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Gyeongsang National University

Blue Ocean of
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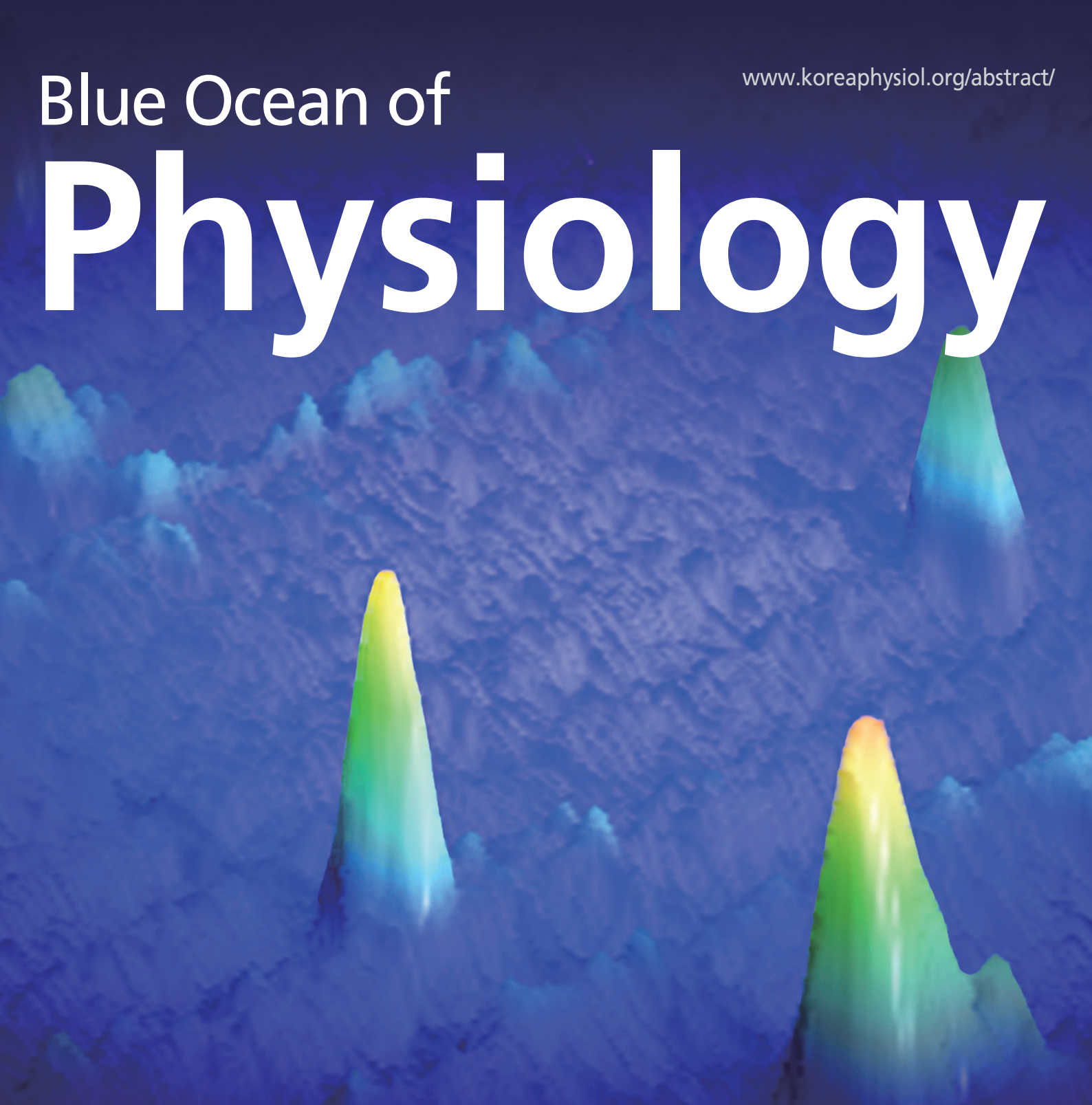

KPS 2014

The 66th Annual Meeting of The Korean Physiological Society

22~24 Oct, 2014
Gyeongsang National University

The Korean Physiological Society
Gyeongsang National University

2014 대한생리학회 임원명단

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

회 장	**서창국**	차기 회장	**박재식**
이 사 장	**나흥식**	차기이사장	**방효원**
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부편집장	**김상정 김성준 안동국 이지희**	학술 위원	**곽효범 강동묵 박규상 배영민 오석배 우선희 임채헌**

이 사	**강동묵 강봉균 강창원 공인덕 권성춘 권혁일 김경년 김동욱 김민선 김보경 김상정 김선희 김성주 김성준 김세훈 김양인 김영미 김용운 김원재 김의용 김재호 김종연 김진혁 김창주 김형찬 나승열 나창수 나흥식 남택상 류판동 박경표 박규상 박명규 박병림 박사훈 박소라 박원균 박재식 박종성 박지호 박진봉 방효원 배영민 배재훈 배혜란 백은주 서덕준 서상원 서석효 서인석 서창국 송대규 신동민 신형철 안덕선 안동국 양훈모 연동수 염재범 염철호 오석배 오우택 우재석 윤신희 윤영욱 이경림 이덕주 이무열 이배환 이상목 이석호 이영만 이영호 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	**염재범 임인자**

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Contents

Invitation (초대의 글)

대한 생리학회 회원 여러분, 안녕하십니까?

유난히 무겁고 더운 공기가 가득했던 지난 시간 속에 건강히 지내셨는지요.

생리학회의 공식학술지인 Korean Journal of Physiology & Pharmacology (KJPP)의 2013년 JCR impact factor가 1.262로 발표되었습니다. 학회지의 이러한 발전은 회원 여러분이 우수한 논문을 투고해주시고 심사를 위해 애써주신 덕분이라고 생각하며 깊은 감사의 인사를 드립니다.

오는 10월 22 (수)～24일 (금)에는 경상대학교에서 제 66회 생리학회 학술대회가 개최됩니다. 기조강연, 다양한 주제의 심포지엄과 포스터 세션을 통해 풍성한 연구 정보들을 새롭게 접하실 수 있습니다. 또한 이번 학술대회에서는 원로 교수님을 모셔서 생리학의 발전사와 그간의 경험을 전해들을 수 있는 소중한 자리를 마련하였습니다. 이제 막 생리학 연구에 발을 들인 대학원생과 신진연구자들에게 뜻 깊은 시간이 될 것으로 생각합니다. 문화와 음악, 교육의 도시 진주에서 열리는 이번 학술대회가 그 동안의 연구 성과를 공유하고 새로운 정보를 교환함으로써 회원 여러분의 친목과 협력을 도모할 수 있는 장이 될 것으로 기대합니다.

학술대회를 준비하기 위해 애쓰신 이사님들, 경상대학교 관계자 여러분을 비롯하여 도움 주신 많은 분께 감사드립니다.

끝으로 회원 여러분들이 첫사랑인 생리학과 함께 건강과 행복하시길 기원합니다.

대한생리학회 회 장 **서 창 국**

대한생리학회 이사장 **나 흥 식**

Schedule (일정표)

Tuesday, October 21

Inje University, Busan

Time	Contents
14:00-20:30	**Satellite Symposium :** Cardiovascular Physiology: Application and Translation **Organizer :** *Nari Kim, Jin Han*
14:00-14:10	Opening Remark
14:10-14:20	Welcome Address
14:20-14:30	Photo Time
14:30-15:50	**Session I :** Cardiovascular Physiology: Application **Chairman :** *Nari Kim*
15:50-16:10	Coffee Break
16:10-17:30	**Session II :** Cardiovascular Physiology: Translation **Chairman :** *Jae Boum Yeom*
17:30-17:50	Open Discussion
17:50-18:00	Closing Remarks
18:20-20:30	Welcome Reception

Wednesday, October 22

Gyeongsang National University, Jinju

Time	Contents
	Hall A, BNIT Room 204
15:00-17:30	**Young Physiologists' Day** **PO-1～PO-12** **Chairs :** *Chae Hun Leem, Young Min Bae*
18:00-18:40	**Special Lecture :** Physiology: Reflections of my Personal Journey Yung E Earm (Seoul National University and Keimyung University, Korea) **Chair :** *Hyoweon Bang*
18:40-20:30	BNIT Lobby (Multiple Purpose, Great Hall)
	Welcome Reception and Poster Presentation

Thursday, October 23

Gyeongsang National University, Jinju

Time	Contents	
08:50-09:00	BNIT, Auditorium (Building # 28)	
	Opening remarks (Hall A, BNIT Room 204)	
09:00-11:00	Hall A, BNIT Room 204	Hall B, BNIT Room 202
	Symposium I: Cardiac Physiology in the Context of Metabolism and Stress **Organizer :** *Zhang Yin Hua, Sun-Hee Woo* **Chair :** *Yung E Earm*	**Symposium II:** Recent Progress in the Physiological Study of Natural Products and Compounds **Organizer :** *Dae-Kyu Song, Young-Ho Jin*

	Chair : *Il-Sung Jang*	
Time	**Contents**	
11:00-11:30	Coffee Break	
11:30-12:30	Hall C, International Language Center, Pioneer Auditorium (Building # 29)	
	Plenary Lecture Donghee Kim (Department of Physiology and Biophysics, Chicago Medical School Rosalind Franklin University of Medicine and Science, USA) **Chair :** *Chang Kook Suh*	
11:00-11:30	Coffee Break	BNIT Room 203
		Advisory Board Meeting
12:30-13:30	**Lunch Time** (Lunch is not provided on this day)	Hall B, BNIT Room 202
		Board of Directors Meeting
13:30-16:00	BNIT Lobby	Hall A, BNIT Room 204
	Poster Presentation	Poster Oral Presentation (14:00–15:00) **PO-13～PO-18** **Chair :** *Tong Mook Kang, Kyu-Sang Park*
16:00-18:00	Hall A, BNIT Room 204	Hall B, BNIT Room 202
	Symposium III: Exercise Physiology Update **Organizer :** *In Deok Kong, Hyo Bum Kwak* **Chair :** *In Deok Kong*	**Symposium IV:** Chronic Abnormal Sensations: Pain & Itch **Organizer :** *Dong-Kuk Ahn* **Chair :** *Jun Kim*
18:30	BNIT, Lobby	
	Dinner	

Friday, October 24

Gyeongsang National University, Jinju

Time	**Contents**	
09:00-11:00	Hall A, BNIT Room 204	Hall B, BNIT Room 202
	Symposium V: Physiological Aspects of Stem Cell Research **Organizer :** *Jae-Ho Kim* **Chair :** *Jae-Ho Kim*	**Symposium VI:** Chemosensation and Ion Channels **Organizer :** *Sun Wook Hwang, Dawon Kang* **Chair :** *Donghee Kim*
11:00-11:15	Coffee Break	
11:15-11:45	**Youdang Scholarship Award Lecture** (Dae-Kyu Song, Keimyung University) **Chair :** *Byung Rim Park*	
11:45-12:30	General Assembly	
12:30-13:30	Lunch Box Provided	
	Photograph and Lunch	
13:30-15:30	Hall A, BNIT Room 204	Hall B, BNIT Room 202
	Symposium VII: Stress & Special Physiology **Organizer :** *Byung-Il Min* **Chair :** *Byung-Il Min, Sun Seek Min*	**Symposium VIII:** Inflammation **Organizer :** *Jihee Lee* **Chair :** *Jihee Lee*
15:30-	Closing remarks	

Venue Guide (학술대회장 안내)

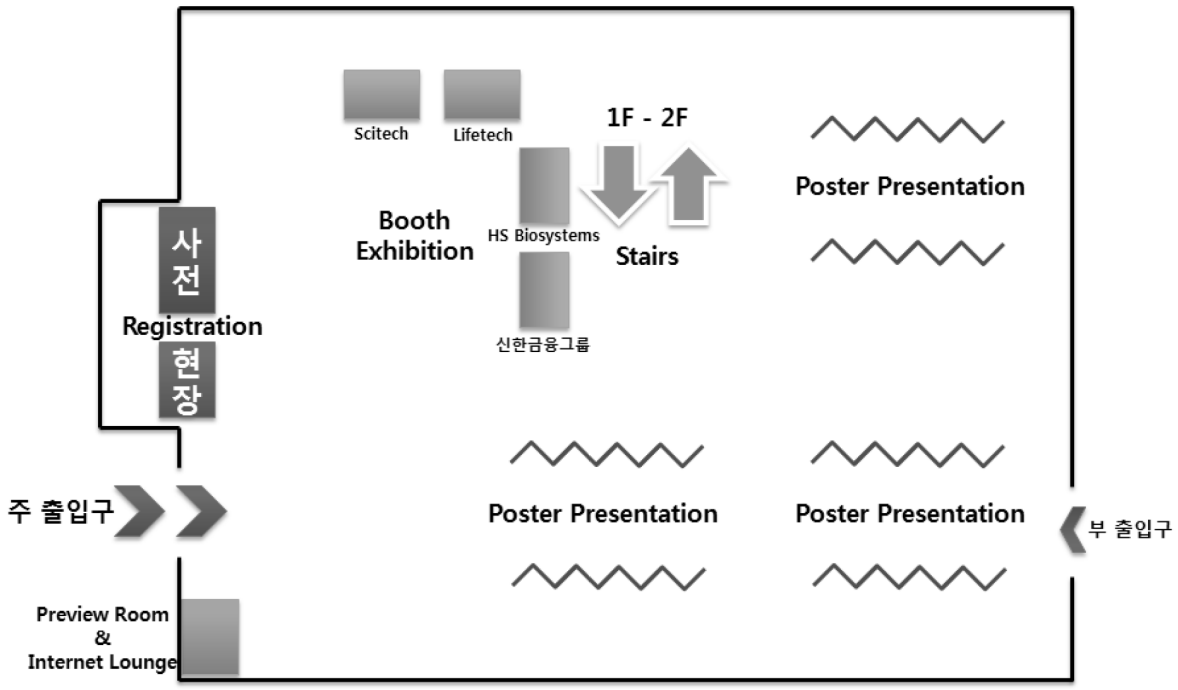
경상대학교 BNIT산학협력관

28동(BNIT 산학협력관) - 22일 ~ 24일 / Hall A, B, 등록데스크, 프리뷰룸, 포스터 전시, 후원 전시

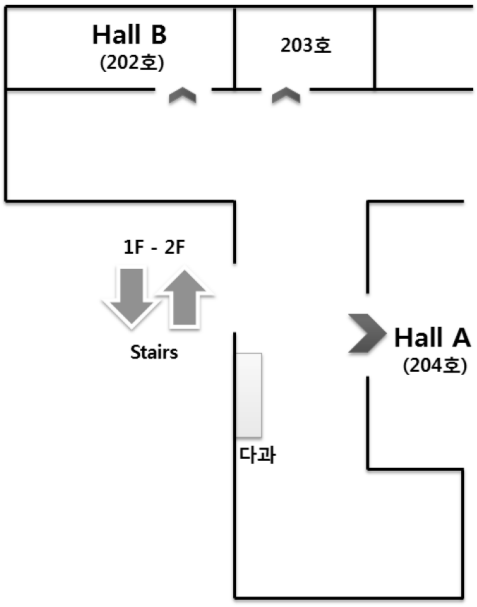
29동(국제어학원) - 23일 / Hall C, Plenary Lecture

3동(학생회관) - 23일 중식가능 장소

BNIT 1F



BNIT 2F



Scientific Program (학술프로그램)

Tuesday, October 21

Inje University, Busan	
14:00-20:30	**Satellite Symposium:** Cardiovascular physiology: Application and Translation **Organizer:** *Nari Kim, Jin Han*
14:30-15:50	**Session I :** Cardiovascular physiology: Application **Chair :** *Nari Kim*
14:30-14:50	▶ Nano-imaging of the cell surface and T-tubules of the atrial cardiomyocytes *Tong Mook Kang (Sungkyunkwan University)*
14:50-15:10	▶ Echinochrome A inhibits phosphorylation of phospholamban Ser16 and Thr17 suppressing cardiac SERCA2A Ca^{2+} reuptake *Hyoung Kyu Kim (Inje University)*
15:10-15:30	▶ Modulation of cardiac calcium signaling by fluid shear force *Sun-Hee Woo (Chungnam National University)*
15:30-15:50	▶ Novel roles of nNOS in cardiac E-C coupling in hypertension *Yin Hua Zhang (Seoul National University)*
16:10-17:30	**Session II:** Cardiovascular physiology: Translation **Chair :** *Jae Boum Youm*
16:10-16:30	▶ The clinical and the angiographic characteristics of the Korean-Chinese nationality and Han nationality with coronary heart disease of Yan Bian area in China *Chun Zi Jin (Yanbian University)*
16:30-16:50	▶ Direct effect of cortisol on the heart *Nari Kim (Inje University)*
16:50-17:10	▶ Hydrogen peroxide as a modulator of arterial function *Young Min Bae (Konkuk University)*
17:10-17:30	▶ Secret life of eNOS at the unexpected second-home; muscular eNOS may set the low resistance of pulmonary artery *Sung-Joon Kim (Seoul National University)*

Wednesday, October 22

BNIT Room 204, Lobby, Gyeongsang National University, Jinju	
15:00-17:30	**Young Physiologists' Day** **PO-1～PO-12**
17:30-18:10	**Special lecture :** Physiology: Reflections of my Personal Journey *Yung E Earm (Seoul National University and Keimyung University, Korea)*
18:10-20:00	Welcome Reception and Poster Presentation

Thursday, October 23

Hall C, International Language Center, Pioneer Auditorium (Building # 29)	
11:30-12:30	**PL:** Ionic mechanisms of chemoreception by the carotid body *Prof. Donghee Kim (Department of Physiology and Biophysics, Chicago Medical School Rosalind Franklin University of Medicine and Science, USA)*
Hall A BNIT Room 204 (Building # 28),	
09:00-11:00	**Symposium I :** Cardiac Physiology in the Context of metabolism and stress **Organizer :** *Yin Hua Zhang, Sun-Hee Woo* **Chair :** *Yung E Earm*
09:00-09:25	▶ Redox-dependent eNOS begets nNOS *Yin Hua Zhang (Seoul National University, China)*
09:25-09:50	▶ Tetrahydrobiopterin enhances metabolic efficiency in type 2 diabetic hearts *Jin Han (Inje University, Korea)*
09:50-10:15	▶ Mitochondrial Ca^{2+} dynamics and metabolism *Chae Hun Leem (Ulsan University, Korea)*
10:15-10:40	▶ Wnt-Fizzled system and arrhythmia *Boyoung Joung (Yonsei University, Korea)*
10:40-11:00	▶ Ion channel remodeling in atrial fibrillation *Tae-Joon Cha (Kosin University, Korea)*
14:00-15:00	**Poster Oral Presentaion** **PO-13～PO-18**
16:00-18:00	**Symposium III :** Exercise physiology update **Organizer :** *In Deok Kong, Hyo Bum Kwak* **Chair :** *In Deok Kong*
16:00-16:20	▶ Physiological adaptations to exercise training *Wook Song (Seoul National University, Korea)*
16:20-16:40	▶ Does interval training improve endothelial function better? *Jung-Jun Park (Pusan National University, Korea)*
16:40-17:00	▶ Effects of aging and exercise training on mitochondrial function and insulin resistance in skeletal muscle *Hyo Bum Kwak (Inha University, Korea)*
17:00-17:20	▶ Effects of exercise training on ER stress *Kyungoh-Doh (Yeungnam University, Korea)*
17:20-17:40	▶ Impacts of Exercise on Type II Diabetic Rats *Sung Ryul Lee (Inje University, Korea)*
17:40-18:00	Q &A
Hall B, BNIT Room 202 (Building # 28)	
09:00-11:00	**Symposium II :** Recent progress in the physiological study of natural products and compounds **Organizer :** *Dae-Kyu Song, Young-Ho Jin* **Chair :** *Il-Sung Jang*
09:00-09:30	▶ Anti-emetic drug screening from herbal phytochemicals *Young-Ho Jin (Kyung Hee University, Korea)*
09:30-10:00	▶ Sleep-promoting effects and GABAergic mechanism of marine polyphenols *Sueng-Mock Cho (Korea Food Research Institute, Korea)*
10:00-10:30	▶ In vivo efficacy of a natural compound on regulation of overweight metabolic stress *Myung-Sook Choi (Kyungpook National University, Korea)*
10:30-11:00	▶ Chemoresistance in ovarian cancer: natural product as a potential modulator *Jung-Hye Choi (Kyung Hee University, Korea)*
16:00-18:00	**Symposium IV :** Chronic abnormal sensations: Pain & Itch **Organizer :** *Dong-Kuk Ahn* **Chair :** *Jun Kim*
16:00-16:30	▶ Chronic itch induced by neonatal capsaicin treatment in rat *Heung-Sik Na (Korea University, Korea)*
16:30-17:00	▶ Inactivation of group I metabotropic glutamate receptor in periaqueductal gray maintains chronic neuropathic pain *Sang-Jeong Kim (Seoul National Univeristy, Korea)*
17:00-17:30	▶ Transitional mechanisms from acute to chronic pain: Can it be prevented? *Dong-Kuk Ahn (Kyungpook National University, Korea)*
17:30-18:00	▶ How to overcome &what to do on neuropathic pain? *Myung-Ha Yoon (Chonnam National University, Korea)*

