw of rats, CMA was evaluated at each time point using von Frey filament. Immunohistochemistry and western blot assay were used to determine the changes of GFAP (marker for astrocyte), Iba-1 (marker for microglia), and IL-1β expression in the spinal cord. CMA was developed day 5 after carrageenan injection in intrathecally (i.t.) vehicle-treated control rats. Furthermore, the GFAP expression was increased with the similar temporal pattern to that of CMA. In contrast, the expression of Iba-1 and IL-1β was immediately increased

after carrageenan injection. Moreover, IL-1$\beta$ was co-localized with Iba-1-positive microglia. Interestingly, i.t. pretreatment of minocycline, a selective inhibitor of microglial activation, or interleukin-1 receptor antagonist (IL-1ra) in day 0-3 resulted in advanced induction time of CMA by dose dependent manner. Moreover, minocycline or IL-1ra pretreatment up-regulated GFAP expression at day 3 as compared to expression of GFAP in vehicle-treated rats. These results demonstrated that blockage of spinal IL-1$\beta$ from activated microglia lead to the astrocyte activation and development of CMA in peripheral inflammatory pain model, suggesting that microglial IL-1$\beta$ plays an important role in the regulation of induction time of MIP.
**Key Words:** Mirror-image pain, Astrocyte, Microglia, Interleukin-1$\beta$

## SY-24

### Peripheral P2Y1 Receptors Contribute to the Induction of Thermal Hyperalgesia via Modulation of p38 MAPK Phosphorylation and TRPV1 Expression in Carrageenan-Induced Inflammatory Pain Rats

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Although previous reports have suggested that P2Y1 receptors (P2Y1Rs) are involved in cutaneous nociceptive signaling, it remains unclear how P2Y1Rs contribute to peripheral sensitization during the pathophysiological state. The current study was designed to delineate the role of peripheral P2Y1Rs in pain behavior and to investigate potential linkages to mitogen-activated protein kinase (MAPK) in DRGs and Transient Receptor Potential Vanilloid 1 (TRPV1) expression in a rodent inflammatory pain model. Following injection of 2 % carrageenan into the hind paw, expressions of P2Y1 and TRPV1 and the phosphorylation rates of both p38 MAPK and ERK were increased and peaked at day 2 post-injection. Blockade of peripheral P2Y1Rs by the P2Y1R antagonist, MRS2500 injection (i.pl, D0 to D2) significantly reduced the induction of thermal hyperalgesia (TH), but not mechanical allodynia (MA). Simultaneously, MRS2500 injections also suppressed upregulated TRPV1 expression and DRG p38 phosphorylation, while pERK signaling was not affected on day 2. To identify the role of p38 MAPK in DRG on inflammatory pain, we inhibited p38 activation in the DRGs by an i.t injection of SB203580 (a p38 inhibitor). As a result, SB-203580 reversed the established TH, but not MA. Furthermore, repeated injection of SB203580 (i.t, D0 to D2) prevented the upregulation of TRPV1. These data demonstrate a sequential role for P2Y1R, p38 MAPK and TRPV1 in inflammation-induced TH; thus, peripheral P2Y-1Rs activation modulates p38 MAPK signaling and TRPV1 expression, which ultimately leads to the induction of TH.
**Key Words:** P2Y1, TRPV1, p38 MAPK, Thermal hyperalgesia, Inflammatory pain

## SY-25

### Sig-1R Dependent Astrocyte Activation through p38MAPK Phosphorylation Leads to the Development of Mechanical Allodynia in CCI Mice