Friday, October 24

Hall A, BNIT Room 204, Hall B, BNIT Room 202, Gyeongsang National University, Jinju	
Hall A	
09:00-11:00	**Symposium V :** Physiological aspects of stem cell research **Organizer, Chair :** *Jae-Ho Kim*
09:00-09:20	▶ Adult Stem Cell Niche and Its Application for Cardiovascular Regeneration *Sang Mo Kwon (Pusan National University, Korea)*
09:20-09:45	▶ Patient-specific iPS cell research *Dong-Wook Kim (Yonsei University, Korea)*
09:45-10:10	▶ Application of biomolecules for enhancement of stem cells functions *Ho Jae Han (Seoul National University, Korea)*
10:10-10:35	▶ Direct lineage conversion from fibroblasts into Oligodendrocyte progenitor cells *Jeong Beom Kim (UNIST, Korea)*
10:35-11:00	▶ Hematopoietic differentiation from human induced pluripotent stem cells *Seok-Ho Hong (Kangwon National University, Korea)*
13:30-15:30	**Symposium VII :** Physiology of Stress **Organizer :** *Byung-Il Min* **Chair :** *Byung-Il Min, Sun Seek Min*
13:30-14:00	▶ Epigenetic modulation of coping strategy to stress: Role of mGluR5 *Dong Goo Kim (Yonsei University, Korea)*
14:00-14:30	▶ Oxytocin protects hippocampal memory and plasticity from uncontrollable stress *Jung-Soo Han (Konkuk University, Korea)*
14:30-15:00	▶ Adrenal stress hormones, amygdala activation, and memory for emotionally arousing experiences *SangKwan Lee (Wonkwang University, Korea)*
15:00-15:30	▶ Microbiota-Brain-Gut axis: A new frontier in the physiology of mind-body interactions *Nobuyuki. Sudo (Kyushu University, Japan)*
Hall B	
09:00-11:00	**Symposium VI :** Chemosenastion and ion channels **Organizer:** *Sun Wook Hwang, Dawon Kang* **Chair :** *Donghee Kim*
09:00-09:20	▶ Regulation of trigeminal chemosensation by two-pore domain K^+ channels *Dawon Kang (Gyeongsang National University, Korea)*
09:20-09:45	▶ Characterization of Drosophila taste receptors *Seok Jun Moon (Yonsei University, Korea)*
09:45-10:10	▶ Differentiated sensory functions of transmembrane channel-like proteins *Sun Wook Hwang (Korea University, Korea)*
10:10-10:35	▶ Interplay of multiple channels in vomeronasal signaling leading to innate behavior *SangSeong Kim (Hanyang University, Korea)*
10:35-11:00	▶ Various faces of ion channels sensing hypoxia: activation of TRPV, inhibition of TASK-1 and upregulation of TASK-2 *Sung-Joon Kim (Seoul National Univeristy, Korea)*
13:30-15:30	**Symposium VIII :** Inflammation **Organizer, Chair :** *Jihee Lee*
13:30-14:00	▶ Molecular mechanisms of apoptotic cell recognition and clearance: New insights into the roles of small GTPases *In-San Kim (Korea Institute of Science and Technology, Korea)*
14:00-14:30	▶ Arhgef16, a novel Elmo binding protein, promotes engulfment of apoptotic cells through RhoG-dependent Rac activation *Daeho Park (Gwangju Institute of Science and Technology, Korea)*
14:30-15:00	▶ Apoptotic cell clearance: Implication in resolution of lung inflammation and fibrosis *Jihee Lee (Ewha Womans University, Korea)*
15:00-15:30	▶ Resolution in Epithelial Mesenchymal Transition and More *Chang Hoon Lee (Dongguk University, Korea)*

Youn Physiologists'day (October 22)

Hall A

15:00-15:10	▶ PO-1: The Central Governor Model of Maximal Exercise Performance Regulation: Review Article *Seung Bo Park (Chung-Ang University)*
15:10-15:20	▶ PO-2: The possibility of irisin as a biomarker of sarcopenia *Jae Seung Chang (Yonsei University)*
15:20-15:30	▶ PO-3: The effect of substrate and osmolarity on mitochondrial respiration *Eun Seok Park (Ulsan University)*
15:30-15:40	▶ PO-4: Sensitization of cardiac Ca^{2+} release sites by protein kinase C signaling: evidence from action of murrayafoline A *Joon-Chul Kim (Chungnam National University)*
15:40-15:50	▶ PO-5: Plant homeodomain finger protein 2 promotes bone formation by demethylating and activating Runx2 for osteoblast differentiation *Hye-Jin Kim (Seoul National University)*
15:50-16:00	▶ PO-6: Motor cortex stimulation activates the incertothalamic pathway in an animal model of spinal cord injury *Myeounghoon Cha (Yonsei University)*
16:20-16:30	▶ PO-7: Down-regulation of THIK-1 expression by inflammatory mediators in rat dorsal root ganglion neurons *Ji Hyeon Ryu (Gyeongsang National University)*
16:30-16:40	▶ PO-8: Echinochrome A Increases Mitochondrial Mass and Function by Modulating Mitochondrial Biogenesis Regulatory Genes *Jubert Marquez (Inje University)*
16:40-16:50	▶ PO-9: Structural and functional significances of T-tubules in rat atrial cardiomyocytes *Jieun An (Sungkyunkwan University)*
16:50-17:00	▶ PO-10: RalBP1 hypomorphic mice display antidepressant-like behaviors *Sang Ho Yoon (Seoul National University)*
17:00-17:10	▶ PO-11: Glutathionylation and Activation of TRPC5 by Altered Glutathione Homeostasis Lead to Neuronal Damage in Huntington's Disease *Chansik Hong (Seoul National University)*
17:10-17:20	▶ PO-12: Sparfloxacin slows Ca^{2+}-dependent inactivation of L-type Ca^{2+} current, inducing action potential prolongation in ventricular myocytes *Jae Gon Kim (Sungkyunkwan University)*

Poster Oral Presentation (October 23)

Hall A

14:00-14:10	▶ PO-13:Passive heating improved lipolysis and regulation of fibroblast growth factor-21 *Tae Wook Kim (Soonchunhyang University)*
14:10-14:20	▶ PO-14: Gas6/Mer signaling enhances LXR expression and activity in macrophages *Si Yoon Kim (Ewha Womans University)*
14:20-14:30	▶ PO-15: Heteromeric TRPC3 with TRPC1 formed via its ankyrin repeats regulates the resting cytosolic Ca^{2+} levels in skeletal muscle *Keon Jin Lee (Catholic University)*
14:30-14:40	▶ PO-16: Functional organization of glutamatergic synapses in the dopamine neurons of the substantia nigra pars compacta *Miae Jang (Sungkyunkwan University)*
14:40-14:50	▶ PO-17:Heteromeric TRPC3 with TRPC1 formed via its ankyrin repeats regulates the resting cytosolic Ca^{2+} levels in skeletal muscle *Jeong Beom Lee (Soonchunhyang University)*
14:50-15:00	▶ PO-18: Ginger and Its Pungent Constituents Non-Competitively Inhibit Serotonin Currents on Visceral Afferent Neurons *Sojin Kim (Kyung Hee University)*

Satellite Symposium

Youdang Scholarship Award Lecture

Special Lecture

Plenary Lecture

Symposia

Poster Presentation (Poster Oral Presentation)

P01: Diet, Phytochemicals and Stress Physiology

P02: Endocrine and Metabolic Physiology

P03: Exercise and Applied Physiology

P04: Ion Channels and Transporters

P05: Molecular Physiology and Cell Signaling

P05-09(PO-8) (10/22) Echinochrome A Increases Mitochondrial Mass and Function by Modulating Mitochondrial Biogenesis Regulatory Genes ····· S 83
Seung Hun Jeong[1], Jubert Marquez[1], Hyoung Kyu Kim[1], In-Sung Song[1], Su Jin Noh[1], Kyung Soo Ko[1], Byoung Doo Rhee[1], Nari Kim[1], Natalia P. Mishchenko[2], Sergey A. Fedoreyev[2], Valentin A. Stonik[2], Jin Han[1]
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P05-10 (10/22) NecroX-5 suppresses sodium nitroprusside-induced cardiac cell death through inhibition of JNK and caspase-3 activation ····· S 84
Seonghoon Jeong[1], Sung Ryul Lee[1], Seon Joong Lee[2], Soon Ha Kim[3], Kyung Soo Ko[1], Byoung Doo Rhee[1], Zhelong Xu[4], Nari Kim[1], Jin Han[1,*]
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P05-11 (10/22) Echinochrome A protects mitochondrial function in cardiomyocytes against cardiotoxic drugs ····· S 84
Seung Hun Jeong[1,2], Su Jin Noh[2], Hyoung Kyu Kim[1,2], In-Sung Song[1,2], Seon Joong Lee[1,2], Kyung Soo Ko[1,2], Byoung Doo Rhee[1,2], Nari Kim[1,2], Natalia P. Mishchenko[3], Sergey A. Fedoryev[3], Valentin A. Stonik[3], Jin Han[1,2]
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P05-12 (10/22) SB743921, a kinesin spindle protein inhibitor, induces cell death via the mitochondrial pathway in Multiple Myeloma cells ····· S 84
Bayalagmaa Nyamaa, Yu Jeong Jeong, In-Sung Song, Hyoung Kyu Kim, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Department of Health Sciences and Technology, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

P05-13 (10/22) B7-H4 downregulation induces mitochondrial dysfunction and enhances doxorubicin sensitivity via the cAMP/CREB/PGC1-α signaling pathway in HeLa cells ····· S 85
Hyoung Kyu Kim, In-Sung Song, Seung Hun Jeong, Sung Ryul Lee, Hye Jin Heo, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, and Jin Han
National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Department of Health Sciences and Technology, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea

P05-14 (10/23) Defective Mer signaling enhances local and systemic inflammation and down-regulates of LXR transcriptional activity ····· S 85
Ji-Yeon Choi, Young-So Yoon, Ye-Ji Lee, Jihee Lee
Departments of Physiology, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans University, Seoul 158-710, Korea

P06: Muscle, Cardiovascular, and G-I System

P07: Neurophysiology

P08: Physiome and Systems Biology

P09: Renal, Respiratory and Reproductive Physiology

P10: other area

Satellite S-1

Nano-imaging of the cell surface and T-tubules of the atrial cardiomyocytes

Tong Mook Kang

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The T-tubules are invaginations of the cardiac cell membrane that include important ion channels and transporters devoted to the EC-coupling of the cardiomyocytes. In ventricular myocytes, pivotal role of organized T-tubules on EC-coupling has been well established. However, the presence of T-tubules in atrial myocytes, especially small mammalian animals, has been a matter of debate and was traditionally believed to be absent or very sparse. Therefore, the present study aimed to validate the presence of T-tubules in rat atrial myocytes. Scanning ion conductance microscopy (SICM) is a newly developed SPM technique that enables noninvasive nanoscale tophographical imaging of live cell membrane with a probing glass nanopipette. With SICM and confocal imaging techniques, we found that populations of atrial myocytes have appreciable amount of T-tubule network. SICM imaging of rat atrial myocytes showed that the cells have surface T-tubule openings and unique membrane nano-structures. We categorized atrial surface nano-structures into several groups based on the presence of T-tubule openings, and they were compared with the ventricular myocytes. Intracellular T-tubule membranes were visualized by a confocal imaging of di-8-ANEPPS-stained atrial myocytes that reveals degree of intracellular T-tubule network. Development of intracellular T-tubule network was positively correlated with width and volume of the atrial cells. In addition, the structural and functional consequences of the loss of T-tubules were evaluated by formamide-induced detubulaton of atrial myocytes. These results clearly validate that rat atrial myocytes have an appreciable T-tubule system and the loss of T-tubules can alter atrial EC-coupling. This work was supported by a Korea Research Foundation Grant funded by the Korean Government (KRF-2011-0012478).

Key Words: Atrial myocytes, T-tubule, SICM, nano-structure, heart

Satellite S-2

Echinochrome A inhibits phosphorylation of phospholamban Ser16 and Thr17 suppressing cardiac SERCA2A Ca^{2+} reuptake

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Echinochrome A (Ech A), a marine bio-product isolated from sea urchin eggs, is known to have cardioprotective effects through its strong anti-oxidant and ATP-sparing capabilities. However, the effects of Ech A on cardiac excitation-contraction (E-C) are not known. In this study, we investigated the effects of Ech A on cardiac contractility and Ca^{2+} handling in the rat heart. In ex vivo Langendorff hearts, Ech A (3 μM) decreased left ventricular developing pressure to 77.7±6.5% of basal level. In isolated ventricular myocytes, Ech A reduced the fractional cell shortening from 3.4% at baseline to 2.1%. Ech A increased both diastolic and peak systolic intracellular Ca^{2+} ($[Ca^{2+}]i$). However, the ratio of peak [Ca]i to resting [Ca]i was significantly decreased. Ech A did not affect the L-type Ca^{2+} current. Inhibiting the $Na+/Ca^{2+}$ exchanger with either NiCl2 or SEA400 did not affect the Ech A-dependent changes Ca^{2+} handling. Our data demonstrate that treatment with Ech A results in a significant reduction in the phosphorylation of phospholamban at both serine 16 and threonine 17 leading to a significant inhibition of SERCA2A and subsequent reduced Ca^{2+} uptake into the intracellular Ca^{2+} store. Taken together, our data show that Ech A negatively regulates cardiac contractility by inhibiting SERCA2A activity, which leads to a reduction in internal Ca^{2+} stores.

Key Words: Echinochrome A, negative inotropic effect, SERCA2A inhibition, phospholamban phosphorylation

Satellite S-3

Modulation of cardiac calcium signaling by fluid shear force

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Cardiac myocytes are subjected to fluid shear force (FSF) during each contraction and relaxation. Under pathological conditions, such as valve disease, heart failure and hypertension, FSF in cardiac chamber may increase due to high blood volume and pressure. We explored the effects of FSF on local and global Ca^{2+} signaling in rat atrial and ventricular myocytes, and underlying cellular mechanisms. FSF was applied onto entire single myocyte using pressurized fluid puffing. Confocal Ca^{2+} imaging was performed to measure local and global Ca^{2+} signals. FSF of about 16 dyn/cm^2 elicited proarrhythmic global Ca^{2+} waves in atrial myocytes, which also significantly altered Ca^{2+} signaling on depolarization. Proarrhythmic longitudinal Ca^{2+} waves in atrial cells under FSF were resistant to blockers for stretch-activated cation channel (SAC), Na^+-Ca^{2+} exchange or Ca^{2+}-activated cation channel (TRPM4), and to removal of external Ca^{2+}. Interestingly, this FSF-induced atrial Ca^{2+} wave was eliminated by inositol 1,4,5-trisphosphate receptor (IP_3R) blockers, ryanodine, or by phospholipase C (PLC) inhibitor, and were absent in the type 2 IP_3R (IP_3R2) knockout cells. Furthermore, the FSF-induced atrial Ca^{2+} waves were suppressed by blockade of P2 purinergic receptors or by inhibition of gap junction that can secrete ATP, suggesting an autocrine action of secreted ATP during FSF. In ventricular myocytes, the same level of FSF produced only enhancement of Ca^{2+} spark frequency. This effect was also resistant to the blockade of SAC or Ca^{2+} entry, but was only partly suppressed by IP_3R- or PLC-blocker. Our data demonstrate a novel Ca^{2+} regulatory mechanism activated by fluid shear in cardiac myocytes involving PLC-IP_3R2 signaling, and larger contribution of this mechanism in atrial myocytes compared to ventricular myocytes.
Key Words: cardiac myocytes, fluid shear force, Ca^{2+} signaling

Satellite S-4

Novel roles of nNOS in cardiac E-C coupling in hypertension

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Neuronal type of constitutive nitric oxide synthase (nNOS or NOS1) is functionally expressed in the myocardium and is involved in a variety of functions especially cardiac inotropy and lusitropy. nNOS exerts these effects by regulating ion channels, intracellular Ca^{2+} homeostasis and signaling pathways (protein kinases/protein phosphatases or oxidase-dependent reactive oxygen species downstream signaling). Recently, we have shown that nNOS is up-regulated in the myocardium from angiotensin II-induced hypertensive rats. Mechanistically, research from our laboratory indicates that the downstream targets of nNOS are shifted during the disease progression (Zhang et al., Journal of Physiology, 2014). Here, I will present novel molecular targets of nNOS in the myocardium of hypertension.

Satellite S-5

The clinical and the angiographic characteristics of the Korean-Chinese nationality and Han nationality with coronary heart disease of Yanbian area in China

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Objective: To compare the clinical and the angiographic characteristics of the Korean-Chinese and Han nationality with coronary heart disease (CHD) in Yanbian area in China. **Methods:** The risk factors and distribution of culprit vessels of coronary artery were retrospectively analyzed in 753 cases of CHD. The levels of plasma nitric oxide (NO) and the protein of endothelial nitric oxide synthase (eNOS) were measured by Elisakit (Cusabio). **Results:** Alcohol consumption was significantly higher in Korean-Chinese than in Han patients. No difference was observed in other risk factors between twogroups. The numbers of stenotic coronary arteries were significantly higher in Korean-Chinese patients. Plasma NO and eNOS were significantly lower in Korean-Chinese patients. **Conclusion:** There are significant differences in the angiographic characteristics and the levels of plasma NO and eNOS between Korean-Chinese and Han patients with CHD in Yanbian area in China. Reduced eNOS may be responsible for increased stenotic coronary arteries in Korean-Chinese CHD patients compared to Han in Yanbian area in China. Mechanistic link between alcohol consumption and eNOS/NO deficiency or angiographic abnormality needs further investigation.

Satellite S-6

Direct effect of cortisol on the heart

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Cortisol is essential steroid hormone for many biological processes including of homeostasis, development, metabolism, and cognition as well as anti-inflammatory and immunosuppressive actions. However, the direct and rapid effect of cortisol on cardiac function is still unknown. To get an insight into understand the complex behavior of cortisol in the heart, we examined the rapid actions of cortisol on cardiac function using ex vivo Langendorff system study and phosphoproetomic anslysis. Cortisol (10 μM) significantly decreased coronary flow, diastole duration and left ventricular developing pressure (LVDP). These resulted from the alteration of phosphos status that cortisol increased phosphorylation of protein kinase C (PKC). We found that the decrease in LVDP was restored in the presence of PKC inhibitor. The activation of PKC by cortisol could phosphorylate both the Ser23 and Tyr10 sites of sodium/potassium ATPase (Na/K ATPase), which increases its activity. When applied with ouabain (10 μM), cortisol suppressed ouabain-mediated increase in cytosolic calcium level. In addition, cortisol also could suppress the ouabain-induced increase in LVDP on ex vivo hearts. Taken together, cortisol at excessive high dose could rapidly influence contractility through the activation of PKC and Na/K ATPase, which suggests to the suppression of calcium handling in rat heart.

Key Words: cortisol, cardiac contractility, PKC, Na/K

Satellite S-7

Hydrogen peroxide as a modulator of arterial function

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Hydrogen peroxide (H2O2) is an endothelium-derived hyperpolarizing factor. Since opposing vasoactive effects have been reported for H2O2 depending on the vascular bed and experimental conditions, this study was performed to assess whether H2O2 acts as a vasodilator in the rat mesenteric artery and if so, to determine the underlying mechanisms. H2O2 elicited concentration-dependent relaxation in mesenteric arteries precontracted with norepinephrine. The vasodilatory effect of H2O2 was reversed by treatment with dithiothreitol. H2O2-elicited vasodilation was significantly reduced by blocking 4-aminopyridine (4-AP)-sensitive Kv channels, but it was resistant to blockers of big-conductance Ca2+-activated K+ channels and inward-rectifier K+ channels. A patch-clamp study in mesenteric arterial smooth muscle cells (MASMCs) showed that H2O2 increased Kv currents in a concentration-dependent manner. H2O2 speeded up Kv channel activation and shifted steady-state activation to hyperpolarizing potentials. Similar channel activation was seen with oxidized glutathione (GSSG). The H2O2-mediated channel activation was prevented by glutathione reductase. Consistent with S-glutathionlyation, streptavidin pull-down assays with biotinylated glutathione ethyl ester showed incorporation of glutathione (GSH) in the Kv channel proteins in the presence of H2O2. Interestingly, conditions of increased oxidative stress within MASMCs impaired the capacity of H2O2 to stimulate Kv channels. Not only was the H2O2 stimulatory effect much weaker, but the inhibitory effect of H2O2 was unmasked. These data suggest that H2O2 activates 4-AP-sensitive Kv channels, possibly through S-glutathionylation, which elicits smooth muscle relaxation in rat mesenteric arteries. Furthermore, our results support the idea that the basal redox status of MASMCs determines the response of Kv currents to H2O2. Supplementary mechanics data from hypertensive rat that are supporting this basal redox status hypothesis would be discussed.

Key Words: H2O2, Kv channel, mesenteric artery, S-glutathionylation, oxidative stress, hypertension

Satellite S-8

Secret life of eNOS at the unexpected second-home; muscular eNOS may set the low resistance of pulmonary artery

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The critical role of endothelial type NO synthase (eNOS/NOS3) expressed in vascular endothelium has been well known as vasorelaxing molecule released under variety of stimuli including shear stress. In the conventional model, $[Ca^{2+}]i$ increase in endothelial cells actvate eNOS, activating guanylate cyclase in smooth muscle; the vascular smooth muscle is a passively regulated by endothelial NO. However, recent reports provide evidences that arterial smooth muscle cells (ASMCs) also express NO synthases including eNOS. Since the activation of eNOS is triggered by $[Ca^{2+}]i$, such expression in ASMCs would mitigate the contractile signals. The apparently contradictory effects might play a role in balancing excessive contraction or provide an additional signaling target for boosting contractile responses, i.e. via inhibition of eNOS in ASMCs. In this presentation, we show our recent results from isometric tension studies using skeletal and pulmonary arteries from rats. We found that the eNOS expressed in ASMCs actually participate the determination of arterial tone under specific conditions. In the skeletal artery, alpha-adrenergic contraction is attenuated by eNOS in ASMCs while hypoxia alleviate the eNOS activity, significantly augment the adrenergic contractions. In the pulmonary artery, mechanical stretch combined with vasoactive agonist stimulate eNOS in ASMCs, preventing their excessive contraction. Such a safe-break like influence from myogenic eNOS might play a role in setting the low resistance of pulmonary circulation and prevent pulmonary hypertension.