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Spinal astrocytes have emerged as important mechanistic contributors to the genesis of mechanical allodynia in nerve-injury induced neuropathic pain. We have recently demonstrated that spinal Sigma-1 receptor (Sig-1R) modulation of p38 MAPK phosphorylation (p-p38 MAPK) plays a critical role in the induction of mechanical allodynia (MA), but not thermal hyperalgesia (TH) in chronic constriction injury (CCI) rats. However, the precise role of Sig-1R on the development of MA, particularly in relation to astrocyte activation, has not been examined. The present study was designed to investigate: (1) the precise location of Sig-1R and p-p38 MAPK in spinal dorsal horn using specific antibodies; (2) whether the CCI-induced astrocyte activation could be modulated by Sig-1R activation related with p-p38 MAPK. The expression of Sig-1Rs was significantly increased in the ipsilateral spinal cord dorsal horn on day 3 after CCI surgery. Double immunofluorescence studies showed that Sig-1R co-localized with GFAP, a specific marker of astrocyte, in CCI mice. Sustained i.t treatment with BD1047, Sig-1R antagonist, during the induction phase attenuated increased the number of GFAP-immunoreactive (ir) astrocytes in dorsal horn induced by CCI. Moreover, i.t injection of fluorocitrate (Fc), astrocyte metabolic inhibitor, in combination with BD1047 also reduced CCI-induced development of MA, but not TH. In addition, i.t treatment with SB203580 during the induction phase also attenuated CCI-induced astrocyte activation. Double staining studies with specific p38 MAPK antibodies showed a number of p-p38 MAPK was in the astrocytes and i.t treatment with BD1047attenuated increased the p-p38 MAPK located in astrocytes. Collectively these findings demonstrate that the spinal Sig-1R signaling to p38 MAPK phosphorylation induces astrocyte activation, which may contribute to MA development after CCI. These results suggest a potential therapeutic role for Sig-1R antagonists in the clinical management of MA associated with neuropathic pain.
**Key Words:** Sigma 1 receptor, Astrocyte activation, p38 MAPK phosphorylation, Mechanical allodynia, Chronic constriction injury

## SY-26

### The Effects of Caffeine Ingestion before Passive Heating on Serum Leptin Level

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We assessed the effects of ingesting caffeine before passive heating on serum leptin and sweating responses, which are both physiological responses associated with energy expenditure. The subjects were nine male university students (age, 24.1±3.5 years; height, 173.4±7.6 cm; weight, 69.2±5.7 kg; maximal oxygen consumption, 48.6±4.7 ml・kg$^{-1}$・min$^{-1}$). This study used a within-subject, random, crossover design. Tests were performed twice at the same time (2-5 p.m.) at a 1-week interval following 3 mg・kg-1 caffeine ingestion or not. Passive heating included a half bath in hot water (42±0.5°C for 30 minutes) in a thermoneutral climate chamber (25±0.5°C, 60±3% relative humidity, ＜1 m/second air velocity). Leptin, free fatty acids, waist size, mean whole body output volume and mean active sweat gland density increased significantly after a single passive heating session ($p < 0.05$). Leptin and all other parameters were significantly higher than those after a single passive heating session and caffeine ingestion before passive heating ($p < 0.05$). A single passive heating session not only increased leptin secretion, lipolysis, and the sweating response but also increased energy expenditure via changes in sympathetic nerve activity. The results suggest that ingesting caffeine before passive heating is more energy efficient than that of a single passive heating session.

**Key Words:** Caffeine, Passive heat loading, Leptin, Sympathetic nerve, Energy expenditure

## SY-27(PO-11)

### Seasonal Acclimatization to the Summer in the Republic of Korea Suppresses Sweating Sensitivity

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The main objective of this study was to determine the central mechanisms involved in suppression of thermal sweating after seasonal acclimatization (SA) during passive heating (immersing the legs in 43°C hot water for 30 min). Testing was performed in July (before-SA) and August (after-SA) [25.2±2.2ºC, 73.9±10.3% relative humidity (RH), Cheonan (Chungnam,126º 52'N, 33.38'E), in the Republic of Korea. All experiments were carried out in an automated climatic chamber (25.0±0.5°C and RH 60.0±3.00%). Twelve healthy men participated. The local sweat onset time was delayed in the after-SA compared to that in the before-SA ($p < 0.001$). The local sweat rate and whole body sweat loss volume decreased in the after-SA compared to those in the before-SA ($p < 0.001$). In addition, evaporative loss volume decreased significantly in the after-SA compared to that in the before-SA [chest, upper-back, thigh and forearm ($p < 0.001$)]. Changes in tympanic temperature and mean body temperature were significantly lower ($p < 0.05$) and the basal metabolic rate decreased significantly in the after-SA compared to those in the before-SA ($p < 0.001$). These results suggest that maintenance of a lower body temperature and basal metabolic rate can occur and blunt the central sudomotor mechanisms following seasonal acclimatization, which suppresses sweating sensitivity.