Key Words: nitric oxide, eNOS, smooth muscle, pulmonary artery, stretch

Youdang Scholarship

Functions in energy metabolism of Epac2A as a cAMP target

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The second messenger cyclic AMP (cAMP) is an essential signaling molecule for regulated exocytosis in response to activation of G-protein coupled receptors (GPCRs). The stimulatory G protein (Gs) activates adenylate cyclase, thus generating cAMP. In mammals, the majorities of cAMP functions are mediated by cAMP-dependent protein kinase (PKA) and exchange proteins directly activated by cAMP (Epacs). Epac bound to cAMP can change GDP to GTP on small G proteins. Two isoforms of Epac, namely Epac1 and Epac2 have been identified so far. In particular, Epac1 mRNA is expressed ubiquitously, whereas Epac2 mRNA is predominantly expressed in the brain and endocrine tissues. The three splicing variants of Epac2 (A, B, C) have been further demonstrated. Recently, we found that orexin A, a hypothalamic neuropeptide, augmented glucose-stimulated insulin secretion in pancreatic beta cells through the beta-cell GPCR orexin receptor 1 (OXR1) and Epac2A-dependent mechanism. This finding is well consistent with glucose intolerance of the narcoleptic patients who have low expression levels of hypothalamic orexin-secreting neurons and low blood orexin levels. Glucagon-like peptide-1 (GLP-1) also induces the amplification of insulin secretion in response to high glucose via its beta-cell GPCR and Epac2A. Therefore, Epac2A is critical for beta-cell insulin secretory function amplified by some important endogenous hormones. In addition, it was found that whole-body Epac1 deficiency in mice alleviated body weight gain and insulin resistance in response to high calorie diet. We further explored the physiological functions of Epac2A by using Epac2A-deficient mice. Weight gain, insulin sensitivity, metabolic rate, and liver lipid content were compared between Epac2A-deficient and wild-type mice (WT). In high-fat diet, Epac2A-deficient mice exhibited greater body weight and white adipose tissue mass, had reduced energy expenditure, and displayed premature onset insulin resistance and leptin resistance. Interestingly, the obese tendency and insulin resistance of Epac2A-deficient mice might be due to the defects of meals-induced atrial natriuretic peptide secretion. We reviewed the characteristics of the Epac2A knock-out mice in energy metabolism.

Key Words: Diabetes, Epac2A, Obesity, Orexin, Atrial natriuretic peptide

SL

Physiology: Reflections of my Personal Journey

Yung E Earm

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In this presentation I would like to share 45 years of my personal experience. Initially, my research focus was on exercise physiology. Then I studied acid-base balance during my graduate courses which was interrupted by military service for 3 years and 3 months. After the military duties, electrophysiology fascinated me most. No facilities were available in physiology department of SNU, or anywhere else in Korea. However, I could manage to record intracellular action potential from frog atrium. Since then most of my research were focused on ion channel activities of cardiovascular system until my retirement in 2009. From late 1990's I introduced Physiome Project of IUPS, which provided hints to systemic approach of Physiology. Consequently, I was involved in launching Korean Physiome Society. Also, I am continuing with editorial activities in physiology journals. The aim of this talk is not just about my past activities but also on the importance of integrative approaches in physiology (I believe physiology is integrative by definition). Hopefully, the new horizon can be opened up with physiology flourishing again. My perspectives can be summarized as follows; Physiology should be pursued through an explicit search for a proper biological observable, present at the right level of organization. Hence, a profound re-thinking of biological paradigm is now underway and it is likely that such a process will lead to a 'conceptual revolution' emerging "from the ashes of reductionism". Much of the logic of living systems is located at the higher levels, it is imperative to focus on those general principles.

Key Words: Korea, Physiology, History, Perspective

PL

Ionic mechanisms of chemoreception by the carotid body

Donghee Kim

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A decrease in arterial O2 pressure (pO_2) elicits a peripheral chemosensory reflex that increases ventilation and sympathetic nerve activity. This reflex is initiated at the carotid body glomus (Type 1) cells where the reduced pO_2 alters the activities of various ion channels and causes cell depolarization. The depolarization opens the voltage-dependent Ca^{2+} channel, leading to elevation of intracellular [Ca^{2+}] ($[Ca^{2+}]_i$) and increased transmitter secretion from glomus cells. The transmitters such as acetylcholine and ATP then alter the carotid sinus afferent nerve activity and the respiratory brainstem neurons to adjust ventilation and help restore arterial pO_2. This general sequence of events is widely accepted by researchers studying carotid body chemosensory reflex mechanisms. However, the cellular and molecular details of how O_2 is sensed and what signals modulate various ion channels within the carotid body are not well defined. I present and discuss the latest view on this subject.

Key Words: carotid body glomus cell, intracellular Ca^{2+}, ion channel, oxygen sensing,

CURRICULUM VITAE

Name: Donghee Kim

Education and Training:

1974-1978	B.S. Massachusetts Institute of Technology Cambridge, MA. Major: Chemistry and Biochemistry
1978-1982	Ph.D. Michigan State University. East Lansing, MI. Pharmacology and Toxicology
1982-1984	Research Fellow in Medicine (cardiovascular), Harvard Medical School and Brigham and Women's Hospital

Academic Appointments:

1984-1985	Instructor in Medicine, Harvard Medical School
1985-1988	Assistant Professor of Medicine, Department of Medicine, Harvard Medical School
1987-1989	Research fellow, Mayo Clinic (Pharmacology: Dr. David E. Clapham)
1989-1998	Associate Professor of Physiology and Biophysics, Chicago Medical School
1996-1997	Visiting Professor, Osaka University (Pharmacology: Dr. Y. Kurachi)
1998-present	Professor, Chicago Medical School, Rosalind Franklin University

Awards:

1978	National Chemistry Honor Society
1982-1984	NIH Postdoctoral Fellowship Award
1987-1988	New Investigator Research Award (NIH)
1988-1993	First Award (NIH)
1990-1995	Established Investigator Award of AHA
1993	Board of Trustees Award for Research (Chicago Med)
2001	Morris Parker Award for Excellence in Basic Research

Memberships:
American Heart Association (1982-1992),
Biophysical Society (1989-present)
Society of Neuroscience (1990-present)
Experimental biology (1990-2008)

Contribution to GenBank (First cloning of K+ channels)
TASK1 K+ channel subunit
TASK3 K+ channel subunit
TREK2 K+ channel subunit
TASK5 K+ channel subunit)
TALK-1b-d spliced variants

Publications
98 peer-reviewed papers and 8 book chapters

S-I-1

Redox-dependent eNOS begets nNOS

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Endothelial and neuronal nitric oxide synthases (eNOS & nNOS) are constitutively expressed in distinct subcellular locations within cardiomyocytes and exert diverse functions. In particular, nNOS protein expression and activity are significantly increased whereas eNOS protein expression is reduced in diseased heart or in the myocardium under stress. nNOS, in turn, protects the myocardium from pathologic stimuli and functional deterioration. Importantly, the work from our work have shown that nNOS exerts its functions by shifting towards more efficient downstream targets in the myocardium under stress (Zhang et al., Journal of Physiology, 2014). So far, mechanism of nNOS up-regulation upon pathogenic stimuli is not fully understood. Recently, we have shown that a pathogenic peptide angiotensin II (Ang II) increases nNOS protein expression in rat left ventricular (LV) myocytes in vitro. The primary effects of Ang II are to activate NADPH oxidase via type 1 Ang II receptor (AT1R) and, to less extent, eNOS via type 2 Ang II receptor (AT2R). Therefore, we hypothesized that Ang II-dependent redox status induces cardiac protective nNOS via crosstalk between AT1R and AT2R. Combining immunoblotting, surface biotinylation and immunocytochemistry techniques, we provide novel evidences to demonstrate that Ang II up-regulates nNOS protein expression via AT1R/NADPH oxidase/ROS dependent-membrane translocation of AT2R. eNOS links AT1R regulation of AT2R membrane expression and activity. Our results suggest a novel crosstalk between eNOS and nNOS following interplay between AT1R and AT2R in the myocardium under pathogenic stimuli.

Key Words: nNOS, eNOS, Angiotensin II, cardiac myocyte, ROS

S-I-2

Tetrahydrobiopterin enhances metabolic efficiency in type 2 diabetic hearts

Hyoung Kyu Kim, Tae Hee Ko, In Sung Song, Sung Hun Jeong, Hae Jin Heo, Sung Ryul Lee, Vu Thi Thu, Nari Kim, Kyung Soo Ko, Byoung Doo Rhee, Jin Han

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Diabetic cardiomyopathy is the major cause of mortality and morbidity in diabetes mellitus patients. Mitochondrial dysfunctions in type 2 diabetes play significant role in the development of diabetic cardiomyopathy. Tetrahydrobiopterin (BH4) is a multifunctional co-factor having potential to regulate mitochondrial function including its biogenesis and oxidative phosphorylation. The aim of this study is to test the mitochondria mediated therapeutic potential of BH4 in the treatment of diabetic cardiomyopathy. Fifty weeks aged LETO and OLETF rats were used as control and type 2 diabetes animal models respectively. Onset of diabetes was confirmed by intravenous glucose tolerance test (IGTT). Randomly selected OLETFs were administrated BH4 20mg/kg/day bolus i.p. during 2 weeks (OLETF/BH4). BH4, total biopterin and BH4/total biopterin ratio was examined in heart and mitochondria. Cardiac functions were monitored by echocardiography. Mitochondria oxygen consumption, complex activities assay and ROS generation analysis were performed to figure out mitochondrial functional modulations. Administration of BH4 did not altered IGTT, body weight or blood component of OLETF. The mitochondrial BH4/ total biopterin ration was significantly decreased in OLETF while, BH4 administration restored it. Echocardiography revealed contractile dysfunction in OLETF compared to LETO. BH4 treatment significantly increased left ventricular contractility in OLETF resulting in enhanced ejection fraction and fractional shortening. BH4 treatment also attenuated the left ventricular hypertrophy and fibrosis in OLETF. Proteomic analysis revealed intensive modulation of mitochondria respiratory chain complex and proteasome activity related proteins in OLETF and OLETF/BH4. Mitochondrial membrane potential, electron transport chain complex activity and ATP concentration were decreased in OLETF model and BH4 treatment successfully restored those. Interestingly, increased oxidative stress in OLETF heart tissues were significantly attenuated by BH4 treatment. Proteasome activity was significantly increased in OLETF while, BH4 treatment significantly attenuated it. BH4 has therapeutic potential which corrected mitochondrial dysfunction resulting enhancement of LV contractility and structural remodeling in diabetic cardiomyopathy.

Key Words: Tetrahydrobiopterin, diabetic cardiomyopathy, mitochondria, left ventricular contractility

S-I-3

Mitochondrial Ca^{2+} dynamics and metabolism

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Mitochondrial Ca^{2+} regulation is closely related to cellular function including energy production and cell viability. Intramitochondrial Ca^{2+} overload is eventually destined to cellular death, therefore, the modulation of the mitochondrial Ca^{2+} uptake is important to regulate cellular function. In this presentation, we would like to overview the general characteristics related to mitochondrial Ca^{2+} dynamics in ventricular myocytes. And also, the effect of various metabolites including inorganic phosphate (Pi), pH, adenosine phosphates and Mg^{2+} on mitochondrial Ca^{2+} uptake kinetics with newly developed quantitative method. The mitochondrial substrates are essential for maintaining the function, however, it has not been clear what conditions could cause what effects. Mitochondria contains very complicated system for converting chemical energy to usable chemical energy that is ATP. Various physicochemical environment change may affect the mitochondrial function, however, there have been no systematic approach to elucidate mitochondrial functional changes. In this presentation, we would like to show the importance of mitochondrial functional status for the cellular function and to show the basic conceptual approach to understand mitochondrial functional changes by physicochemical environmental changes. **Acknowledgement:** This work was supported by NRF grant (No 2011-0010965 and 2012-0009829)
Key Words: Mitochondria, Ca^{2+}, metabolism

S-I-4

Receptor for Advanced Glycation End Products Silencing Suppress Arrhythmia and Improve Electrical Conduction by Increasing Connexin 43 via Wnt 1 Activation in Rat Ischemia Reperfusion injury model

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Background: Expression of receptor for advanced glycation end products (RAGE) plays a crucial role to mediate cardiac ischemia/reperfusion (I/R) injury. Although the detail mechanism is unknown, RAGE silencing has been suggested as a cardioprotective therapy. I/R injury is related with increased GSK 3β, and Wnt family of genes is one of the regulators of gap junction expression and function. This study evaluated the effect of RAGE silencing on electrical conduction, arrhythmia, connexin43 (Cx43) and Wnt activation in rat I/R injury model. **Methods:** I/R injury rats (I/R, n=11) were produced by the ligation of left anterior descending artery for 1 hour and reperfusion for 2 hours in Sprague-Dawley rats. RAGE-siRNA/PEI-DA nanocarrier were treated after I/R injury for RAGE silencing (I/R+RAGE, n=10), and scrambled RNA for the discrimination of RNA effect (I/R+scRNA, n=9). Hearts were perfused, mapped optically to analyze action potential durations, intracellular Ca^{2+} transients, and restitution kinetics (RK), and tested for VF vulnerability. **Results:** Compared with control (n=10), I/R rats had shorter action potential duration (APD), slower conduction velocity (CV; $p<0.01$) and steeper CV-RK, higher level of transcripts for tumor necrosis factor-α and interleukin 6, and increased RAGE. RAGE-siRNA/PEI-DA nanocarrier treatment reversed the transcripts for inflammation and the level of RAGE, increased CV ($p<0.01$), flattened CV-RK, and increased APD at 90% recovery to baseline. Programed stimulation triggered VT/VF in in I/R (71%), but not in control (0%) and I/R+RAGE (0%, $p<0.001$ versus I/R). The I/R injury increased the expression of GSK 3β, and reduced Wnt1, β-catenin and Cx43. However, RAGE silencing decreased the expression of GSK 3β, and enhanced Wnt 1, β-catenin and Cx43 ($p<0.05$). **Conclusion:** RAGE silencing therapy suppressed VT/VF in I/R rat hearts by increasing CV from a combination of the inhibition of GSK 3β and enhancing Wnt1, β-catenin and Cx43 in rat I/R injury model.
Key Words: receptor for advanced glycation endproducts, ischemia/reperfusion injury, arrhythmia, connexin43, Wnt

S-I-5

Ion channel remodeling in atrial fibrillation

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Atrial fibrillation (AF) is the most common sustained arrhythmia. Most serious complication of AF was stroke due to contractile dysfunction of left atrium especially left atrial appendage. Prevalence of AF is continuously increase because of aging population. AF is major health problem in aging society. Atrial cardiomyocyte ion remodelings were developed in AF. The most important electrophysiological changes of AF were action potential duration (APD) shortening. Ionic basis of APD shortening were developed by membrane hyperpolarization due to increased inward rectifier current such as IK1 and acetylcholine activated potassium current (IKACh), decreased L type calcium current (ICa,L,), increased or no changes of slow rectifying potassium current (IKs). Dysfunction of Calcium handling process especially ryanodine dysfunction was important cause of intracellular calcium elevation during diastolic period. Leaky ryanodine receptor cause decreased contractility during systole and increased calcium level induced delayed after depolarization (DAD) in diastole. Abnormalities of calcium handling process and constitutively active acetylcholine dependent potassium current were related to increased PKC epsilon. Elevation of PKC epsilon were reported in carcinogenesis in other organs but there are no cancer development in heart. Elevation of PKC epsilon was associated with AF maintenance and contractile dysfunction. Acetylcholine dependent potassium current were elevated in hypercholesterolemic rabbit carddiomyocytes. hypercholesterolemic culture media also increased IKACh in cardiomyocytes. We recently reported lipophilic statin simvastatin decrease IKACh but not hydrophilic statin pravastatin. This effect was due to statin's effect on membrane acetylcholine ligand binding site, after activation of M2 receptor by intracellular gamma GTP, statin's IKACh blocking effects were no longer existed.

Key Words: atrial fibrillation, ion channel

S-II-1

Anti-emetic drug screening from herbal phytochemicals

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Recent progress in biochemical and neuronal science enabled to find out the undelaying mechanisms of many diseases in molecular levels. In spite of such greater progress in pathophysiological knowledge of somatic and psychiatric diseases, the corresponding progress for searching new medicine is fall behind. The plant kingdoms comprise over 300,000 species and synthesize more than 200,000 secondary metabolite compounds. Those secondary metabolites mostly composed with three common structures (the phenolics, the terpenes, and the alkaloids) and affect animal functions including humans. Plants synthesize secondary metabolites for protect against herbivores or attract pollinator insects. Fruit fly has genome that encodes 14,000 genes, surprisingly, among these are identifiable homologues for over 70% of human disease-related genes. Also, insects and human share common neurotransmitters that include; acetylcholine, dopamine, serotonin, glutamate, GABA, opioids, and even steroid receptors. Therefore, similarity between human and insect in cellular, neuronal signal pathways may make human susceptible for plant synthesized secondary metabolites. Here we show an example experiments conducted to find anti-emetic constituents from natural herbal medicine by targeting selective neuronal ion channel. Nausea and vomiting commonly occur in many pathophysiological conditions including early pregnancy, motion sickness, food poisoning, radiation, and chemotherapy with cytotoxic drugs. Selective serotonin type 3 receptor (5-HT3) antagonist has been used for inhibit emesis, however, over 70% of the properly treated patients still experience nausea. The 5-HT3 non-competitive antagonist known as inhibits emesis that observed in patients who treated with anti-emetic drugs including competitive 5-HT3 antagonist. Thus, we screened 5-HT3 antagonist from 20 natural herbal medicines. We find that some of the constituents blocked 5-HT3 receptor responses in visceral afferent neurons.

Key Words: nausea & Vomiting, 5-HT3 receptor, anti-emetic, Herbal medicine, phytochemical

S-II-2

Sleep-promoting effects and GABAergic mechanism of marine polyphenols

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Polyphenols are distributed in a large variety of plants, and the most common polyphenol classes are phenolic acids, flavonoids, and tannins. In particular, terrestrial polyphenols have been considered as one of the most important sedative-hypnotic compounds. Studies on the biological properties of the marine polyphenol phlorotannins have increased markedly over the past decades. However, scientific information on the hypnotic effects and action mechanisms for phlorotannins is limited. Therefore, we investigated whether phlorotannins have sleep-promoting effects similar to those for polyphenols from terrestrial plants. The effects of phlorotannins on the sleep-wake profiles were evaluated by recording electroencephalograms (EEG) and electromyograms (EMG) in mice. HP-PRT was orally administered to C57BL/6N mice. To demonstrate hypnotic mechanism of HP-PRT, flumazenil (a $GABA_A$-benzodiazepine receptor antagonist) was injected. HP-PRT (250 and 500 mg/kg) produced a significant decrease in sleep latency and an increase in the amount of non-rapid eye movement sleep (NREMS) without changes in EEG power density. The hypnotic effect of HP-PRT was completely blocked by flumazenil. These findings support the idea that the hypnotic effects of HP-PRT should be attributed to the positive allosteric modulation of GABAA receptors at the BZD-binding site. Six major phlorotannin constituents from HP-PRT (eckstolonol, triphlorethol A, eckol, fucodiphlorethol G, 6,6'-bieckol, and dieckol) increased sleep duration, and their hypnotic effects were antagonized by flumazenil like HP-PRT. These results provide important clues to the development of a dietary supplement with a new structure because it is structurally different from the polyphenols of terrestrial plants and hypnotic chemicals.

Key Words: Polyphenols, Phlorotannins, Sleep, EEG, NREM

S-II-3

In vivo efficacy of a natural compound on regulation of overweight metabolic stress

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Overweight and obesity have been linked to a low-grade chronic inflammatory response and an increased risk of developing metabolic syndrome including insulin resistance, type 2 diabetes mellitus, cardiovascular disease and certain types of cancers. The aim of the present study was to reveal the effect of luteolin in diet-induced obesity using "Omics" tool (transcriptomics and metabolomics). C57BL/6J mice were fed a normal diet (ND), a high-fat diet (HFD, 20% fat), and a HFD containing 0.005% (w/w) luteolin for 16 weeks. Body weight, all white adipose tissue (WAT) depot weights and adipocyte size were significantly decreased by luteolin supplementation compared to HFD. In addition, luteolin was effective for lowering the plasma and hepatic lipid levels in diet-induced obese mice by altering the hepatic lipid metabolizing enzyme activities and fecal lipids contents. IPA revealed upstream regulator on significant networks targeted by luteolin was consisted of genes related to lipid synthesis and oxidation (Ppara and Ppard) in liver and inflammatory response (Lep, Insr and Il6) in epididymal adipose tissue. In liver, luteolin down-regulated gene expression associated with lipid and cholesterol synthesis, while it up-regulated gene expressions associated with fatty acid oxidation. In adipose tissue, luteolin up-regulated gene expressions related to fatty acid oxidation and TCA cycle, and it down-regulated gene expression of Adrp which involves in formation of lipid droplet. Luteolin also suppressed inflammatory response by down-regulating pro-inflammatory chemokine and cytokine genes as well as its upstream regulator genes such as TLRs, IRF in adipose tissue. Our results indicate that luteolin supplement exhibits positive effects on obesity and certain obesity-related metabolic disease by modulating the gene expressions and metabolites associated with lipid metabolism and inflammation in diet-induced obese mice. Anti-obesity effect of luteolin could help to ameliorate the deleterious effects of HFD-induced obesity and its complication.

Key Words: Obesity, Metabolic Regulation, Adipokines, Transcriptomic Profile

S-II-4

Chemoresistance in ovarian cancer: natural product as a potential modulator

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Paclitaxel and cisplatin are currently used as the front-line chemotherapeutic agents for several cancers including ovarian carcinoma; however, the drugs frequently induce drug resistance through multiple mechanisms. The new strategy of using natural compounds in combination therapies is highly attractive because those compounds may enhance the efficacy of chemotherapy. In the present study, we found that tectorigenin, an isoflavonoid isolated from flower of Pueraria thunbergiana, enhanced the growth-inhibitory effect of paclitaxel in paclitaxel-resistant ovarian cancer cells as well as their naive counterparts. The combination of tectorigenin with paclitaxel resulted in a synergistic apoptosis compared with either agent alone. Mechanism studies revealed that tectorigenin could sensitize paclitaxel-resistant human ovarian cancer cells through inactivation of the Akt/IKK/I κ B/NF κ B signaling pathway. We also investigated the effect of a phlorotannin-rich extract from the edible brown alga Ecklonia cava (PREC) and its major phlorotannin (dieckol) on cisplatin responsiveness and side effects. We found that PREC enhanced the tumor growth-inhibitory effect of cisplatin and diminished cisplatin-induced nephrotoxicity and weight loss in SKOV3-bearing mice. PREC augmented cisplatin-induced apoptosis by activating caspases in SKOV3 and A2780 ovarian cancer cells. In addition, a combination of PREC and cisplatin induced ovarian cancer cell apoptosis by downregulating the Akt and NF κ B pathways. These data suggest that polyphenol tectorigenin and dieckol promise a new intervention to chemosensitize paclitaxel/cisplatin-induced cytotoxicity in ovarian cancer.

Key Words: ovarian cancer, chemoresistance, polyphenols

S-III-1

Physiological Adaptations to Exercise Training

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Physiological adaptations that occur in response to both endurance exercise training and resistance training will be explained and discussed through this lecture. Firstly, beneficial physiological adaptations in response to endurance training (ET) will cover following topics: How does ET improve VO2max (maximal oxygen consumption). ET-induced changes in fiber type and capillary density. ET-induced increase in mitochondrial content in skeletal muscle fibers. Biochemical adaptations and improvement of antioxidant capacity and acid-base balance. Furthermore, intracellular signal transduction pathways mediating ET-induced skeletal muscle response will be briefly discussed. Secondly, in contrast to ET, mechanisms responsible for resistance training (RT) induced increases in strength will be discussed. Adaptations to RT will include following topics: RT-induced increases in skeletal muscle size. RT-induced changes both in the nervous system and in muscle fiber type. In addition, signaling events leading to RT-induced muscle growth will be discussed compared to ET-induced signaling pathways. Lastly, detraining effects following both endurance training and resistance training will be also introduced.

Key Words: adaptation, endurance training, resistance training

S-III-2

Does interval training improve endothelial function better than regular exercise?

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It is well known that regular exercise is effective treatment and preventive means for hypertension. Over the past few decades, many expert groups such as American College of Sports Medicine (ACSM) have highly recommended continuous moderate-intensity aerobic exercise (CME) for hypertensive patients (ACSM, 2004). One of the systemic review papers based on more than 100 RCT studies reported that aerobic exercise generally reduces systolic and diastolic blood pressure about 6.9 mm Hg and 4.9 mm Hg in hypertensives, 3.1 mm Hg and 1.7 mm Hg in pre-hypertensives, 2.4 mm Hg and 1.6 mm Hg in normotensives, respectively. Furthermore, one bout of exercise has shown to reduce systolic and diastolic blood pressure about 15 mm Hg and 4 mm Hg up to 4 to 22 hours (HHS, 2008). As a result, aerobic exercise is now considered to reduce the risk of hypertension about 30-40%. Recently, an interest in high-intensity interval exercise (HIE) for hypertensive patients is increasing because it has been speculated that HIE could maximize the effect of shear stress on endothelium due to frequent stimulation. Indeed, an animal study using hypertensive rats found that reductions in resting blood pressure were greater in HIE than CME after 8 weeks of training (Haram et al., 2009). Human studies also showed that HIE decreased even exercising blood pressure more than CME after 16 weeks of training (Ciolac et al., 2010). In addition, increases in nitric oxide production and bioavailability and decreases in endothelin-1 at rest and during exercise were greater in HIE than CME (Tjønna et al., 2008; Ciolac et al., 2010). Our recent unpublished data also demonstrated that HIE increased more endothelial progenitor cells than CME in hypertensive patients after 10 consecutive days of exercise. These results suggest that HIE is more effective than CME on improvements in endothelial function. Therefore, it is possible that HIE could be a better exercise program than CME for hypertensive patients

Key Words: Hypertension, Interval exercise training, Endothelial function

S-III-3

Effects of aging and exercise training on mitochondrial function and insulin resistance in skeletal muscle

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Aging is characterized by a progressive loss of muscle mass and muscle force called 'sarcopenia'. Age-related muscle atrophy is due to loss of muscle fibers associated with mitochondrial dysfunction in skeletal muscle. Mitochondria regulate a multitude of different metabolic and signaling pathways. Accumulating evidence shows that impairment in skeletal muscle mitochondria with aging is thought to play a primary role in insulin resistance. The development of insulin resistance, particularly in skeletal muscle (organ of insulin-mediated glucose disposal), is closely associated with type II diabetes. Furthermore, mitochondrial reactive oxygen species (ROS), a byproduct of mitochondrial oxidative phosphorylation, has been also implicated in insulin resistance in skeletal muscle. The purpose of this study was to determine the effects of aging and exercise training on mitochondrial function, mitochondrial H2O2 emission, and insulin resistance in rat skeletal muscles. Four and 20 month old Fischer 344 rats were randomly assigned to young sedentary (YS), young exercise (YE), old sedentary (OS), or old exercise (OE) groups. Exercise training groups ran on a treadmill at 15 m/min (young) or 10 m/min (old), 45 min/day, 5 day/week for 8 weeks. The skeletal muscles such as soleus (SOL, Type I fiber) and white gastrocnemius (WG, Type IIb fiber) were permeabilized by sarponin for determination of mitochondrial respiratory capacity, Ca^{2+} retention capacity, and mitochondrial H2O2 emission. Exercise attenuated age-induced decrease in WG mass. Insulin resistance by oral glucose tolerance test was elevated in OS rats. However, exercise preserved whole body insulin sensitivity in OE rats. The mitochondrial respiratory capacity and Ca^{2+} retention capacity were decreased by aging and protected by exercise in both SOL and WG. In addition, exercise attenuated age-induced mitochondrial H2O2 emitting potential in both SOL and WG. These data demonstrate that the increase in energy expenditure induced by exercise training prevents the impairment of mitochondrial function in skeletal muscles and development of insulin resistance induced by aging, supporting the concept that the exercise regulation of mitochondrial dysfunction with aging is a primary factor improving insulin sensitivity in skeletal muscle [2012R1A1A1042383].

Key Words: Aging, Exercise, Mitochondrial Function, Insulin Resistance, Skeletal Muscle

S-III-4

Effects of exercise training on ER stress

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Endoplasmic reticulum (ER) stress, unfolded protein response (UPR), and mitochondrial biogenesis were assessed following varying intensities of exercise training. The animals were randomly assigned to receive either low-(LIT, n=7) or high intensity training (HIT, n=7), or were assigned to a control group (n=7). Over 5 weeks, the animals in the LIT were exercised on a treadmill with a 10^{o} incline for 60 min at a speed of 20 m/min group, and in the HIT group at a speed of 34 m/min for 5 days a week. No statistically significant differences were found in the body weight, plasma triglyceride, and total cholesterol levels across the three groups, but fasting glucose and insulin levels were significantly lower in the exercise-trained groups. Additionally, no statistically significant differences were observed in the levels of PERK phosphorylation in skeletal muscles between the three groups. However, compared to the control and LIT groups, the level of BiP was lower in the HIT group. Compared to the control group, the levels of ATF4 in skeletal muscles and CHOP were significantly lower in the HIT group. The HIT group also showed increased PGC-1α mRNA expression in comparison with the control group. Furthermore, both of the trained groups showed higher levels of mitochondrial UCP3 than the control group. In summary, we found that a 5-week high-intensity exercise training routine resulted in increased mitochondrial biogenesis and decreased ER stress and apoptotic signaling in the skeletal muscle tissue of rats.

Key Words: ER stress, Exercise, Mitochondria, Skeletal muscle, Unfolded protein responses

S-III-5

Impacts of Exercise on Type II Diabetic rats

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Type 2 diabetes mellitus (T2DM) is one of major public health problems. Blood glucose lowering strategy through Oral hypoglycemic agents or insulin is a leading type of treatment. Although the beneficial effects of exercises on health promotion or disease prevention have been extensively under investigation, their underlying mechanisms are largely unknown. Our study aimed to determine the effects of aerobic (EXA, treadmill running) and resistance exercise (EXR, ladder climbing) on the cardiac alterations caused by T2DM in Long-Evans Tokushima fatty rats OLETF). OLETF rats were experienced treadmill running (n=8) or 85 degree of ladder climbing (n=8) with 5 days/week. After 12 weeks exercise, intraperitoneal glucose tolerance tests (IGTTs) and lipid profiles were determined. Heart morphological and functional parameters were measured by echocardiography. Mitochondrial changes were determined by electron microscopy and oxygen consumption rate. Both exercise groups significantly improved blood glucose status and lipid profiles (n=5～6, $P<0.05$). Both types of exercise were effective on improving cardiac function and integrity of heart mitochondria. However, ladder climbing was more potent than treadmill running in improving glucose and lipid profile. Taken together, exercises significantly ameliorated cardiac damage, blood glucose, and lipid profile followed by T2DM. However, their underlying mechanisms are still unclear and thus more extensive works should be performed to tackle the beneficial effects of exercise on treating T2DM.

Key Words: diabetes, exercise, heart, mitochondria

S-IV-1

Chronic itch induced by neonatal capsaicin treatment in rat

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Chronic itch can be categorized into four subtypes: pruriceptive, neurogenic, neuropathic, and psychogenic. Pruriceptive itch arises from diseases of the skin, such as atopic dermatitis. Neurogenic itch is induced by the blockage of pain such as an increase in opioidergic tone. Neuropathic itch results from central sensitization after nerve injury such as postherpetic itch. Recently, we developed a rat model of chronic itch induced by neonatal capsaicin treatment. This model showed the obvious characteristics of atopic dermatitis. However, given that neonatal capsaicin treatment can destruct the capsaicin-sensitive pain fibers, we could not rule out the possibilities that the itch in this model is neurogenic or neuropathic. In the present study, we examined whether the itch signs in a rat model of chronic itch induced by neonatal capsaicin treatment contained neurogenic or neuropathic components. Capsaicin (50 mg/kg) was given to rat pups subcutaneously within 48 h after birth, and then scratching behavior was investigated. A bout of consecutive scratching strokes by hind paw was regarded as one scratch. In the previous study, the number of scratches showed peak value at 6 weeks after the treatment, and was maintained for 3 weeks. Thus, to clearly characterize the itch signs of capsaicin-treated rats, we compared the scratching behaviors before and after the intraperitoneal injections of ketanserin (5 mg/kg, 5-HT2 receptor antagonist), naloxone (1 mg/kg, μ-opioid receptor antagonist) and gabapentin (30 mg/kg, $\alpha 2\delta 1$ antagonist) at 6 weeks after neonatal capsaicin treatment. And, ketotifen (1 mg/kg, mast cell stabilizer) was intraperitoneally injected twice a day from 3 to 6 weeks after neonatal capsaicin treatment. Intriguingly, all of these drugs significantly alleviated the scratching behaviors. These results suggest that chronic itch induced by neonatal capsaicin treatment may involve neurogenic and neuropathic as well as pruriceptive components. This study was supported by the Nation Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012-0009675 and NRF 2013R1A1A2011913) and a Korea University Grant.
Key Words: chronic itch, atopic dermatitis, capsaicin, neurogenic itch, neuropathic itch

S-IV-2

Inactivation of group I metabotropic glutamate receptor in periaqueductal gray maintains chronic neuropathic pain

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Neuropathic pain is a pathological pain caused by damage to the peripheral or central nervous system. Being characterized by allodynia, hyperalgesia and persistent pain, symptoms of neuropathic pain are often intractable. However, mechanism of chronification and maintenance of neuropathic pain is still elusive. Aim of this study is to investigate altered state of endogenous pain modulation system during neuropathic pain and underlying mechanism involving maintenance of chronic pain. Among various pain-related area in the brain, periaqueductal gray (PAG) is one of the most important area in terms of activating endogenous analgesic system. Ventrolateral PAG (VL-PAG) integrates pain modulation signals from various brain areas and sends signal to rostral ventromedial medulla (RVM), thus modulates pain signals from spinal cord. To see possible change of endogenous analgesic system following neuropathic pain, brain metabolism was measured using positron emission tomography (PET) and metabolic relationship between VL-PAG and RVM was analyzed. Result showed that activities of VL-PAG and RVM were negatively correlated to each other in neuropathic pain group, but not in control group. Another interesting observation from PET scan was the different metabolic response between pain animals and control animals following activation of group I mGluR within VL-PAG. In pain group, strong metabolic increase was observed from VL-PAG and RVM. However, weak or no metabolic increase was observed from VL-PAG and RVM in control animals. One of the possible explanations is that mGluR signaling within VL-PAG is constitutively active already in normal state to maintain normal sensory perception, and is lost following chronic neuropathic pain so that neuropathic symptoms are maintained. To address this issue, mGluR5 within VL-PAG of naive animals was deactivated and the behavioral change was observed. Administration of mGluR5 inverse agonist into bilateral VL-PAG reduced paw withdrawal threshold of naive animals, inducing long-lasting mechanical allodynia. We could also observe that deactivation of mGluR5 reduces neuronal excitability of VL-PAG neurons in control group but not in pain group, indicating mGluR5 signaling is already deactivated in pain condition. Overall, these data suggest that persistently active mGluR5 signaling in VL-PAG plays a key role in normal sensory transmission, and a disturbance of it causes induction and maintenance of neuropathic pain.
Key Words: metabotropic glutamate receptor, periaqueductal gray, neuropathic pain

S-IV-3

Transitional mechanisms from acute to chronic pain: Can it be prevented?

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It has been well known that acute post-operative pain is strongly correlated with chronic post-operative pain in the previous clinical studies (Katz et al., 1996; Perttunen et al., 1999; Poleshuck et al., 2006). The major finding of these studies showed that the incidence and severity of chronic post-operative pain can be predicted by experimental pain assessment. Acute postoperative pain is followed by persistent pain in 10～50% of individuals after common operations, such as groin hernia repair, breast and thoracic surgery, leg amputation, coronary artery bypass surgery (Kehlet et al., 2006). Several risk factors, including nerve injury, genetics factor, age and gender, psychological factor and severity of preoperative pain, are involved in the development of transition from acute to chronic pain. The present study demonstrated that central transitional mechanisms from acute to chronic pain. Especially the present data showed important roles of glia cells involved in the development of chronic pain conditions. First experiment demonstrated that role of glia cells in the transitional mechanisms. Predisposing pain produced glia cell activation resulted in the central sensitization. Moreover, glial cell activation plays a role of key factor in the transition to chronic pain condition. Second experiment examined effects of preemptive analgesia on the development of neuropathic pain. For this purpose, the mechanical allodynia has been evaluated in rats with chronic constriction injury of infraorbital nerve after perineural application of 2% QX-314 in the infraorbital nerve. These results suggest that QX314-induced long lasting preemptive analgesia produces inhibition of development of neuropathic pain through a regulation of the satellite glial cells and neuronal p-p38 expression in the trigeminal ganglion. Importantly, these results provide a potential preemptive therapeutic strategy for the treatment of neuropathic pain following nerve injury. In the third experiment, we investigated the antinociceptive effects of botulinum toxin type A (BoNT-A) in a rat model of trigeminal inflammatory and neuropathic pain. The present data provide possible mechanisms involved in BoNT-A-induced anti-nociception. Also these results suggest a potential new therapeutic strategy for treatment of chronic trigeminal pain (supported by No. 2012M3A9B6055414).

Key Words: pain, trigeminal, glia, neuropathic pain, botulinium

S-IV-4

How to overcome & what to do on neuropathic pain?

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Neuropathic pain is a complex, chronic pain state typically accompanied by disease of the somatosensory system that is associated with abnormal sensations, such as allodynia, hyperalgesia, and spontaneous pain. These abnormal sensations may greatly influence on the quality of life, cause problems with mood and sleep, and cause patients to withdraw from social activities. Moreover, commonly used analgesics are often inadequate in treating neuropathic pain. Present pharmacologic therapy is not enough effective in approximate 50% of neuropathic pain patients. Thus, there is a continuing need for the development of effective analgesic therapies for neuropathic pain. Significant increases of knowledge about nociceptive information have been made at the spinal level. Thereafter, multiple molecules have been reported to be involved in the modulation of nociception in the spinal cord. They are amino acids, neuropeptides and other channels, which may play a key role in the nociceptive pathways. Followings are pronociceptives; excitatory amino acids acting on ionotropic/metabitropic receptors, substance-P, CGRP, bombesin, cholecystokinin, VIP, neuropeptide YY, ATP, nitric oxide, nerve growth factor, cytokines, prostaglandin. On the other hand, following substances are antinociceptive; GABA, glycine, opioids, serotonin, noradrenaline, nociceptin, vanilloid receptor. Therefore, agents blocking nocicepive property and augmenting antinocieptive activity contribute to modulate the nociception. Despite of vigorous researches, unfortunately most physicians have not met the magic bulletin to conquer the neuropathic pain. Now where we are in dealing with the neuropathic pain? And also, how we can solve these peculiar phenomena? Both doctors and scientists are responsible for overcome these neuropathic pain. Today, I'll share the information on the neuropathic pain mechanisms and strategy. And I hope to offer the possibility of highly-effective and well-tolerated analgesics development on neuropathic pain.

Key Words: neuropathiv pain, mechanisms, analgesia, allodynia, hyperalgesia

S-V-1

Adult Stem Cell Niche and Its Application for Cardiovascular Regeneration

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Stem cells provide fresh cells to replenish blood, bone, epithelial, nervous system, muscle, and various tissues. Stem cells are regulated and maintained by local tissue microenvironments, or niches. Since the coining of the concept of the stem cell niche, it has been possible to identify stem cells and niches with increasing precision. However, the importance of the stem cell niche has not been completely recognized because models of stem cell behavior are not well understood. Thus, it is challenging to predict how self-renewal is influenced by external factors. Asahara et al. revealed that CD34+ hematopoietic stem cells (HSCs) from peripheral blood mononuclear cells (PB-MNCs) can differentiate into endothelial lineage cells, and many researchers have shown that these cells, endothelial progenitor cells (EPCs), play a pivotal role in neovascularization; however, the identity of the EPCs is not clear. Here, I summarize stem cell niche issue for developing therapeutic approaches for vascular diseases. We investigated the functional properties of 2 types of EPCs, also known as endothelial colony forming cells (ECFCs), CD34-/CD34+ cell-derived ECFCs (hybrid-dECFCs) and CD34+ cell-derived ECFCs (stem-dECFCs), isolated using different methods, to elucidate the role of CD34- cell populations as cell-supporting niches. Using EPC colony-forming and insert coculture assays, we found that CD34- accessory cells dynamically modulate hematopoietic stem cell-derived endothelial cell progenitor commitment via angiogenic cytokines secreted by CD34-/CD11b+ macrophages. On the basis of these findings, we isolated 2 types of ECFCs and investigated their bioactivities. In a murine hindlimb ischemia model, hybrid- dECFCs showed significantly enhanced blood perfusion, capillary density, transplanted cell survival and proliferation, and angiogenic cytokine secretion compared with stem-dECFCs. CD34- accessory cells of hybrid-dECFCs might be niche-supporting cells that facilitate cell survival,increase the secretion of angiogenic cytokines, and increase incorporation. This study provided important insight into blood vessel formation and repair in ischemic diseases for ECFC based cell therapy.

Key Words: endotheal progenitor cells, stem cells,ischemia, vascular regeneration, therapy

S-V-2

Patient-specific iPS Cell Research: Gene and Cell Therapy

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iPS cells provide a useful tool to model human diseases, screen for new drugs and test candidate drugs for toxicity. In addition, in case of familial diseases, the mutations can be corrected using patient-derived iPS cells for gene and cell therapy. Hemophilia A, one of the most common genetic bleeding disorders, is caused by various mutations in the blood coagulation factor VIII (F8) gene. Among the genotypes that result in hemophilia A, two different types of chromosomal inversions that involve a portion of the F8 gene are most frequent, accounting for almost half of all severe hemophilia A cases. In this study, we used a TALEN pair to invert a 140-kbp chromosomal segment that spans the portion of the F8 gene in human induced pluripotent stem cells (iPSCs) to create a hemophilia A model cell line. In addition, we reverted the inverted segment back to its normal orientation in the hemophilia model iPSCs using the same nucleases. Importantly, we detected the F8 mRNA in cells derived from the reverted iPSCs lines but not in those derived from the clones with the inverted segment. Thus, we showed that TALENs can be used both for creating disease models associated with chromosomal rearrangements in iPSCs and for correcting genetic defects caused by chromosomal inversions. This strategy provides an iPSC-based novel therapeutic option for the treatment of hemophilia A and other genetic diseases caused by chromosomal inversions. This research was supported by grants from the National Research Foundation of Korea (the Bio & Medical Technology Development Program, 2012M3A9B4028631 and 2012M3A9C7050126) and from Korean Ministry of Health & Welfare (A120254).

Key Words: iPS Cells

S-V-3

Application of lipid metabolites for enhancement of stem cells functions

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Embryonic stem cells and various adult stem cells are thought to reside in niches characterized by low oxygen levels, which play an important role on control of stem cells functions through alteration of stem cell metabolism. Although it is likely that lipid metabolism alteration in hypoxic condition is thought to be a key factor in controlling stem cell fate and functions, the interaction between hypoxia and the metabolic and functional changes to stem cells is incompletely described. Thus, we investigated the physiological changes of stem cell under hypoxic condition and the effect of lipid metabolites on regulation of stem cells functions including proliferation and migration. In this study, H_2O_2 stimulated MSCs motility by increasing MMP12-dependent degradation of COL-5 and FN, which is critical for providing a suitable microenvironment for MSCs transplantation and re-epithelialization of skin wounds in mice. Hypoxia stimulates hMSC proliferation, and expression of two lipogenic enzymes: fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD1). FASN but not SCD-1 is a key enzyme for regulation of hMSC proliferation and migration. Hypoxia-induced FASN expression was controlled by the HIF-1α/SCAP/SREBP1 pathway. Hypoxia-induced mTORC1 activation regulated cell cycle regulatory proteins and F-actin expression as well as that of c-myc, p-cofilin, profilin, and Rho GTPase. In next, we investigated the effect of lipid metabolites such as arachidonic acid (AA), oleic acid (OA), lysophosphatidic acid (LPA), and sphingosine-1-phosphate (S1P) on regulation of stem cells functions. AA, OA, and LPA accelerated wound healing by increasing the migration of hMSCs into a wound site in a mouse skin wound healing model. LPA stimulated migration through LPA receptor 1/3-dependent dissociation of cellular adherent junction and cytoskeleton rearrangement. AA-induced mTORC2-dependent phosphorylation of PKC and Akt activated Sp1, which increased MMP-16 and fibronectin degradation, and subsequently increased hMSCs migration. And OA-induced transactivation of Flk-1 stimulates EphB2 expression through GSK3β/β-catenin pathway. In addition, S1P stimulated both VEGF-dependent and -independent Flk-1 activation through β-arrestin and c-Src activation, which involved in mESC proliferation. Taken together, enhancement of stem cells functions through either modulation of lipid metabolism or targeting the signaling molecules and enzymes involved in lipid metabolism may improve effectiveness of cell-based therapy in regenerative medicine.