**Key Words:** Seasonal acclimatization, Passive heating, Central sudomotor, Sweating sensitivity

## SY-28

### Anti-Platelet and AntI-Thrombotic Agents from Marine Algae

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An exaggerated platelet aggregation response at sites of atherosclerotic plaque rupture can lead to the development of vascular occlusion, precipitating diseases such as the acute peripheral artery disease, acute coronary syndromes, and ischemic stroke. Although anti-platelet drugs (APD) have been widely used in the treatment of atherothrombotic diseases, these agents are associated with significantly high risks including fatal or nonfatal bleeding, neutropenia, agranulocytosis, recurrent ischemic events, and thrombocytopenia. In the present study, thus, we screened inhibitors of platelet aggregation from marine natural products. Sargahydroquinoic acid (SHQA) and sargaquinoic acid (SQA) isolated from Sargassum yezoense strongly inhibit platelet aggregation induced by collagen, adenosine-diphosphate, and thrombin with SQA being more potent than SHQA. To determine the anti-platelet aggregation activity of SHQA and SAQ, rat platelets were pre-incubated with SQA, SHQA, and aspirin as a positive control, and then exposed to collagen (2 $\mu$g/ml) to induce a platelet aggregation. SQA and SHQA inhibited the collagen-induced platelet aggregation in a dose-dependent manner. In addition, the inhibitory potency of SQA is much stronger than the aspirin-mediated collagen-induced platelet aggregation, whereas SHQA has similar effect to the aspirin. To evaluate in vivo anti-thrombotic effect of SQA and SHQA, pulmonary thromboembolism model experiment induced by intravenous injection of collagen and epinephrine was performed. The death or paralysis effect of collagen-induced pulmonary thrombosis was known to be caused massive occlusion of the pulmonary microcir-

culation via platelet thromboembolism. SQA/SHQA and aspirin (5 mg/Kg) as a positive control were intravenously administrated in tail vein to ICR mouse, and after 10 min, a mixture solution of collagen (8 mg/Kg) and epinephrine (160 μg/Kg) was injected into the tail vein to induce pulmonary thrombosis, which result in mouse paralysis over 40 min or death. In the present study, antithrombotic effect was evaluated to the recovery time (RT) from muscle paralysis. The RT of SQA, SHQA, and aspirin were 6.8±6.2, 14.7±7.2, and 10.3±5.2 min (n=10), respectively, and showed fast recovery compared to the control (54.3±8.3 min, n=10). These results suggested that the SQA and SHQA may be using the treatment or prevention of atherothrombotic diseases caused by platelet-mediated pathogenic thrombi. Also, SQA and SHQA will be promising lead structure of an anti-platelet agent.

**Key Words:** Antiplatelet, Antithrombosis, Thromboembolism, Sargaquinoic acid, Aspirin

## SY-29

### Prediction Equation of $VO_2$ Peak from Anthropometric and Simple Fitness Measurement in Paraplegic Men

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Peak oxygen uptake ($VO_2$ peak) has been described as an important parameter of aerobic performance. Although graded exercise testing (GXT) with direct laboratory measurement provides the most widely used and accurate $VO_2$ peak assessment, since equipment, expense, time and personal requirements make the laboratory procedure prohibitive for large populations, prediction equation for $VO_2$ peak have been developed for disabled persons such as the individuals with spinal cord injury. Thus, if there is a easy methods to assess $VO_2$ peak of such individuals, it may be useful for the prescription by a safe and effortless assessment. The aim of the present study was to provide a predictive peak oxygen uptake ($VO_2$ peak) equation in paraplegic men using anthropometric and simple fitness test. Thirty-three paraplegic men (mean age=44.9±8.7 years) with a traumatic cord lesion (T1-L2) performed anthropometric measurement (body surface area; BSA), fitness tests (handgrip strength, biceps muscle endurance, shoulder flexibility), and GXT until volitional exhaustion using arm-ergometer (crank) and breath-by-breath gas analyzer. After warming-up for 3 minutes with freeload, GXT started with an initial workload of 30 watts, with an increment of 15 watts per 2 minutes, and pedaling cadence controlled at 60 rpm. The $VO_2$ peak was negatively correlated with age (r=-0.374*) and BSA (r=-0.418*), whereas positively correlated with muscle strength (r=0.585**), muscle endurance (r=0.399*) and flexibility (r=0.407*). In a multivariate regression analysis, 64.8% of $VO_2$ peak variance ($r^2$=0.648) was explained by age, BSA, muscle strength, muscle endurance and flexibility. Prediction equation for $VO_2$ peak = 35.081 - 0.23×age - 13.227×BSA + 0.205 × muscle strength (adjusted with body weight) + 0.171 × muscle endurance + 0.129 × flexibility We suggest that $VO_2$ peak in wheelchair-dependent individuals was predictable using the equation of the present study and the described accessible and convenient measurements. *p＜0.05, *p＜0.01. **Acknowledgement:** This research was supported by the R&D grant of rehabilitation services by Korea National Rehabilitation Center Research Institute, Ministry of Health & Welfare.