Key Words: Stem Cells, Hypoxia, Lipid Metabolites, Proliferation, Migration

S-V-4

Reprogramming of cell fate into Pluripotency and Multipotency

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Reprogramming of somatic cells to induced pluripotent stem (iPS) cells by ectopic expression of the four transcription factors (OCT4, SOX2, MYC and KLF4) represents a potential tool regarding regenerative medicine. Recently, we reported that single transcription factor Oct4 is sufficient to directly reprogram adult mouse neural stem cells (NSCs) into iPS cells. Furthermore, we found that the generation of one-factor (1F) human iPS cells from human NSCs is possible by the ectopic expression of OCT4 alone. Reprogramming between iPS cells from somatic stem cells by the overexpression of a single transciption factor, even avoiding oncogene integration into host genome, offer a new strategy in the field of regenerative medicine. Recent advances have suggested that direct conversion of fibroblasts into multipotent stem cells with lineage-specific transcription factors could provide an alternative to derivation from somatic tissues or pluripotent cells. Here we show direct derivation of stably expandable multipotent stem cells from fibroblasts through overexpression of certain factors. induced multipotent stem cells uniformly display morphological and molecular features of them, as well as in vitro and in vivo functionality similar to those of wild-type stem cells. We conclude that somatic cells can be reprogrammed directly into adult stem cells by certain factors.

Key Words: Reprogramming, Direct conversion, Stem cells, Pluripotency, Multipotency

S-V-5

Hematopoietic differentiation from human induced pluripotent stem cells

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Human induced pluripotent stem cells (hiPSCs) are undifferentiated cells that can self-renew and potentially differentiate into all hematopoietic lineages, such as myeloid and lymphoid cells. Therefore, hiPSCs hold huge promise for the generation of hematopoietic stem cells (HSCs) for use in blood diseases such as anemia and leukemia. Nowadays, successful transplantation of HSCs derived from bone marrow and umbilical cord blood has been achieved, especially in malignant hematopoietic diseases. However, due to lack of donors and a limited amount of HSCs, hiPSCs have been regarded as an alternative source of HSCs and mature blood cells. In addition, hiPSCs are an important tool for better understanding in hematopoietic development and the mechanisms of hematopoietic disease. My research group focuses on dissecting the functional and phenotypic heterogeneity within hiPSC cultures for the enrichment of cells primed to hematopoietic lineage and the development of a robust hematopoietic induction system. This system will provide an efficient way of predicting the cell fate potential and generating enough number of transplantable HSCs. This work was supported by Grant for "the Center for Evaluating Next-Generation Stem Cell-based Therapeutics (CENST)" supported by National Institute of Food and Drug Safety Evaluation, an affiliate of the Ministry of Food and Drug Safety (14172 CENST 974).
Key Words: iPS, hematopoiesis, leukemia, HSC

S-VI-1

Regulation of trigeminal chemosensation by two-pore domain K^+ channels

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The trigeminal somatosensory system plays a fundamental role in chemosensation. Sensory terminals of the trigeminal nerve, which innervate the face, the eye and the nasal oral cavities, contain multiple types of chemosensory receptors, including ion channels. Activation of the nerve by physical (mechanical forces and temperature) and chemical stimuli evokes sensations of touch, temperature and pain. In this presentation, I introduce the role of two-pore domain K^+ (K_{2P}) channels in trigeminal chemosensation. It is well-known that TASK channels, members of K_{2P} channel family, are involved in the central and peripheral chemosensation. A number of K_{2P} channels are expressed in trigeminal ganglion (TG) neurons. THIK-1-like, TREK-2-like and TRESK-like channels contribute to the background K^+ conductance of TG neurons. Changes in temperature, which affect the sensation evoked by many chemical agents, modulate the activity of THIK-1-like and TREK-2-like channels. In addition, THIK-1, TREK-2 and TRESK-like channels are modulated by noxious chemicals, such as formaldehyde and hydrogen sulfide, and pro-inflammatory mediators. The excitability of trigeminal terminals increased by noxious stimuli is reduced by overexpression of K_{2P} channels expressed in TG neurons. Therefore, K_{2P} channels are likely to be involved in trigeminal chemosensation.
Key Words: Background K^+ channel, Chemosensation, Trigeminal ganglion neuron

S-VI-2

Characterization of Drosophila taste receptors

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Many of naturally occurring insecticides or repellents are produced by plants to protect themselves from predator. Insects show repulsive response to these compounds because they might be harmful. However, how insect can sense these chemicals are enigmatic. In Drosophila, there are 68 gustatory receptors (GRs) which are predicted as seven transmembrane domain proteins. Recently, Ors are reported as channels. According to their similarity to Drosophila olfactory receptors (ORs), it is believed that GRs are also channels rather than G-protein coupled receptors (GPCRs). The function of some of GRs in vivo has been identified. However, it is not clear what the molecular identity of GRs is, because the functional bitter receptor complex was not identified so far. Recently we reported that Gr8a and Gr66a were required for the detection of naturally occurring insecticide, L-canavanine, but Gr8a and Gr66a were not sufficient to confer L-canavanine response. We take advantages of RNAi screen to identify functional complex for L-canavanine receptors and found that additional GR is required for the detection of L-canavanine. We will present our progress on L-canavanine receptors.

Key Words: Drosophila, Taste, Taste receptors, L-canavanine

S-VI-3

Differentiated sensory functions of transmembrane channel-like proteins

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Transmembrane channel-like (tmc) genes constitute a family of broadly-conserved genes encoding integral membrane proteins of animals. Tmc genes are evolutionarily conserved, for example, with at least eight members in vertebrates, two in the nematode Caenorhabditis elegans (C. elegans), one in the fruit fly D. melanogaster, etc. multiple number of putative membrane spanning amino acid domains when translated, and the alteration of cellular ion conductions when overexpressed, have suggested that the proteins encoded by tmc genes are critical components of an ion channel complex. Furthermore, a certain TMC protein members were detected in sensory organs such as stereocilia of mammalian cochlear hair cells and nociceptive sensory neurons of C. elegans, which implicates those TMC proteins may not only be ion channel components but also possibly sensory receptors for chemical or mechanical sensations. Indeed, our recent study demonstrated that C. elegans TMC-1 (cTMC-1) mediates ion conduction in response to increased environmental sodium concentrations. Interestingly, a series of hearing studies have suggested that mammalian TMC1 and TMC2 are indispensible in mechanosensory electrical transduction of cochlear hair cells for hearing and balance. Therefore, individual TMCs may play sensory roles by possessing differentiated sensitivities to physical or chemical stimuli. Here we examined the sensory functions of an unexplored TMC member, C. elegans TMC-2 (cTMC-2) in this regard. Various chemical and mechanical stimulations were tested in terms of cTMC-2-mediated ion flux. As a result, we found that cTMC-2 have distinctive sensitivities different from those of its closest cousin, cTMC-1.

Key Words: transmembrane channel-like genes, cTMC-1, cTMC-2, chemosensation, mechanosensation

S-VI-4

Intracellular Signaling in Vomeronasal Neuron through Multiple Ion Channel Activation

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The vomeronasal organ (VNO) is essential for intraspecies communication in many terrestrial vertebrates. The ionic mechanisms of VNO activation remain unclear. We found that the calcium-activated potassium channel SK3 and the G protein-activated potassium channel GIRK are part of an independent pathway for VNO activation. In slice preparations, the potassium channels attenuated inward currents carried by TRPC2 and calcium-activated chloride channels (CACCs). In intact tissue preparations, paradoxically, the potassium channels enhanced urine-evoked inward currents. This discrepancy resulted from the loss of a high concentration of lumenal potassium, which enabled the influx of potassium ions to depolarize the VNO neurons in vivo. Both Sk3 (also known as Kcnn3) and Girk1 (also known as Kcnj3) homozygous null mice showed deficits in mating and aggressive behaviors, and the deficiencies in Sk3(-/-) mice were exacerbated by Trpc2 knockout. Our results suggest that VNO activation is mediated by TRPC2, CACCs and two potassium channels, all of which contributed to the in vivo depolarization of VNO neurons.

Key Words: Vomeronasal Neuron, SK3 ,GIRK ,CACCs

S-VI-5

Various faces of ion channels sensing hypoxia: activation of TRPV, inhibition of TASK-1 and upregulation of TASK-2

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Cells encounter hypoxia (1～3% Po_2) as physiological as well as pathophysiological internal environments. O_2-sensitive ion channels play important roles in hypoxic pulmonary vasoconstriction (HPV) and immune cell responses. In this presentation, our experiences of hypoxic O_2-sensing mechanisms of ion channels in rat pulmonary artery and mouse B cells will be introduced. Pulmonary arterial contraction study indicates that membrane depolarization under hypoxia is critical for HPV. Consistently, hypoxia combined with TXA2 treatment activates nonselective cation channels with properties consistent with TRPV2 in pulmonary arterial smooth muscle cells (PASMCs). In addition, hypoxia inhibits TASK-like background K^+ channels as well as voltage-gated K^+ channels in PASMCs. Hypoxic inhibition of TASK-1 current requires co-expression of NOX4. However, both pharmacological assay and response of mutated NOX4 indicate that not an ROS production but a putative direct interaction between NOX4 and TASK-1 underlie the signaling mechanisms. Mouse B cells express multiple types of K^+ channels; TREK-2, TASK-2 and Kv1.3. Among them, TASK-2 channels are largely upregulated by B cell receptor stimulation. Interestingly, sustained hypoxia (3% Po_2, ＞12 h) also induces TASK-2 current in HIF-1 dependent translational upregulation. The increased background K^+ conductance in B cells facilitates Ca^{2+} influx via augmenting electrical driving force. In contrast, T cells do not express background-type K2P channels such as TASK-2. Since hypoxia inhibits Kv1.3 in both B and T cells, the hypoxic upregulation of TASK-2 might be associated with unique proliferative responses of B cells under hypoxic environment.

Key Words: hypoxia, ion channel, TASK-1, TASK-2, TRPV2

S-VII-1

Epigenetic modulation of coping strategy to stress: Role of mGluR5

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Being repeatedly exposed to the same stressful event can produce a wide range of long-lasting behavioral responses. PTSD is one of these long-lasting conditions. Current treatments are not definitive and a substantial portion of PTSD patients continue to suffer. In light of the urgent need for the development of new treatment options, the regulatory mechanisms of extinction and reconsolidation of fear memory hold large potential for clinical relevance and has not yet been extensively studied. In this study, we investigated mGluR5 expression levels in rats and how they played a role in determining an individual's response to stressful stimuli at the reconsolidation-extinction boundary. We found that repeated exposure to restraint stress resulted in mGluR5 protein expression levels in the hippocampus that varied greatly from rat to rat. The low mGluR5 protein expression group showed increased methylation sites on the CpG island of the mGluR5 gene, decreased mGluR5 mRNA expression, and unaltered basal theta electroencephalogram power and corticosterone blood concentrations, suggesting positive behavioral adaptation. In contrast, the high mGluR5 protein expression group displayed the exact opposite results, suggesting negative adaptation. These alterations in mGluR5 expression were closely linked to MR, BDNF, and EGR1 mRNA expression levels. In addition, the individual differences in mGluR5 expression among the rats disappeared following infection with the shmGluR5 lentivirus. This study suggests that the epigenetic modulation of the mGluR5 gene can in turn modulate the reconsolidation and extinction process in the face of a stressor, and that the regulation of mGluR5 activity can be a key target in the treatment of traumatic disorders like PTSD. This work is supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-7-2008-0409).

Key Words: Stress, mGluR5, Epigenetics, Reconsolidation, PTSD

S-VII-2

Oxytocin protects hippocampal memory and plasticity from uncontrollable stress

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The hippocampus is vulnerable to uncontrollable stress and enriched with oxytocin receptors, but their interactive influences on hippocampal functioning are unknown. Here, we present that intranasal administration of oxytocin reduced stress effects on hippocampal synaptic plasticity and memory in rats via acting on the oxytocin receptors and regulating the extracellular signal-regulated kinase signaling. Exogenous oxytocin then may be therapeutically effective means to counter the detrimental neurocognitive effects of stress.

Key Word : Oxytocin, Stress, Hippocampus

S-VII-3

Adrenal stress hormones, amygdala activation, and memory for emotionally arousing experiences

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Extensive evidence indicates that stress hormones released from the adrenal glands are critically involved in memory consolidation of emotionally arousing experiences. Epinephrine or glucocorticoids administered after exposure to emotionally arousing experiences enhance the consolidation of long-term memories of these experiences. Our findings indicate that adrenal stress hormones influence memory consolidation via interactions with arousal-induced activation of noradrenergic mechanisms within the amygdala. In turn, the amygdala regulates memory consolidation via its efferent projections to many other brain regions. In contrast to the enhancing effects on consolidation, high circulating levels of stress hormones impair memory retrieval and working memory. Such effects also require noradrenergic activation of the amygdala and interactions with other brain regions.

Key Words: Glucocorticoids, Emotionally Arousing Memory, Amygdala

S-VII-4

Microbiota-Brain-Gut axis: A new frontier in the physiology of mind-body interactions

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Early postnatal life represents a period of bacterial colonization, a time when a previously sterile milieu is inhabited by microorganisms that are likely to remain as residents throughout the life of the animal. The human intestine is more densely populated with microbes than any other organ, and 1014 bacteria inhabit the gastrointestinal tract of adult humans, which exceeds the number of eukaryotic cells (1013) of which the human body is constituted. Therefore, it seems natural that such colonizing bacteria would play a principal role in the postnatal maturation of the mammalian immune system. In addition, these bacteria aid in the digestion and absorption of macromolecules and act as a barrier to gut pathogens by blocking attachment to gut binding sites, which is the first step of bacterial pathogenicity. Thus, there is no doubt that most of our bacterial symbionts have several beneficial effects on host physiological functions; however, until recently, little has been known about whether or not such microbes can affect the development and function of the central nervous system. In 2004, we demonstrated for the first time that commensal microbes can influence the hypothalamic-pituitary-adrenal (HPA) stress response of the host. Namely, plasma ACTH and corticosterone elevation in response to restraint stress was substantially higher in germ-free (GF) mice than in specific pathogen free (SPF) mice. The exaggerated HPA stress response by GF mice was reversed by reconstitution with Bifidobacterium infantis. In contrast, monoassociation with enteropathogenic Escherichia coli, but not with its mutant strain devoid of the translocated intimin receptor gene, enhanced such a response to stress. Importantly, the enhanced HPA response of GF mice was partially corrected by reconstitution with SPF feces at an early stage, but not by any reconstitution exerted at a later stage, which thus indicates that exposure to gut microbes is a critical environmental determinant that regulates the development of the HPA stress response and also the set point for this axis. Recently, we developed a novel method for evaluating mouse behavior inside a contamination-free environment using a closed population of an inbred strain of mice with a common genetic background. Based on this method, behavioral analyses were conducted in gnotobiotic mice that were kept contamination-free even at the time of behavioral testing. As a result, EX-GF mice, the gnotobiotic mice reconstituted with normal SPF microbiota, were less anxious and active than GF mice using open-field and marble-burying tests. The norepinephrine, dopamine and serotonin turnover rates were higher in the EX-GF mice than in the GF mice in most regions of the brain, suggesting that monoaminergic neurotransmission might increase in the EX-GF mice comparing the GF mice. Mono-association with Clostridium (Brautia) coccoides reduced the anxiety level, but it did not affect the locomotor activity. In contrast, colonization with Bifidobacterium infantis decreased the locomotor activity, while having little effect on the anxiety level. These results taken together support the current view that gut microbiota

modulate brain development and behavior. Moreover, they also provide evidence for the existence of the "microbiota-gut-brain axis", an elaborate interaction among the microbiota, the gut and the brain. Given our data demonstrating normal gut microbiota to exert protective effects against impaired HPA stress response, commensal bacteria may play a crucial role against developing stress-related disorders such as anxiety and depression, through providing the host with "stress resilience" necessary for adapting to a rapidly changing external environment.

S-VIII-1

Molecular mechanisms of apoptotic cell recognition and clearance: New insights into the roles of small GTPases

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The deletion of unwanted or damaged cells is achieved by finely regulated cleaning process, efferocytosis1. It has been established that RhoA and Rac1 GTPases are antagonistically involved in cytoskeleton organization for clearance of apoptotic cells. Overall, Rac1 and its upstream activators facilitate engulfment of apoptotic cells, whereas RhoA and its downstream effector Rho kinase has an inhibitory role. However, the coordinate actions of Rac1 and RhoA at the individual phagocytic cup would sophisticatedly regulate phagocytic process, which are not fully addressed before. Using FRET biosensors to visualize the spatiotemporal dynamics of Rho family GTPases, here we showed that Rac1 and RhoA coordinately regulate critical points of phagocytic process through turning on/off their activities at precise timing and site. RhoA, a negative regulator, known to be down-regulated during phagocytosis, was transiently up-regulated at the phagocytic cup right before the ingestion of apoptotic cells. Rac1 was up-regulated around phagocytic cup during engulfment then immediately down-regulated ensuing actin disassembly prior to phagosome maturation. Demolition of these dynamic activities of RhoA and Rac1 leaded to uncontrolled engulfment and delayed phagosome maturation, respectively. Our results revealed distinct regulatory roles of Rho family GTPases, RhoA as an engulfment holder and Rac1 as a maturation holder during phagocytosis. We believe our findings provide insight how the phagocytes control determine when and where to eat apoptotic cells as well as to digest. Furthermore, understanding of regulatory mechanism of phagocytosis could be applied to therapeutic approaches based on phagocytosis of immune cells.

Key Words: apoptosis, phagocytosis, Rac1, RhoA

S-VIII-2

Arhgef16, a novel Elmo1 binding partner, promotes clearance of apoptotic cells via RhoG-dependent Rac1 activation

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Elmo is an evolutionarily conserved mammalian ortholog of Caenorhabditis elegans CED-12 with proposed roles during the removal of apoptotic cells, cell migration, neurite outgrowth, and myoblast. Elmo mediates these cellular processes by interacting with various proteins located in the plasma membrane, cytoplasm and nucleus, and by modulating their activities although it has no intrinsic catalytic activity. Because there are a limited number of proteins known to interact with Elmo, we performed a yeast two-hybrid screen using Elmo1 as bait to identify Elmo1-interacting proteins and to evaluate their mode of regulation. Arhgef16 was one of the proteins identified through the screen and subsequent analyses revealed that Arhgef16 interacted with Elmo1 in mammalian cells as well. Expression of Arhgef16 in phagocytes promoted engulfment of apoptotic cells, and engulfment mediated by Arhgef16 increased synergistically in the presence of Elmo1 but was abrogated in the absence of Elmo1. In addition, Arhgef16-mediated removal of apoptotic cells was dependent on RhoG, but independent of Dock1. Taken together, this study suggests that the newly identified Elmo1-interacting protein, Arhgef16, functions synergistically with Elmo1 to promote clearance of apoptotic cells in a RhoG-dependent and Dock1-independent manner.

Key Words: Arhgef16, Elmo, GEF, engulfment, apoptotic cell

S-VIII-3

Apoptotic cell clearance: Implication in resolution of lung inflammation and fibrosis

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The clearance of apoptotic cells by tissue macrophages and non-professional phagocytes is an essential process in tissue homeostasis, immunity, and resolution of inflammation. Apoptotic cell recognition actively leads to the production of anti-inflammatory mediators such as TGF-β, IL-10, and PGE_2. Recently, apoptotic cell recognition was shown to induce production of tissue cell growth factors or other stimuli that may normally contribute to the replenishment of damaged cells. In addition, changes in macrophage phenotype have been implicated in apoptotic cell-mediated immune modulation via induction of peroxisome proliferator-activated receptor gamma (PPARγ). Our results demonstrate that in vivo exposure to apoptotic cells enhances transcriptional HGF production and COX-2/PG_2 through a positive feedback loop in bleomycin-stimulated lungs, resulting in attenuation of lung injury and fibrosis. Moreover, apoptotic cell instillation contributes to anti-inflammatory and anti-fibrotic responses via upregulation of PPARγ expression and subsequent activation, leading to enhancement of efferocytosis and coordinated regulation of TGF-β, IL-10, and HGF. Our study supports the concept that Thus, early apoptotic cell instillation and/or strengthening apoptotic cell recognition and clearance system may be a novel potent strategy for prevention and intervention for fibrotic lung diseases.

Key Words: apoptotic cell clearance, alveolar macrophages, inflammation, fibrosis

S-VIII-4

Resolution in Epithelial Mesenchymal Transition and More

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Epithelial-mesenchymal-transition (EMT) is a critical step for tumor cells to initiate process of metastasis. Resolvins promote the resolution of inflammation and phagocytosis of macrophages. However, the role of resolvins in EMT of cancer is not fully studied. We investigated effects of resolvins on transforming growth factor, beta 1 (TGF-β1)-induced EMT. Expression of E-cadherin and N-cadherin in A549 lung cancer cells was examined by Western blot and confocal microscopy. Involvement of lipoxin A4 receptor/formyl peptide receptor 2 (ALX/FPR2) was investigated by gene silencing. TGF-β1 induced expression of N-cadherin in A549 lung cancer cells, and resolvin D1 and D2 suppressed the expression of N-cadherin even at low concentrations (1 ~100 nM). Resolvin D1 and D2 also reduced the expression of zinc finger E-box binding homeobox 1 (ZEB1). The effects of resolvin D1 and D2 were also confirmed in other lung cancer cell lines including H838, H1299, and H1703 cells. Resolvin D1 and D2 did not induce the proliferation of A549 lung cancer cells. Resolvin D1 and D2 also blocked the TGF-β1-induced morphological change. Resolvin D1 and D2 also suppressed the TGF-β1-induced migration and invasion of A549 cells. Resolvin D1 acts via ALX/FPR2 and GPR32. Thus, we studied the involvement of ALX/FPR2 and GPR32 in the inhibitory effects of resolvin D1 on TGF-β1-related EMT. Gene silencing of ALX/FPR2 and GPR32 suppressed the action of resolvin D1. Ectopic expression of ALX/FPR2 or GPR32 increased the effects of resolvin D1. The mechanism of action of GPR32 is not clear. To understand the action of GPR32, we investigated the binding partner of GPR32 using yeast two hybrid method. Several new partners of GPR32 were identified. Our results suggest that resolvin D1 suppressed TGF-β1-induced EMT via ALX/ FPR2 and GPR32 by reducing the expression of ZEB1. These will help us to understand the role of resolution in EMT.