**Key Words:** Paraplegic men, Prediction equation of $VO_2$ peak, Simple fitness measurement

## SY-30

### Modulation of Neuronal Activities in the Subthalamic Nucleus of Hemiparkinsonian Rats by Noisy Galvanic Vestibular Stimulation

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Several clinical trials have shown that noisy galvanic vestibular stimulation (noisy GVS) can alleviate symptoms of Parkinson's disease (PD). However, the mechanism of relieving symptoms of PD is unclear. Because PD shows abnormal neuronal activities in subthalamic nucleus (STn) and motor cortex, we investigated whether noisy GVS can modulate their neuronal activities in hemiparkinsonian rat model. To make an animal model of PD, 6-hydroxydopamine (6-OHDA) was injected to the medial forebrain bundle in right hemisphere of Sprague Dawley rats. Three to four weeks after 6-OHDA lesioning, we simultaneously recorded extracellular single-unit activity and local field potential (LFP) of the ipsilesional STn and motor cortex in urethane-anaesthetized rats before and after noisy GVS on bilateral mastoid. Neuronal activities of baseline were measured for 2 minutes before noisy GVS. Noisy GVS was continued for 2 minutes and then a recording was resumed for 2 minutes. Recording the single-unit activity and LFP revealed that noisy GVS leaded to a mild suppression of beta rhythm (13~35 Hz) power of STn and motor cortex considered as abnormal neuronal activities caused by PD as well as the decreased correlation between STn and motor cortex. Furthermore, noisy GVS tended to decrease neuronal activities and irregularity of STn. We propose that modulation of neuronal activities may be a potential mechanism for alleviating symptoms of PD. This study was supported by Korea basic science institute's high field NMR research program grant T3022B.

**Key Words:** Parkinson's disease, Galvanic vestibular stimulation, Subthalamic nucleus

## SY-31

### 18F- FDG PET Imaging of Functional Connection of Central Vestibular System in Rat Brain

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The central vestibular nuclei in the brain stem, a key center for regulation of vestibular function has a massive neural network with higher centers within the brain. So, the activation of the vestibular nuclei can affect activity of higher brain centers. Recent clinical studies demonstrate activation of the vestibular system resulted in a relief of clinical symptoms in central pain, post-stroke hemineglect, and phantom limb illusion. There a little information about functional activation of central vestibular network by stimulation of the vestibular nuclei in rodent brain. The purpose of present study was to evaluate functional connection of central vestibular system in the brain following unilateral activation of the medial vestibular nucleus (MVN) of Sprague-Dalwey rats. Animals received continuous electrical stimulation of unilateral MVN with 200Hz of frequency for 20minutes. After stimulation activity of glucose uptake in the brain were acquired using [F-18] Fluorodeoxyglucose micro PET scanner. Unilateral VS stimulation resulted in a strong activation of ipsilateral anterior pertectal nucleus (APtN) of the midbrain and bilateral subthalamic nuclei. There was also the bilateral activation of the somatosenory cortex and retrosplenial cortex with ipsilateral predominance. These results suggest that activation of the central vestibular system can take part in modulation of somatosensory inputs by alteration of thalamus or APtN activity in rats. This study was supported by Korea basic science institute's high field NMR research program grant T3022B.
**Key Words:** Medial vestibular nucleus, FDG-PET, Cortex, Functional connection, Vestibular neuromodulation