Key Words: Resolvin, Resolution, EMT, GPR32, A549 cells

P01-01

Inhibitory effect of cyanidin-3-glucoside on proliferation and induces cell death in human prostate cancer LNCaP cells through calcium homeostasis of endoplasmic reticulum

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The level of Ca^{2+} in the endoplasmic reticulum is one of critical factor for cell growth and death. Cyanidin-3-glucoside (C3G) is a member of anthocyanins which belong to the flavonoid family. In this study, we investigated the effects of C3G extract on proliferation, cell death and calcium homeostasis of ER in human prostate cancer LNCaP cells. Treatment with C3G (1 μg/ml to 100 μg/ml) for 24 h～72 h significantly inhibited cell proliferation in the absence and presence of epidermal growth factor (EGF) (2 ng/ml), in time- and concentration-dependent manner. C3G (1 μg/ml to 100 μg/ml) for 24 h - 72 h also induced cell death in the absence and presence of EGF (2 ng/ml) in time- and concentration-dependent manner. C3G (15 μg/ml, 24 h to 72 h) significantly reduced the increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) induced by treatment of thapsigargin (1 μM, 24 h to 72 h) in the absence and presence of EGF. Under Ca^{2+}-free condition, C3G (15 μg/ml, 24 h to 72 h) also reduced the $[Ca^{2+}]i$ following treatment of thapsigargin (1 μM, 24 h to 72 h). Treatment with C3G (15 μg/ml, 24 h to 72 h) significantly reduced the Ca^{2+} concentration in the ER in the absence and presence of EGF. Our data suggest that cyanidin-3-glucoside inhibits proliferation and induces cell death in human prostate cancer LNCaP cells through calcium homeostasis of endoplasmic reticulum.

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Key Words: Cyanidin-3-glucoside, ER calcium, Flavonoid, LNCaP cells, Proliferation

P01-02

Cyanidin-3-glucoside protects cultured hippocampal neurons against glutamate-induced neurotoxicity by inhibiting Ca^{2+}-induced mitochondrial depolarization and formation of reactive oxygen species

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Glutamate-induced excitotoxicity is widely thought to be a key event of neuronal damage, ranging from acute insults such as ischemia and traumatic injury to chronic neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Cyanidin-3-glucoside (C3G), a member of the anthocyanin family, is a potent natural antioxidant. However, less is known about how C3G affects glutamate-induced cell death in neurons. We studied effects of C3G on glutamate-induced cell death in cultured rat hippocampal neurons from embryonic day 17 maternal Sprague-Dawley rats using a MTT assay for cell survival and digital imaging methods for Zn^{2+}, Ca^{2+}, ROS and mitochondrial membrane potential. Reproducible $[Zn^{2+}]i$ and $[Ca^{2+}]i$ increases were elicited by applying glutamate (100 μM) for 7 min at 35 min interval. Pretreatment with C3G (100 ng/ml to 1 mg/ml) for 30 min inhibited the glutamate-induced $[Zn^{2+}]i$ and $[Ca^{2+}]i$ response in a concentration-dependent manner. Two membrane permeable antioxidants such as trolox (100 μM) and DTT (50 μM) significantly inhibited $[Zn^{2+}]i$ responses, but they did not affect $[Ca^{2+}]i$ response. C3G blocked glutamate-induced formation of ROS. Trolox and DTT also blocked glutamate-induced formation of ROS. C3G moderately inhibited glutamate-induced mitochondrial depolarization. Intracellular Zn^{2+}-chelator TPEN (5 μM) slightly inhibited glutamate-induced mitochondrial depolarization. However, trolox and DTT did not affect glutamate-induced mitochondrial depolarization. Treatment with C3G (15 μg/ml), DTT (50 μM) and trolox (100 μM) for 24 h attenuated glutamate-induced neuronal cell death in cultured rat hippocampal neurons. Taken together, these results suggest that cyanidin-3-glucoside inhibits glutamate-induced cell death in cultured rat hippocampal neurons by inhibiting Ca^{2+}-induced mitochondrial depolarization and formation of reactive oxygen species.

Acknowledgements: This work was carried out with the support of "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022014)" Rural Development Administration, Republic of Korea.

Key Words: Flavonoid, glutamate, mitochondrial membrane potential, reactive oxygen species, Zn^{2+}

P01-03

Adolescent mice suffered from neonatal maternal separation express emotional disorder and defects of long term-potentiation in hippocampal synapse

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Exposure to maternal separation (MS) during early life is an identified risk factor for emotional disorder such as anxiety, depression later in life. MS has been used as an animal model to study changes in behavior and neurochemistry. Recently, it has been suggested that GABAergic dysfunction was involved in depression. This study investigated the effects of neonatal maternal separation on the behavior and long term potentiation (LTP) at hippocampal

Mossy fiber (MF)-CA3 synapse which was strongly regulated by GABAergic modulation After MS procedure for 19 days, we measured anxiety, depression, aggression level, using elevated plus maze, 3 consecutive days open field test, forced swim test, social interaction test, and tube dominance test. As results, daily body weight gain in the MS group did not significantly different in postnatal day (PND) 1～22 compared with handling group (HG). However, MS mice exhibited an increased immobility time in forced swim test and spent more time in closed arms in elevated plus maze, when compared to HG. MS mice also showed more aggressive behavior in tube dominance test than HG. In addition, the magnitude of LTP in the MS group was reduced, compared HG. Our results indicate that early life stress by MS cause later emotional disorder, and these effects are association with synaptic plasticity at the hippocampal MF-CA3 synapse.
Key Words: neonatal maternal separation, depression, anxiety, aggression, long term potentiation

P02-01

Oxidative stress mediates high phosphate-induced secretory defects in rat pancreatic β-cells

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Inorganic phosphate (Pi) is an essential constituent of the body, playing an important role in cell signaling and energy metabolism. In insulin releasing cells, Pi transport into mitochondria is critical for the generation of ATP, a signaling factor in metabolism-secretion coupling. However, excessive Pi load elicits osteogenic differentiation in vascular smooth muscle cells and cytotoxicity in different cell types, of which the molecular mechanism remains elusive. In this study, we characterized the plasmalemmal and mitochondrial Pi transport and its detrimental effects on pancreatic β-cell function. Electrophysiology and fluorescence imaging experiments were performed to estimate ionic currents, cytosolic and mitochondrial pH, ion concentrations, superoxide levels, and mitochondrial membrane potential (ψm). In rat pancreatic β-cells and its clonal cell line INS-1E cells, a type III NaPi cotransporter, PiT-1 was predominantly expressed and responsible for cytosolic Pi uptake. PiT-1-mediated Pi influx was dependent on extracellular pH and led to alkalinization of cytosol and mitochondrial matrix. Mitochondrial superoxide generated during hyperpolarization of the ψm by Pi uptake into mitochondria was accelerated by cytosolic alkalinization. Mitochondrial oxidative stress and matrix alkalinization with high Pi level can cooperatively stimulate the opening of permeability transition pore resulting in mitochondrial dysfunction. High Pi exposure decreased cytosolic insulin content without transcriptional changes, and diminished glucose-stimulated ATP synthesis, cytosolic Ca^{2+} oscillation and insulin secretion. All these defects were recovered by pretreatment with mitochondrial antioxidant. We suggest that mitochondrial superoxide mediates high Pi-induced secretory defects in pancreatic β-cells, which could explain the reduction of insulin content and secretion in hyperphosphatemic conditions. This study was supported by the Myung Sun Kim Memorial Foundation (2014).
Key Words: Inorganic phosphate, oxidative stress, pancreatic beta-cells, permeability transition pore, insulin secretion

P02-02

Protective effect of GLP-1 on pancreatic beta-cells via KATP channel-mediated pathway

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Chronic glucose stimulation of beta-cells imposed by insulin resistance is a risk factor for the development of beta-cell dysfunction and type 2 diabetes mellitus (T2DM) since it causes depletion of islet insulin stores and beta cell loss. It has been known KATP channel openers bind to KATP channels in the plasma membrane, leading to hyperpolarization and inhibition of Ca^{2+} influx and resulting in inhibition of insulin secretion. Thus, treatment with KATP channel openers may lead to metabolic beta cell “rest,” protecting beta cells against exhaustion and making them less prone to apoptosis. It has been known that GLP-1 play a central role in the homeostasis of pancreatic beta cell mass as well as function. In normal condition, GLP-1, as a physiologic regulator of insulin secretion, has been demonstrated to enhance glucose-induced insulin secretion by facilitating closure of KATP channels. However, the effect of GLP-1 on KATP channel activity in specific conditions, like beta cell exhaustion and glucotoxicity has not been studied. In this study, we would like to elucidate whether GLP-1 also facilitate closure of KATP channels in specific conditions, like beta cell exhaustion. Resting membrane potential was measured at using a perforated whole cell patch clamp technique. In normal condition, GLP-1 facilitated closure of KATP channels, resulting in enhancement of glucose-induced insulin secretion in rat pancreatic beta cells. For inducing beta cell exhaustion, cells were treated with high glucose (17 mM glucose) for 2h. After 2 h, 20 nM GLP-1 was treated with high glucose for 30 min. In contrast to previous finding, GLP-1 induced membrane hyperpolarization via KATP channel activation (opening). This GLP-1-induced KATP channel activation was dependent on intracellular PI3K activity. These results suggest that GLP-1 might induce beta cell "rest" by activating KATP channel in the state of beta cell exhaustion. And it can protect pancreatic beta cell against overwork-induced cell apoptosis and prevent the development of diabetes. As a physiological modulator, GLP-1 can control beta cell excitability by controlling KATP channel activity.
Key Words: GLP-1, beta-cell, KATP channel, insulin resistance

P02-03

Stimulation of high atrial stretch-induced anp secretion by angiotensin IV through IRAP by activating the PI3K/Akt/mTOR signaling pathway

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Angiotensin IV (Ang IV) is formed by aminopeptidase N (APN) from angiotensin III (Ang III) by removing the first N-terminal amino acid. Recently, we reported that angiotensin II (Ang II) inhibits atrial natriuretic peptide (ANP) secretion via angiotensin II type 1 receptor (AT1R). In contrast, Angiotensin-(1-7) [Ang-(1-7)] and Ang III stimulate ANP secretion via Mas receptor and angiotensin II type 2 receptor (AT2R), respectively. However, it is not known whether there is any relationship between Ang IV and ANP secretion. Therefore, the aim of the present study was to determine the effect of Ang IV on ANP secretion and its downstream signaling pathway using in isolated perfused beating atria and in vitro study. Ang IV (0.1, 1 and 10 μM) stimulated high atrial stretch-induced ANP secretion and ANP concentration in a dose-dependent manner. The augmented effect of Ang IV (1 μM) on high atrial stretch-induced ANP secretion and concentration was attenuated by pretreatment with insulin-regulated aminopeptidase (IRAP) antagonist but not by AT1R or AT2R antagonist. Pretreatment with inhibitors of downstream signaling pathway which phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (mTOR) blocked Ang IV-induced ANP secretion and concentration. These signaling pathway was confirmed by phosphorylated protein in H9C2 cardiomyocyte cell line. Therefore, these results suggest that Ang IV stimulates ANP secretion and concentration via IRAP-PI3K-Akt-mTOR pathway.

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Key Words: Angiotensin IV, ANP, Insulin-Regulated Aminopeptidase (IRAP), PI3K, mTOR, H9C2

P02-04

TonEBP/NFAT5 suppresses adipocyte differentiation of 3T3-L1 cells

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Obesity resulting from excessive accumulation of white adipose tissue is closely related to the chronic diseases such as diabetes, hyperlipidemia, and hypertension. White adipose mass is determined by the number and size of adipocytes. The differentiation of adipocyte can be divided into two broad stages. Determination phase results in the conversion of the stem cell to a preadipocyte. The committed cells undergo terminal differentiation manifested by formation of lipid droplets as well as adipocyte specific protein. Mouse 3T3-L1 cell line is widely used as an in vitro model for studying terminal adipocyte differentiation. TonEBP/NFAT5 belongs to the Rel family of transcription factors and plays important roles in the development and maintenance of kidney. However, recent reports suggest that TonEBP/NFAT5 function is not limited to the renal medulla. Although the functions of TonEBP/NFAT5 during chondrogenesis and myogenesis are reported recently, its role in the adipogenesis is not known yet. TonEBP/NFAT5 protein expression was dramatically reduced during adipocyte differentiation of 3T3-L1 cells. RNAi-mediated knock down of TonEBP/NFAT5 facilitated adipogenesis. Whereas sustained expression of TonEBP/NFAT5 using adenovirus suppressed the formation of lipid droplet and the expression of FABP4, marker for terminally differentiated adipocytes. TonEBP/NFAT5 inhibited not only the expression of PPARγ, master regulator of terminal adipocytes but mitotic clonal expansion, prerequisite for the adipogenic differentiation of 3T3-L1 cells. These result suggest that TonEBP/NFAT5 may be an important regulatory factor in the differentiation of adipocytes.

Key Words: TonEBP/NFAT5, terminal adipocyte differentiation, PPARr, FABP4

P02-05

Relation of RCAN1 (regulator of calcineurin 1) to osteoclast differentiation in vitro

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Purpose: RCAN1 (regulator of calcineurin, type 1) is known as an endogenous calcineurin inhibitor. It plays an important role on pathogenesis of diseases related with a calcineurin-NFATc1 signaling pathway such as Down syndrome. RCAN1-4, one of RCAN1 isoforms, shows the characteristics of NFATc1-dependent regulation and is induced by Ca^{2+} stimulus. During RANKL-stimulated osteoclastogenesis, a calcineurin-NFATc1 pathway is critical. However, there is little information about the role of RCAN1 on the calcineurin-NFATc1 pathway and thus osteoclast differentiation. This study was undertaken to investigate whether the change in RCAN1 expression is related to osteoclast differentiation in vitro. **Materials and methods:** To stimulate osteoclast differentiation of a mouse monocytic cell line, RAW 264.7 and primary cultured bone marrow monocytes (BMMs), the cells were treated with 50 ng/ml of RANKL and/or M-CSF for 24 hr or 72 hr. The expression levels of NFATc1, calcineurin, and RCAN1 determined by RT-PCR and Western blotting. Endogenous level of RCAN1, RCAN1-4, and calcineurin mRNAs was quantitated by real-time RT-PCR, and the osteoclast differ-

entiation was examined by TRAP staining. To evaluate the effect of RCAN1 overexpression on osteoclastogenesis, BMMs were transfected with a mouse RCAN1-4 cDNA plasmid and osteoclast differentiation was analyzed after transfection for 24 hr and 72 hr. **Results:** Twenty four hr after RANKL stimulation, mRNA and protein expression of NFATc1, RCAN1, and calcineurin was not affected. After 72 hr, the expression of NFATc1 and RCAN1 was greatly increased at the levels of mRNA and protein, while calcineurin protein expression was not changed despite an increase in its mRNA level. TRAP staining showed more prominent osteoclastogenesis at day-5 than day-4. When a construct of mouse RCAN1-4 gene was transfected to RAW264.7 cells and BMMs, the expression of RCAN1-4 and RCAN1 mRNAs was increased several-fold, suggesting that effective transfection has occurred in both cells. RCAN1 protein, however, was not increased. BMMs were differentiated into distinct osteoclasts after transfection for 24 hr and 72 hr and apparently not different from mock controls. There seemed to be no difference in osteoclast differentiation between 24 hr and 72 hr transfection. **Conclusion:** These results suggest that during RANKL-stimulated osteoclast differentiation, a calcineurin-NFATc1 pathway is not hindered despite increased RCAN1 expression and the overexpression of RCAN1-4 gene does not seem to affect osteoclast differentiation.

Key Words: Bone marrow monocytes, NFATc1, Transfection, RCAN1-4, Calcineurin-NFATc1 pathway

P02-06

Functional characterization of MTERF3 binding protein, MBP

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MTERF3 binding protein (MBP) is a novel mitochondrial protein which binds storngly to MTERF3, a major negative regulator of mitochondrial DNA transcription. Our preliminary results shows that reduction of MBP cause severe deregulation of mitochondrial transcription. It is very likely that MBP, together with MTERF3, play a very important role in the regulation of mitochondrial transcription. In this study, we are aiming to decipher in vivo function of MBP and its molecular mechanism.

Key Words: mitochondria, metabolism, respiration

P02-07

Orexin A time-dependently regulates plasma insulin and leptin levels in response to high glucose loading

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Orexin A (OXA) is a major orexin implicated in the regulation of energy metabolism. Orexin receptors (OXRs) are located in the endocrine pancreas and adipose tissue; however, little is known regarding their physiological function. This study investigated the roles of OXA in glucose homeostasis; furthermore, the effects of OXA on insulin secretion and intracellular target molecules were identified in mouse pancreatic beta cells. During high glucose loading, exogenous OXA acutely enhanced plasma insulin levels and caused a delayed but prolonged increase in plasma leptin levels as a negative feedback to insulin secretion. This OXA effect was also observed in high fat-diet mice. OXA significantly augmented the first phase of glucose-stimulated insulin secretion, which increased intracellular Ca^{2+} levels through adenylated cyclase and ryanodine receptor activation. This Ca^{2+}-dependent insulinotropic effect of OXA was blocked in Epac2 knockout (KO) beta cells. However, the OXA-induced leptin increase was maintained in Epac2 KO mice. These results suggest that OXA is a critical regulator of both insulin and leptin secretion in response to high glucose via different cellular mechanisms for blood glucose homeostasis during prandial and post-prandial period. Thus, OXA might have therapeutic potential to improve blood glucose control in patients with type 2 diabetes.

Key Words: orexin, insulin, leptin, pancreatic beta cells, Epac2

P03-01(PO-1)

The Central Governor Model of Maximal Exercise Performance Regulation: Review Article

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The purpose of this study is to provide basic data for the improvement of athletic performance in elite athletes and to expand the field of brain research in Korea by reviewing the relationship between maximal exercise performance and the brain. In 1923, Archibald Vivian Hill, a muscle physiologist, reported that muscle fatigue during exercise was determined by “peripheral fatigue” caused by biological changes and that the central nervous system could not directly cause fatigue. He also reported that the maximum capacity of the heart at the end of exercise was a pivotal factor in causing muscle fatigue. However, with the introduction of the “Central Governor Model(CGM)” by Timothy David Noakes in 1997, the theory that maximal exercise performance was controlled by the brain was systematically established. According to the CGM, the central nerves of the brain voluntarily suspend the continuation of maximal exercise performance in order to maintain homeostasis. however, because this mechanism involves overcompensation by the brain to protect the body, it prevents demonstration of the potential to sustain maximal exercise performance. Therefore, we propose that if domestic athletes can fully understand and manage this phenomenon while exercising in the field, it can play

an effective role in maximizing exercise performance during training or competition. Moreover, we need continued studies for establishing new protocols to increase the accuracy of maximum oxygen uptake measurement methods.
Key Words: Central governor, Maximal exercise test protocols, Central nervous system, Fatigue

P03-02(PO-2)

The possibility of irisin as a biomarker of sarcopenia

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Sarcopenia is a gradual decrease of skeletal muscle mass and function during aging. Due to the absence of clear clinical indicator, it is required to develop useful biomarkers that can be quantified in a feasible, accessible manner and can be served the diagnosis and therapy of pathological muscle atrophy in general clinical practices and trials. Recently, skeletal muscles have been established as an endocrine organ. Irisin is one of the myokines derived from skeletal muscles and is known to be induced upon vigorous exercise to facilitate thermogenesis of adipocytes. The aims of this study are to assess the association of serum irisin level with sarcopenia in human, and to determine whether irisin affects muscle growth as a anti-atrophic factor in vitro. In cross-sectional human study, we measured serum levels of irisin, as well as muscle mass and function in 715 Korean persons. Firstly, we divided subjects into 3 age groups: young, middle aged adults and elderly. Meanwhile, the subjects were classified as sarcopenia using cutoff values of muscle atrophy and muscle strength regarding to gender. Serum irisin levels were significantly lower in elderly groups compared to young adults in both men and women. In addition, the serum irisin level was positively correlated with muscle mass and strength. This study also revealed that the serum irisin levels were significantly lower in the subjects with sarcopenia than that of normal group, regardless of gender (normal vs. sarcopenia, mean±SD, 1491±501 vs. 1178± 399 ng/mL, $p < 0.001$). The cut-off values of serum irisin to detect sarcopenia were determined as 1069.9 ng/ml (AUC: 0.72, sensitivity: 0.836, specificity: 0.633, $p < 0.001$). On the other hand, to investigate the function of irisin as a trophic factor, we applied DEX-mediated myotube atrophy model using C2C12 muscle cells in vitro. Irisin treatment increased phosphorylation of IGF-1 receptor and Akt through enhancing IGF-1 expression in a time-dependent manner. Moreover, irisin attenuated DEX-induced downregulation of FoxO3α phosphorylation, upregulation of atrogin-1 and elevation of proteasome activity in C2C12 myotubes. These results demonstrate a novel anti-atrophic function of irisin in muscle cells through suppression of FoxO/atrogenes-mediated ubiquitin proteasome pathway. Taken together, the irisin not only might be related to muscle mass and function in human aging, but also could exert a noticeable benefit to inhibit myotube atrophy in culture condition. Therefore, it may be considered as a potential biomarker for muscular atrophy/sarcopenia.
Key Words: Irisin, Sarcopenia, Muscle mass, Muscle strength, DEX-mediated myotube atrophy

P03-03

Somatotype Analysis of Korean Youth Soccer Players According to Playing Position

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The purpose of this study was to show somatotype and body composition differences between playing position of Koran youth soccer. In this study, we observed 22 Korean youth soccer players. We are divided playing position as follow: forward (FW), mid-fielder (MF), defender (DF), and goalkeeper (GK). The participants were measured with the modified somatotype method of Heath-Carter, resulting in three kinds of somatotypes (endomorph, mesomorph, and ectomorph) and a balanced type (central type). Body composition such as lean body mass (LBM), fat free mass (FFM), fat mass (FM), basal metabolic rate (BMR) of participants were measured using a precision body composition analyzer (InBody 520, Biospace, Korea). The youth soccer players consisted of twelve ectomorphic, eight mesomorphic, and two central predominant types. Subdividing the youth soccer player's somatotypes resulted in seven balanced ectomorph, four mesomorphic ectomorph, three ectomorphinc mesomorph, three balanced mesomorph, two central, one endomorphic ectomorph, one endomorphic mesomorph, and one mesomorph-endomorph type, respectively. Just DF position player was taller than other field players (FW, MF), but almost similar in physical characteristics of field players. GK players were very taller and heavier than other position players. However, somatotype components were not different significantly in all position players. LBM, FFM, FM, BF, and BMR were not different significantly in field players. However, LBM, FFM, BMR were significantly higher in GK players than FW, MF players. LBM, FFM, BMR value among the GK players and DF players were not statistically significant, but was a large difference. Our study provides, in part physical characteristics of youth soccer players to establish a reference for the study of sports health sciences.
Key Words: Somatotype analysis, Youth soccer player, Sports rehabilitation

P03-04

Cofilin Phosphorylation Decreased by Serum-Free Starvation with Low Glucose in the L6 Myoblasts for Physiotherapy Research

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Many studies have been using cell culture models of muscle cells with exogenous cytokines or glucocorticoids to mimic atrophy in in vivo and in vitro tests. However, the changes in the phosphorylation of atrophy-related cofilin are still poorly understood in starved skeletal muscle cells. In this study, we first examined whether or not phosphorylation of cofilin is altered in L6 myoblasts after 3, 6, 12, 24, 48, and 72 hours of serum-free starvation with low glucose. We used Western blotting to exam protein expression and phosphorylation in atrophied L6 myoblasts. L6 cell sizes and numbers were diminished as a result of serum-free starvation in a time-dependent manner. Serum-free starvation for 3, 6, 12, 24, 48, and 72 hours significantly decreased the phosphorylation of cofilin, respectively. Although cofilin is essential for maintenance of skeletal muscle mass, it has not been reported that phosphorylation of cofilin is related to atrophy caused by serum-free starvation in the area of physical therapy. However, further systematic studies in the area of physical therapy such as electrotherapy, neurotherapy, hydrotherapy, and others are needed to confirm the mechanism of cofilin under atrophic conditions. These results suggest that starvation-induced atrophy may be in part related to changes in the phosphorylation of cofilin in L6 myoblasts.
Key Words: Cofilin, Serum-free Starvation, L6 myoblasts

P03-05

Analysis of Pulmonary Function in Korean Youth Soccer Players for Sports Rehabilitation

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Pulmonary function is very important on soccer players. However, the changes in the respiration of soccer plyers are not fully understood in terms of forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1). Therefore, the purpose of this study was to analysis of pulmonary function in Korean youth soccer players. Pulmonary function was measured in twenty Korean young soccer players while they were in the sitting position using Spirobank G spirometer. The FVC of youth soccer players was higher compared with the Korean predictive equation value. The FEV1 of youth soccer players also was significantly higher compared with the Korean predictive equation value. These results suggest that soccer affects the sensitivity of pulmonary function in Korean youth soccer players. Therefore, the present study may contribute to our understanding of importance of pulmonary function in soccer game from the perspective of health science research.
Key Words: Pulmonary function, Korean youth soccer players, Health science research

P03-06

Somatotype Analysis of Freestyle Wrestlers Compared with Nonathletes for Sports Rehabilitation

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The purpose of this study was to show somatotype and physical characteristic differences between freestyle wrestlers and nonathletes. Differences among weight categories for freestyle wrestlers were also examined. In this study, we first observed thirteen elite wrestlers and thirteen nonathletes. The participants were measured with the modified somatotype method of Heath-Carter, resulting in 3 kinds of somatotypes (endomorphic, mesomorphic, and ectomorphic) and 1 balanced type (central type). The nonathletes consisted of 4 endomorphic, 5 mesomorphic, 2 ectomorphic, and 2 central types. The wrestlers consisted of 12 mesomorphic types and only 1 ectomorphic type. Subdividing wrestler somatotypes resulted in 8 endomorphic mesomorph, 3 balanced mesomorph, and 2 mesomorph-ectomorph types. Wrestlers had higher weight, body mass index, and mesomorphic component values than did nonathletes. However, the wrestlers' endomorphic and ectomorphic component values were lower than in the nonathletes. Furthermore, wrestlers in the heavy class tended to have higher endomorphic, very high mesomorphic, and lower ectomorphic component values. The data from our study provides in part physical characteristics of freestyle wrestlers that can be used to establish a reference for systemic study of

sports health sciences.
Key Words: Somatotype analysis, Freestyle wrestlers, Sports health sciences

P03-07

Resistance exercise improves cardiac function by modulation of mitochondrial biogenesis and uncoupling proteins in type 2 diabetic hearts

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Type 2 diabetes switches energy substrate utilization with reduced cardiac efficiency, which causes metabolic and mitochondrial abnormality, leading to pathological cardiac remodeling. Here, we assessed the effect of resistance exercise on the mitochondrial biogenesis and activation of uncoupling proteins in type 2 diabetic hearts. Twenty eight-week-old Otsuka Long Evans Tokushima Fatty (OLETF) rats were divided into 2 groups; sedentary (SED) and resistance exercise (EXR). EXR rats were exercise trained for 12 weeks and the effect of resistance exercise on OLETF diabetic rats were investigated by glucose tolerance tests, lipid profiles, echocardiography, and mitochondrial functional studies. Resistance exercise successfully improved hyperglycemia and hyperlipidemia in OLETF rats. In their cardiac function, SED rats showed lower stroke volume (SV), decreased left ventricular chamber size and higher relative wall thickness ratio (RWT), indicating pathological cardiac remodeling, EXR rats improved SV by increased LV chamber and cardiac contractility. SED rats also showed disrupted mitochondrial morphology and impaired mitochondrial function, whereas resistance exercise training improved of those abnormalities with higher protein expression, including Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and mitochondrial transcription factor A (TFAM). Importantly, resistance exercise increased cardiac glucose transporter 4 (GLUT 4), glucose utilization protein level, whereas decreased fatty acid metabolism regulated proteins, including carnitine palmitoyltrasferase 1 (CPT1), UCP2 and UCP3 compared to SED group. These data demonstrate that resistance exercise training restored mitochondrial and cardiac dysfunction in type 2 diabetic hearts via shifting energy utilization and enhancing cardiac energy efficiency.
Key Words: Type 2 diabetes, resistance exercise, mitochondrial function, ROS, uncoupling protein

P03-08

Ursolic acid-induced elevation of serum irisin augments muscle strength during resistance training in men

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Ursolic acid (UA), a type of pentacyclic triterpenoid carboxylic acid purified from natural plants can promote skeletal muscle development. We measured the effect of resistance training (RT) with/without UA on skeletal muscle development and related factors in men. Sixteen healthy male participants (age, 29.37±5.14 years; body mass index= 27.13±2.16 kg/m2) were randomly assigned to RT (n=7) or RT with UA (RT+UA, n=9) groups. Both groups completed 8 weeks of intervention consisting of 5 sets of 26 exercises, with 10～15 repetitions at 60～80% of 1 repetition maximum and a 60～90-s rest interval between sets, performed 6 times/week. UA or placebo was orally ingested as 1 capsule 3 times/day for 8 weeks. The following factors were measured pre-and post-intervention: body weight, insulin, insulin-like growth factor-1 (IGF-1), irisin, and skeletal muscle strength. Body fat percentage was significantly decreased ($P < 0.001$) in the RT+UA group, despite body weight, body mass index, lean body mass, glucose, and insulin levels remaining unchanged. IGF-1 and irisin were significantly increased compared with baseline levels in the RT+UA group ($P < 0.05$). Maximal right and left extension ($P < 0.01$), right flexion ($P < 0.05$), and left flexion ($P < 0.001$) were significantly increased compared with baseline levels in the RT+UA group. These findings suggest that UA-induced elevation of serum irisin may be useful as an agent for the enhancement of skeletal muscle strength during RT.
Key Words: Ursolic acid, Irisin, IGF-1, Muscle strength, Resistance training

P04-01(PO-3)

The effect of substrate and osmolarity on mitochondrial respiration.

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Mitochondrial respiration is the essential process of the electron transport chain (ETC) to consume the oxygen and to produce carbon dioxide. Mitochondrial respiration is the source of ATP production and approximately 85% to 90% of the total oxygen consumption is used for the synthesis of ATP. We have shown the combination of the mitochondrial substrates, malate and pyruvate, could maximize NADH level compared to the NADH level with single substrate. We would like to measure TCA cycle activity with mitochondrial oxygen consumption in different substrate concentrations. The substrates (malate+pyruvate) concentration (μM) of 10, 30, 100, 300, 1000, and 5000 were used. To

maximize ETC activity, we added FCCP, an uncoupler of ETC. We found the mitochondrial oxygen consumption was increased as the mitochondrial substrate concentration was increased. In parallel, NADH level was measured and the level was increased in a dose dependent manner and maximized around 300 μM. However, oxygen consumption was further increased when the substrates concentration was 5 mM. This effect may be caused by the osmolarity changes. We tested the effect of the different osmolarity on mitochondrial oxygen consumption. In the presence of 80 mM KCl, the basal osmolarity was set to 200 mOsm and the osmolarity was increased by the addition of mannitol. When osmolarity was 400 mOsm, the oxygen consumption was suddenly increased. These results suggest that the increase of osmotic pressure could activate ETC. When we checked the NADH consumption rate by changing osmolarity, the increase osmolarity also increase NADH consumption. Both results suggest that the change of osmolarity could affect the ETC activity. The above results showed that the mitochondrial substrates around 300 μM may be enough to maintain mitochondrial respiration and the increase of osmolarity could activate the ETC. Cytosolic osmolarity increases are frequently seen in ischemic condition. This mechanism might be related to ischemic preconditioning mechanism.

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Key Words: Mitochondria, Respiration, Osmolarity, NADH

P04-02

Effect of cytosolic K^+ on mitochondrial function

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Mitochondrial function is maintained in cytosolic environment. We have shown the cytosolic mitochondrial substrates such as malate affect mitochondrial function. It is well known the increase of cytosolic Ca^{2+} induces the increase of intra-mitochondrial Ca^{2+}, which, in turn, activates tricyclic acid (TCA) cycle to generate NADH. Cytosolic Na+ may reduce intra-mitochondrial Ca^{2+} via Na+-Ca^{2+} exchanger. However, the functional role of the cytosolic K^+ was not understood very well. We have shown the removal of cytosolic K^+ could attenuate Na+-Ca^{2+} exchange activity. However, any other metabolic consequences by changing cytosolic K^+ have not been shown. Recently, we found the substitution of the cytosolic K^+ with sucrose or mannitol could eliminate O2 consumption in mitochondria. These results surprised us because most mitochondria isolation procedure used a sucrose environment. We raise the questionnaire what are the roles of cytosolic K^+ environment in mitochondrial function such as NAHD level, mitochondrial membrane potential, etc. Mannitol or sucrose instead of KCl could swell the mitochondrial, even though the same osmolar solution was used. The NADH level was decreased. When KCl solution was perfused, the NADH was rapidly recovered even overshoot. From the above results, without K^+, mitochondria TCA cycle or the electron transport chain may be blocked. Interestingly, when we substituted K^+ with N-methyl-d-glucamine (NMDG), the mitochondrial potential was more hyperpolarized in a dose dependent manner. The NMDG substitution induced mitochondrial swelling, which may link to the hyperpolarization, however, the exact mechanism need to be pursued. The above preliminary results were very important to understand mitochondrial physiology, which has never been pursued in terms of K^+ environment. Lots of further studies are necessary to see the various aspects of mitochondrial function related to K^+ environment.

Acknowledgements: This work was supported by the grant from NRF (NRF-2014M3A9D70 34366)
Key Words: Mitochondria, Potassium, NADH, Oxygen

P04-03(PO-12)

Sparfloxacin slows Ca^{2+}-dependent inactivation of L-type Ca^{2+} current, inducing action potential prolongation in ventricular myocytes

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Introduction: The proarrhythmic effects of quinolone antibiotics used clinically have been assessed by measuring the rapidly activating delayed-rectifier K^+ current (IKr) antagonist potency. However, the gaps between clinically reported proarrhythmic effects and IKr antagonist potency remain unexplained. We sought to determine the relevance of L-type Ca^{2+} current (ICaL) in arrhythmogenic effects of quinolone antibiotic using sparfloxacin. **Methods and Results:** In the present study, we investigated possible involvement of ICaL in the sparfloxacin-induced action potential duration (APD) prolongation in neonatal rat ventricular myocytes using patch clamp technique. Even in the presence of IKr blocker, E4031, APD was concentration-dependently prolonged by sparfloxacin at 2 Hz stimulation frequency, indicating the involvement of additional channel in sparfloxacin-induced proarrhythmic properties. The APD prolongation was associated with a slowing of inactivation of ICaL. In contrast to sparfloxacin, four other quinolones including ciprofloxacin, enoxacin, ofloxacin, and levofloxacin, which are with little proarrhythmic potentials, did not affect ICaL. Further analysis showed that sparfloxacin reduced Ca^{2+}-dependent of inactivation (CDI). Consistent with modulation of CDI by sparfloxacin, replacement of extracellular Ca^{2+} with Ba^{2+} abolished sparfloxacin action on ICaL inactivation. In addition, we found that sparfloxacin modulated Ca^{2+}-dependent inactivation of ICaL in a use-dependent way. **Conclusion:** The present findings demonstrate the role of ICaLin sparfloxacin-induced APD prolongation. We

further provide the evidence that sparfloxacin modified the shape of ICaL by altering CDI. This study suggests that quinolones causing delay of ICaL inactivation combined with IKr block may have more adverse effects than those with purely selective IKr block.
Key Words: sparfloxaicn, arrhythmia, cardiomyocyte, inactivation, L-type Ca^{2+} channel

P04-04

NALCN ion channel is regulated by arginine methylation

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NALCN (Sodium leak channel, non-selective) is predominantly expressed in neurons where it regulates the resting membrane potential and neuronal excitability. Both in mammals and invertebrates, animal models revealed an involvement of NALCN in many processes such as locomotor behaviors, sensitivity to volatile anesthetics, and respiratory rhythms. NALCN was activated by M3 muscarinic receptor and enhanced by lowering $[Ca^{2+}]_e$. However the role of post-translational modifications in NALCN function remains largely unknown. Here we show that in HEK cells, arginine methylation regulates NALCN activity. Blocking arginine methylation with 5-deoxy-5-(methylthio) adenosine (MTA, 100 μM) enhanced NALCN currents by 130% (n=9). MTA-activated current was blocked by 10 μM Gd^{3+}. Consistent with previous study, lowering $[Ca^{2+}]_e$ to 0.1 mM activates a NACLN by 150 % (n=9). Subsequent application of MTA further enhanced NALCN currents (MTA in 0.1 mM $[Ca^{2+}]_e$, 280 %, n=4, $P<0.05$ vs. MTA alone or 0.1 mM $[Ca^{2+}]_e$ alone), suggesting synergism between low $[Ca^{2+}]_e$ and the MTA in NALCN activation. The activation of NALCN currents by lowered $[Ca^{2+}]_e$ and MTA was completely blocked by PIP_2 loading, indicating that the change in PIP_2-channel interaction underlies NALCN regulation by calcium-sensing receptor and arginine methylation. This is the first evidence for the role of methylation in NALCN and opens the door to explore NALCN methylation as a novel mechanism for the cell-type specific constitutive activity of NALCN.
Key Words: NALCN, Methylation, PIP_2

P04-05

Orai1 and STIM1 are Critical for Tumor Progression in Clear Cell Renal Cell Carcinoma

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The ubiquitous second messenger Ca^{2+} regulates various cellular processes such as proliferation and migration. The Ca^{2+}-mediated signaling pathways have been implicated either directly or indirectly in tumorigenesis and tumor progression. Notably, store-operated Ca^{2+} entry (SOCE) is a major Ca^{2+} entry mechanism in non-excitable cells including epithelial cells which are the most common origin of cancer. However, the expression and biological role of SOCE have not been investigated in clear cell renal cell carcinoma (ccRCC) whose origin is mostly kidney epithelial cells including proximal and distal tubular cell. Here, we demonstrate that Orai1 and STIM1, not Orai3, are crucial components of SOCE in the progression of ccRCC. The expression levels of Orai1 in tumor tissues were significantly higher than those in the adjacent normal parenchymal tissues. The overall survival rate of ccRCC patients with high Orai1 was significant lower than that of patients with low Orai1 expression. Functionally, native SOCE was blunted by inhibiting SOCE or by silencing Orai1 and STIM1 in ccRCC cell lines. Pharmacological blockade or knockdown of Orai1 or STIM1 also significantly inhibited RCC cell migration and proliferative capability. Taken together, Orai1 is highly expressed in ccRCC tissues illuminating that Orai1-mediated SOCE may play an important role in ccRCC development. Indeed, Orai1 and STIM1 constitute a native SOCE pathway in ccRCC by promoting cell proliferation and migration.
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Key Words: Orai1, Orai3, STIM1, clear cell renal cell carcinoma, migration

P04-06

Klotho Protects Podocytes via Inhibiting TRPC6 Channel

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An anti-aging protein Klotho exerts organ protection that is predominantly expressed in the kidney. Despite there is no doubt that Klotho is renoprotection protein, to date, little insight has been provided into Klotho function on glomerular filtration barrier including podocytes which are highly susceptible to intracellular Ca^{2+}. Overactivation of Ca^{2+}-permeable TRPC6 channel is involved in proteinuric glomerular diseases. Here, we examine the protective role of Klotho on podocyte against rearrangement of actin cytoskeleton by TRPC6-mediated Ca^{2+} overload. Klotho in-

hibits TRPC6-evoked Ca^{2+} influx and currents in cultured podocytes and HEK293 cells, respectively, without affecting intrinsic channel properties. Mechanistically, Klotho decreases cell surface abundance of TRPC6 via blocking serum-stimulated VAMP2-dependent exocytosis of the channel as well as TRPC3 indicating Klotho can regulate diverse TRPC channels. Functionally, in vitro, Klotho attenuates TRPC6-mediated actin cytoskeletal rearrangement and albumin permeability in podocytes leading to proteinuria. Moreover, overexpression of TRPC6 using in vivo gene delivery in mice causes albuminuria and Klotho ameliorates basal and TRPC6-induced albuminuria supporting that Klotho has a remarkable protective effect on podocyte. Altogether, these results provide novel perspectives on glomerular barrier function and glomerulopathies and offer new therapeutic strategies for treatment of proteinuria.

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Key Word : TRPC3, TRPC6, Klotho, podocyte, proteinuria

P04-07

Loss of the Shh coreceptor Cdo Leads to Alteration in Connexins With Dilated Cardiomyopathy

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Shh signaling is required for various cardiac developmental processes including the looping of heart tube and specification of cardiomyogenesis. Consistently, Shh mutant mice display the abnormalities in heart tube formation and specification of the myocardial and outflow tract. A multifunctional receptor Cdo functions as a Shh coreceptor to fully activate Shh signaling and Cdo deficient mice exhibit multiple defects in embryonic development, associated with the decreased Shh activity. However its role in heart development is entirely unknown. This study examined the role of Cdo in heart development and cardiomyogenesis using Cdo deficient ($Cdo^{-/-}$) mice. Histological and morphological analyses revealed development of dilated cardiomyopathy (DCM) with a frequent death around 3 weeks of age. Echocardiographic analyses of $Cdo^{-/-}$ mice at 2 weeks confirmed the dilated phenotype of the heart with a significant decrease in the left ventricular fractional shortening. Patch clamp experiments revealed an increase in I_{to} and reduction in I_{K1}, without any significant changes in action potential duration (APD) in Cdo-/- cariomyocytes. A major gap junctional protein Connexin 43 (Cx43) appears to be localized at the lateral border of the $Cdo^{-/-}$ cardiomyocytes whereas Cx43 remained predominately at the intercalated disk in wildtype cardiomyocytes. Furthermore the phosphorylation status of Cx43 was altered in $Cdo^{-/-}$ hearts. Taken together, these data suggest that Cdo signaling may influence the phosphorylation status and distribution of Cx43 thereby regulating intercellular communication and functionality of cardiomyocytes.

Key Words: Sonic Hedgehog (Shh), Cdo, Connexin, Inwardly rectifying potassium channel (IRK)

P04-08

Activation of Orai1 in Podocytes Causes Proteinuria

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The Ca^{2+}-mediated signaling pathways have been implicated either directly or indirectly in podocyte dysfunction leading to proteinuria. Both TRPC5 and TRPC6 channels have been involved in pathogenesis of podocyte. Nevertheless, store-operated Ca^{2+} entry (SOCE) is a major Ca^{2+} influx pathway in non-excitable cells like podocyte. However, the expression and functional role of SOCE have not been investigated in podocyte. Moreover, pathophysiology of Orai-mediated Ca^{2+} handling also remains largely elusive. Here, we show that overexpression of Orai1 causes proteinuria and insulin exaggerates Orai1-induced podocyte dysfunction. Orai1 channel is a predominant molecular component of SOCE in podocyte. Insulin stimulates cell surface abundance of Orai1 via activating its exocytosis in a PI3K-Akt-dependent manner. Exaggerated Ca^{2+} influx by Orai1 augments cytoskeletal rearrangement and contractility of podocytes leading to increased albumin permeability. Transient in vivo gene delivery of Orai1 into mice causes proteinuria that is augmented by insulin treatment via NFAT-dependent mechanism. Furthermore, in db/db mice, hyperinsulinemic type II diabetic model, podocyte dysfunctions such as foot process effacement and proteinuria are significantly ameliorated by blockade of Orai1 or its downstream effector calcineurin supporting that insulin-mediated activation of Orai1 may involve podocyte injury and proteinuria. Altogether, Orai1 is a novel Ca^{2+} influx pathway in podocyte and its overexpression and/or activation cause podocyte dysfunction and actin cytoskeleton remodeling leading to foot process effacement, disruption of slit diaphragm, and proteinuria. These results provide a new perspective on pathogenesis and treatment of glomerular diseases.