## SY-32(PO-12)

### Genome as an Operating System of Living Cells for Implementing Phenotypes

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Genome of higher eukaryotes is special in that it is not a mere storage of protein templates activated at the needs of proteins but the software as an operating system of living cells by producing proteins on its own initiative for implementing phenotypes. This was demonstrated in this study exploring the genome activity of breast cancer with four sets of breast cancer microarray gene expression data collected by different institutions and methods. A malignant tumor is a well organized system, composed of not only abnormally growing cancer cells but also several kinds of stromal cells, expressing its own phenotypes totally different from its host system regardless of the same genome. It is necessary to focus on that how different states the genome would exhibit to express such phenotypes. The states of a genome should be defined with quantitative measures of the genome activity and one of the most significant measures is the expresion level of mRNA, directly produced by the genome. This study shows that the states of the breast cancer genome can be outlined into a network, shaped as several modules of distinct features. The network modules were scale-free subnetworks that consist of genes known to participate in cell growth, angiogenesis, immune response, extracellular matrix remodeling, and (de) differentiation, respectively. The entropy levels of the network modules substantiate that some modules would be originated from epithelial cells and others would be from stromal cells, such as fibroblasts, macrophages, and lymphocytes. We also found another module isolated from the main network structure, which is assumed to be from a cancer stem cell population. Our results support that cancer cells can modify the microenvironment including stromal cells, utilize the microenvironment for their survival and development, and eventually become an independent organ out of the control of the host system. [This research was supported by the Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (NRF-2013R1A1A2A10013032)].
**Key Words:** Genome, Phenotype, System, Entropy, Cancer

## SY-33

### Involvement of the Hypothalamus in Analgesia Following Electroacupuncture in an Animal Model of Nerve Injury

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Acupuncture and electroacupuncture as complementary medicine have been important therapeutic techniques in oriental medicine. Although they have been widely used to treat acute and chronic pain, underlying mechanisms are unknown. The objective of this study was to investigate the effect of acupuncture and electroacupuncture on the activity of hypothalamic- pituitary-adrenal axis (HPA axis) in an animal model of hypersensitivity such as neuropathic pain induced by peripheral nerve injury. Under pentobarbital anesthesia, the tibial and sural nerves (branches of the sciatic nerve) of adult male Spargue-Dawley rats were tightly ligated and cut. And then, rats were divided into 4 groups; the first group received 1 Hz electrical acupuncture stimulation at Choksamni (ST36), Eumleungcheon (SP9) and the second group was offered 100 Hz electrical acupuncture stimulation at the same sites. The control group received same treatment except that there was no electrical stimulation. The last, naïve group, was not offered any treatment. We assessed mechanical allodynia of the hind paw by using von Frey filament. c-Fos immunohistochemistry in the hypothalamus was performed. The c-Fos gene is known as immediately early gene, i.e., when pain is aroused, c-Fos appears immediately. In behavioral test, acupuncture with electrical stimulation or without it, reduced withdrawal responses but electroacupuncture more

significantly inhibited pain responses. And in immunohistochemistry, c-Fos expression was decreased in electroacupuncture groups as compared with naive group. The difference between group 1 (1 Hz) and group 2 (100 Hz) wasn't seen significantly. The research was supported by a grant from Basic Science Research Program (No. 2005-0049404, 20090076605) through the National Research Foundation funded by the Ministry of Education, Science and Technology.

**Key Words:** Electroacupuncture, HPA axis, Behavior Test, c-Fos, Neuropathic pain

# Exhibition (전시)

| No | 업체명<br>URL | 대표전화 | 주요취급품목 | 기술제휴 회사명 | 부스위치 |
|---|---|---|---|---|---|
| 1 | (주)싸이텍코리아<br>www.scitechkorea.co.kr | 02-986-4413 | Multi Myograph System, Patch Clamp System, Live Cell Imaging System, Electrophysiology Instruments | DMT, MDC, Fluxion Biosciences, ADInstruments, Ltd. | 3 |
| 2 | 에스엔티코퍼레이션 | 02-953-3255 | Fully Automated Patch Clamp System, 3D Piezo Micromanipulator. | Cytocentrics Bioscience GmbH, SensApex Oy | 2 |
| 3 | 상정상사(주)<br>www.sang-chung.co.kr | 02-564-8766 | Multi Organ Bath System, Patch Clamp System, 생리, 약리학, 뇌신경과학용 실험 기자재 등 | HARVARD, WARNER, MED64, PANLAB 등 | 1 |

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