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Key Words: Orai1, TRPC6, podocyte, proteinuria, store-operated Ca^{2+} entry

P04-09

Hypoxic upregulation of TASK-2 channels via HIF-1 in B cells

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The general consensus is that immune cells are exposed to physiological hypoxia in vivo ($PhyO_2$, 2-5% P_{O2}). Despite this physiological hypoxia is well acknowledged, most biological experiments and cell culture processes are performed at atmospheric P_{O2} ($AtmO_2$, 20-21%). Whether hypoxia (especially sustained hypoxia) affects the membrane conductance in B cells and the types of ion channels those are responsible for the effects are unknown. Recently, we reported the expression in mouse B cells of TASK-2, a member of pH-sensitive two-pore domain K^+ channels with background activity. Here, we investigated the response of K^+ channels to sustained $PhyO_2$ (SH, 3% P_{O2} for 24 h) in WEHI-231 mouse B cells. SH induced voltage-independent background K^+ conductance (SH-K_{bg}) and hyperpolarized the membrane potential. The pH-sensitivity and the single channel conductance of SH-K_{bg} were consistent with those of TASK-2. Immunoblotting assay results showed that SH significantly increased plasma membrane and total protein expressions of TASK-2 in B cells. Conversely, SH failed to induce any current by siTASK-2 transfection, confirming that SH induces TASK-2. Both functional increase of TASK-2 activity and protein expression are also increased in the primary splenic B cells incubated in SH. Mechanistically, upregulation of TASK-2 by SH was prevented by siHIF-1 alpha transfection or by YC-1, a pharmacological HIF-1 alpha inhibitor. In addition, TASK-2 current was increased in WEHI-231 cells overexpressed with O_2-resistant HIF-1 alpha. Importantly, $[Ca^{2+}]_c$ increment in response to B cell receptor stimulation was significantly higher in SH-exposed WEHI-231 cells, which was abolished by high K^+-induced de-polarization or by siTASK-2 transfection. Also, in Primary splenic B cells the $[Ca^{2+}]_c$ signal induced by BCR-ligation was augmented in SH, which became indistinguishable by 145 KCl depolarization. The data demonstrate that TASK-2 is upregulated under hypoxia via HIF-1 alpha dependent manner in B cells. This is functionally important in maintaining the negative membrane potential and providing electrical driving force to control Ca^{2+} influx.

Key Words: TASK-2, Hypoxia, B cells, HIF-1 alpha

P04-10

Activation of Ca^{2+}-dependent cation current by fluid shear force in atrial myocytes: possible role of cross-talk between IP_3R2 and TRPM4

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Atrial myocytes are subjected to fluid shear force (FSF) during each contraction and relaxation. Under pathological conditions, such as valve disease, heart failure, and hypertension, fluid shear force may increase in atria due to high blood volume and pressure. So far, ionic currents regulated by shear force and their molecular integrity in cardiac myocytes have not been well understood. We examined whether FSF activates specific current in atrial myocytes and underlying mechanisms for FSF-sensitive ionic current using whole-cell patch-clamp technique. A FSF of ~16 dyne/cm^2 was applied to entire single atrial myocyte using automated micro-puffing apparatus. A FSF-sensitive current (I_{FSF}) was detected in lowly Ca^{2+}-buffered (0.5 mM EGTA) atrial myocytes, but not in highly Ca^{2+}-buffered ($\geq$4 mM EGTA or 10 mM BAPTA) myocytes. The I_{FSF} showed an outward rectification with a reversal potential of about −6 mV. The I_{FSF} was inhibited by high concentrations (20-50 μM) of ryanodine and by replacement of external and internal cation with impermeant $NMDG^+$, suggesting that I_{FSF} is a Ca^{2+} release-dependent cation current. Application of either TRPM4 inhibitor 9-phenanthrol or TRPM4-specific antibodies removed most of inward I_{FSF} and 85% of outward I_{FSF}. However, stretch-activated cation channel blocker GsMTx-4 did not affect I_{FSF}. Interestingly, I_{FSF} was strongly inhibited by inositol 1,4,5-trisphosphate receptor (IP_3R) blockers, 2-APB (2 μM) or xestospongin C. In addition, in atrial myocytes isolated from type 2 IP_3R (IP_3R2) knock-out mouse, I_{FSF} was not detected, although 9-phenanthrol-sensitive I_{FSF} was recorded in wild-type myocytes. Co-immunostaining of TRPM4 and IP_3R2 in rat atrial myocytes revealed peripheral localization of these proteins with significant co-localizations. These results suggest that fluid shear stimuli may activate TRPM4 channels in atrial myocytes via Ca^{2+} releases triggered by the activation of nearby IP_3R2.

Key Words: fluid shear force, atrial myocytes, TRPM4, IP_3R2

P04-11

G α i-mediated TRPC4 activation by PKD1 contributes to cystic disease via STAT1 activation

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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. We have previously reported that TRPC4/C5 channel can be activated by Gαi through direct interaction. Here, we identified that PKD1 dominantly interacts with Gαi3 using co-immunoprecipitation. Thus we recorded the activity of TRPC4/C5 heterologously co-expressed with PKD1 in HEK293 cells. PKD1 activated TRPC4 β channel (4±1 → 41±14 pA/pF) by modulating G-protein signaling without change in TRPC4 translocation. Intracellular 0.2 mM GTPγS-induced TRPC4 activation was not significantly different in the presence or absence of PKD1. 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). Dominant negative Gαi3 (G202T) mutant inhibited PKD1-activated TRPC4 current. TRPC5 also was activated by full-length PKD1 (54±8 → 114±16 pA/pF). We, next, investigated whether TRPC4 induces activation of STAT (signal transduction and transcription) proteins, leading to cell proliferation or death. We observed that STAT1 and STAT3, but not STAT6 activation by PKD1 is independent on Src kinase cascades. Interestingly, TRPC4 promoted STAT1 activation. When PKD1 co-expressed with TRPC4, STAT1 activation was further increased compared to each sole expression, causing cystic cell death. Our findings indicated an important function between PKD1 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.

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Key Words: TRP, TRPC, TRPP, PKD, Gi

P04-12(PO-11)

Glutathionylation and Activation of TRPC5 by Altered Glutathione Homeostasis Lead to Neuronal Damage in Huntington's Disease

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Aberrant Ca^{2+} signaling is associated with the pathogenesis of neurodegenerative disorders. However, the role of Ca^{2+}-permeant TRPC channel in neurodegeneration is not known. Here, we report a mechanism of TRPC5 activation by oxidants and the effect of glutathionylated TRPC5 on striatal neurons in Huntington's disease (HD). Intracellular oxidized glutathione (GSSG) leads to TRPC5 activation through TRPC5 S-glutathionylation at C176-C178 residues. The GSSG-activated TRPC5-like current resulted in sustained increase of cytosolic Ca^{2+}, activated CaM kinase and calpain-caspases pathway, and caused striatal neuronal cell death. Both knockdown and inhibition of TRPC5 significantly attenuated oxidation-induced striatal neuronal cell death. Moreover, TRPC5 blocker ameliorated rearing behavior in HD (YAC128) mice and motor behavioral symptoms in WT mice by improving the survival of striatal neurons. Taken together, these findings reveal that increased TRPC5 S-glutathionylation by oxidative stress contributes to the neuronal damage in the striatum and it may account for the neurodegeneration in HD.

Key Words: TRPC, GSSG, glutathione, glutathionylation, Ca^{2+}, Huntington, Neurodegeneration

P04-13

Alterations of contractions and L-type Ca^{2+} currents by murrayafoline-A in rat ventricular myocytes

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We examined the effects of murrayafoline-A (1-methoxy-3-methylcarbazole, Mu-A), which is isolated from the dried roots of Glycosmis stenocarpa, on cell shortenings and L-type Ca^{2+} currents ($I_{Ca,L}$) in rat ventricular myocytes. Cell shortenings and $I_{Ca,L}$ were measured using the video edge detection method and patch-clamp techniques, respectively. Mu-A transiently increased cell shortenings in a concentration-dependent manner with an EC50 of ~20 μM. The maximal effect of Mu-A, approximately 175% of the control, was observed at ≥100 μM. The positive inotropic effect of Mu-A (25 μM) reached a maximum after ~2-min exposures, and then decayed after a ~1-min steady-state. During the Mu-A- induced positive inotropy, the rate of contraction was accelerated, whereas the rate of relaxation was not significantly altered. To understand the possible mechanism for the Mu-A-induced positive inotropy, the $I_{Ca,L}$ was assessed. Mu-A transiently enhanced the $I_{Ca,L}$. Concentration-dependence of the increase in $I_{Ca,L}$ by Mu-A was similar to that of positive inotropic effect of Mu-A. The maximal effect of Mu-A (25 μM) on $I_{Ca,L}$ was observed at 2-3 min after the application of Mu-A. A partial inhibition of $I_{Ca,L}$ using verapamil (1 μM) induced a right shift of concentration-response curve of the positive inotropic effect of Mu-A and significantly attenuated the effect. These results suggest that Mu-A may transiently enhance contractility, at least in part, by increasing the Ca^{2+} influx through the L-type Ca^{2+} channels in rat ventricular myocytes.

Key Words: Murrayafoline-A (Mu-A), Cell shortening, L-type Ca^{2+} current, Positive inotrophy, Rat ventricular myocytes

P04-14(PO-4)

Sensitization of cardiac Ca^{2+} release sites by protein kinase C signaling: evidence from action of murrayafoline A

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In the present study, we explored the effects of a plant alkaloid compound, 1-methoxy-3-methylcarbazole (murrayafoline A, Mu-A), on focal and global Ca^{2+} signaling, and the underlying cellular mechanisms. Rapid two-dimensional confocal Ca^{2+} imaging and image analysis were used to measure Ca^{2+} signals in rat ventricular myocytes. Application of Mu-A (10-100 μM) significantly enhanced the magnitude and rate of Ca^{2+} release on depolarization with no change in Ca^{2+} transient decay. Focal Ca^{2+} release events (Ca^{2+} sparks) occurred more often, and their duration and size were greater after the application of Mu-A. In addition, sarcoplasmic reticulum (SR) Ca^{2+} loading and fractional release were increased by exposure to Mu-A. All these effects reached steady state within 2-3 min after Mu-A application. The higher occurrence of Ca^{2+} sparks in the presence of Mu-A was resistant to SR Ca^{2+} clamping, removal of extracellular Ca^{2+} and Na^{+}, and blockade of either protein kinase A, Ca^{2+}/calmodulin-dependent protein kinase II, phospholipase C, or inositol 1,4,5-trisphosphate receptors, but it was abolished by the inhibition of protein kinase C (PKC). SR Ca^{2+} clamping prevented the Mu-A-induced Ca^{2+} spark prolongation and enlargement. The Mu-A-induced enhancement of Ca^{2+} transients was also eliminated by PKC blockade. Mu-A enhanced PKC activity in vitro. These results suggest that Mu-A may increase spark occurrence via its direct enhancement of PKC activity and subsequent sensitization of ryanodine receptor clusters and that this mechanism, as well as increased SR Ca^{2+} loading, may partly explain larger and more rapid global Ca^{2+} releases in the presence of Mu-A during depolarization.

Key Words: ventricular myocytes, murrayafoline A, Ca^{2+} transient, Ca^{2+} spark, protein kinase C

P04-15

RASD1 activates TRPC4 through G α i independent 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 Rasd1 is related to GIRK channel and Ca^{2+} channel. However it is unknown whether Rasd1 is associated with TRPC channels. We assumed that Rasd1 might regulate TRPC4 channel, since Rasd1 interacts with Gi/Go and TRPC4 is activated by Gi/o subunits. Here, we measured whole cell current of TRPC4 after the co-expression of TRPC4 with constitutively active form of small GTPases in HEK293 cells. Rasd1 (CA) mutant (Q to L) activated TRPC4 without GTP γ S and independently of GPCR. Pertussis toxin (PTX), G α i specific inhibitor, blocked Rasd1-activated TRPC4 current. When co-expressed with dominant negative G α i protein subtype, Gi1,3 are more effect than Gi2 for TRPC4 activation by Rasd1. With previous report that TRPC4 are activated primarily by selective G α i subunits, these results suggest that Rasd1 activates TRPC4 channel through modulating G α i subunits and Rasd1 is a new activator for TRPC4 channel.

Key Words: TRPC,Rasd1,Galphai

P04-16

Identification of Membrane Targeting Domain of Transient Receptor Potential Canonical (TRPC)4 Channel unrelated to its Formation of Tetrameric Structure

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Canonical transient receptor potential (TRPC) channels are Ca^{2+}-permeable nonselective cation channels that are activated by a wide variety of stimuli, including a family of G protein-coupled receptors (GPCRs). The TRPC4 channel is expressed in a punctate distribution in the membrane. To identify the regulating region of the channel trafficking to the membrane, we generated deletion mutants of the TRPC4 channel. We determined that when either region that was downstream of the 20 amino acids of the N-terminal or the 700-730 amino acids was deleted, the mutants were retained in the endoplasmic reticulum. By coexpression of the wild type of TRPC4 with deletion mutants, we found that the 23-29 amino acids of the N-terminal regulate the membrane trafficking. Additionally, by the fluorescence resonance energy transfer (FRET) method, we found that the regions downstream of the 99 amino acid region of the N-terminal and upstream of the 730 amino acid region in the C-terminal produce assembly of the TRPC4 tetramers. We inferred the candidate proteins that regulate or interact with the 23-29 domain of TRPC4.

Key Words: TRPC4, FRET, membrane expression, Trafficking

P04-17(PO-7)

Down-regulation of THIK-1 expression by inflammatory mediators in rat dorsal root ganglion neurons

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A recent study has demonstrated that THIK-1, a small conductance two-pore domain K^+ (K_{2P}) channel, is expressed in trigeminal ganglion (TG) neurons, a type of pain-sensing neuron. The overall knowledge about the biophysical, pharmacological and physiological properties of THIK-1 channels remains far behind those of other K_{2P} channels. In particular, little is known about the physiological role of THIK channels in DRG neurons. Here, we studied the single-channel properties of THIK-1 in DRG neurons and the effect of proinflammatory mediators on DRG. RT-PCR and Western blot analyses showed that THIK-1 was expressed in DRGs. Immunocytochemistry showed that the THIK-1 channel was localized at the plasma membrane and cytoplasm of DRG neurons. In cell-attached patches with high KCl in the pipette and bath solutions, low conductance (~5-pS) channels with a mild inwardly rectifying current-voltage relationship were present in DRG neurons. Halothane and cold inhibited the single-channel activity of THIK-1 by 50%, whereas arachidonic acid activated this activity. Semiquantitative PCR showed that the THIK-1 expression levels were high compared to those of THIK-2 in DRGs, whereas the expression levels were similar in TGs. After treating DRG neurons with proinflammatory mediators, THIK-1 transcript, but not THIK-2, significantly decreased. Mice overexpressing THIK-1 showed reduced formalin-induced nociceptive behavior during phase II. These results show that proinflammatory mediators alter the expression of THIK-1 expressed in DRG neurons; these changes could affect pain sensation through THIK-1 channels. We suggest that THIK-1 might regulate inflammatory pain by functioning as background K^+ channels in DRG neurons.
Key Words: background K^+ channel, dorsal root ganglion neurons, inflammation, pain

P04-18

Biophysical and pharmacological characterization of TREK-2-like channel expressed in rat trigeminal ganglion neurons

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Trigeminal ganglion (TG), which is linked to the pain pathway in migraine, expresses the mRNA and protein for TREK/TRAAK and TRESK, which are members of two-pore domain K^+ (K_{2P}) channel family. Little is known about the expression and role of K_{2P} channels in TG neurons. This study was performed to determine whether and which type of K_{2P} channels are mainly expressed in rat TG neurons. RT-PCR data showed that TASK-1, TASK-2, TASK-3, TREK-1, TREK-2, TRAAK, and TRESK mRNA transcripts were expressed in TG neurons. Real-time PCR data indicated that the mRNA expression levels of TREK-1, TREK-2, TRAAK and TRESK were higher than those of TASK-1, TASK-2 and TASK-3. Single-channel recordings in the cell-attached and inside-out patch modes showed that TREK-like and TRESK-like channels were mainly expressed in TG neurons. The expression of the TREK-2 protein was confirmed by immunoblotting and immunostaining. The TREK-2-like channels were predominantly expressed at the physiological conditions (37°C and circulation). The single-channel properties of the TREK-2-like channel were similar to those of the cloned rat TREK-2. These channels showed a weakly inward rectified current-voltage relationship. TREK-2-like channels were activated by the application of pressure, riluzole, acidic solution (pH_i6.3) and halothane, whereas they were inhibited by cold (10°C). In addition, the TREK-2 channels were inhibited by amitriptyline, escitalopram and fluoxetine, antidepressants for migraine prophylaxis, and whole-cell currents in TG neurons were also inhibited by these chemicals. These results show that TREK-2-like channel is mainly expressed in TG neurons. We suggest that TREK-2 might contribute to the background K^+ conductance of TG neurons, and TREK-2 could be an appropriate target for migraine prophylaxis and treatments.
Key Words: Antidepressive agents, Background potassium channel, Pain, Trigeminal ganglion

P04-19

Signals governing the trafficking of PKD2L1 to primary cilia

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Primary cilia are solitary organelles that extend from the basal body of the apical surface into the extra cellular matrix of most eukaryotic cells. Dysfunctions of primary cilia underlie a multitude of human disorders, including autosomal dominant polycystin kidney disease (ADPKD), yet membrane targeting to the cilium remains poorly understood. Several ciliar targeting motifs, Ax[S/A]xQ, RVxP, AxEGG, and VxPx have previously reported. Among the aforementioned motifs, arguably the best understood mechanistically is the VxPx motif found in polycystin 2, CNGB1b, and rhodopsin that is necessary for targeting of these proteins to renal cilia, olfactory cilia, and photoreceptor outer segments, respectively. The motif is also detected in PKD2L1, which are reported to play an important role in flow sensing mechanism and calcium regulation in cilia. Here, we investigated electrophysiological

characteristics of hPKD2L1 and mPKD2L1. In whole-cell patch clamp of HEK293 cells transiently transfected with hPKD2L1 and mPKD2L1, each produced outward currents, 134.1±48.7 pA/pF and 47.2±27.5 pA/pF, respectively. Their currents were 4 times more activated with application of 1 μM Calmidazolium. In order to detect their localization, we overexpressed mPKD2 with unique sequences mediates localization to cilia. PKD2 shows similar homology with PKD2L1 and mPKD2L1, and hPKD2L1 on cilia were shown after MDCK cells and mIMCD-3 cells display monolayer structure. Here, we showed that mPKD2, mPKD2L1, and hPKD2L1 are targeted to cilia, respectively. Since patients with PKD show abnormal sensory cilia function, the aim of our current study was to search for a ciliar targeting motif in PKD2L1 and their unknown role in calcium regulation mechanism in cilia. Here, we try to identify a novel ciliary trafficking determinant in PKD2L1 that furthers our understanding of how proteins are selectively targeted to the cilium and their functional role in calcium regulation.
Key Words: ADPKD, Primary Cilia, Targeting Motif

P04-20

Expression profiling of mitochondrial voltage-dependent anion channel-1 associated genes predicts recurrence-free survival in human carcinomas

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Background: Mitochondrial voltage-dependent anion channels (VDACs) play a key role in mitochondria-mediated apoptosis. Both in vivo and in vitro evidences indicate that VDACs are actively involved in tumor progression. Specifically, VDAC-1, one member of the VDAC family, was thought to be a potential anti-cancer therapeutic target. Our previous study demonstrated that the human gene VDAC1 (encoding the VDAC-1 isoform) was significantly up-regulated in lung tumor tissue compared with normal tissue. Also, we found a significant positive correlation between the gene expression of VDAC1 and histological grade in breast cancer. However, the prognostic power of VDAC1 and its associated genes in human cancers is largely unknown. **Methods:** We systematically analyzed the expression pattern of VDAC1 and its interacting genes in breast, colon, liver, lung, pancreatic, and thyroid cancers. The genes differentially expressed between normal and tumor tissues in human carcinomas were identified. **Results:** The expression level of VDAC1 was uniformly up-regulated in tumor tissue compared with normal tissue in breast, colon, liver, lung, pancreatic, and thyroid cancers. Forty-four VDAC1 interacting genes were identified as being commonly differentially expressed between normal and tumor tissues in human carcinomas. We designated VDAC1 and the 44 dysregulated interacting genes as the VDAC1 associated gene signature (VAG). We demonstrate that the VAG signature is a robust prognostic biomarker to predict recurrence-free survival in breast, colon, and lung cancers, and is independent of standard clinical and pathological prognostic factors. **Conclusions:** VAG represents a promising prognostic biomarker in human cancers, which may enhance prediction accuracy in identifying patients at higher risk for recurrence. Future therapies aimed specifically at VDAC1 associated genes may lead to novel agents in the treatment of cancer.
Key Words: VDAC1, cancer, expression profiling, molecular signature, mitochondria

P04-21

Investigation of charged amino acids in c-terminal region for the sensitivity of TREK-2 K^+ channel to pH_i

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TREK-2, a member of two-pore domain K^+ channel (K2P) family, are activated by various chemical and physical stimuli including arachidonic acid (AA) and intracellular acidic pH (pHi). Cytoplasmic c-terminal of TREK-2 is believed to contain the regulatory region sensing pHi. A previous study of TREK-1 pinned down 306Glu as the pHi sensing residue. However, since there are at least seven Glu on the supposedly critical regulatory region in TREK-2 (from 324Val to 398Ser), comprehensive investigation is requested to precisely understand the pHi sensing mechanism. In inside-out (i-o) patch clamp conditions, application of acidic pH (6.0) and alkali pH (8.0) activated and inhibited TREK-2, respectively. Maximum activity was confirmed by applying 10 μM AA for normalization. Site-directed mutations of Glu in the corresponding region showed that the activation of pH 6.0 was eliminated by individual neutralization of multiple sites (E332A, E335A, E350A, and E361A). Surprisingly, neutralization of a cationic Lys (K330A) also eliminated the activation by acidic pHi. These results suggest that multiple sites of charged amino acids in cytoplasmic c-terminal participate in the pHi-dependent activation of TREK-2. It was also supposed that the titration of Glu might interfere with the putative electrostatic interaction with Lys, thereby leading to the conformational changes of TREK-2 for activation.
Key Words: K2P, TREK channel, pH sensitivity, c-terminal

P04-22

TLR4 stimulation induces Kir2.2 trafficking via PKC-dependent pathway and modulates channel activity via reciprocal regulation of PIP2 by PI3K/PTEN in human monocyte

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Lipopolysaccharide (LPS) is an agonist for Toll-like receptor 4 (TLR4). In THP-1 monocytes or human primary monocytes, LPS treatment time-dependently induced inwardly rectifying K^+ channel (Kir) current ($I_{Kir-LPS}$); $I_{Kir-LPS}$ newly appeared from 1 h, peaked at 4 h, and decayed to 27% of peak at 24 h. Single channel conductance of $I_{Kir-LPS}$ (38 pS) and its Ba^{2+}-sensitivity (IC_{50}, 1.42 μM) were consistent with Kir2.2. The functional upregulation of Kir2.2 was also confirmed in primary human monocytes. LPS induced translocation of Kir2.2 to plasma membrane, and $I_{Kir-LPS}$ was attenuated by Exo-1 and Brefeldin A (vesicular trafficking inhibitors) or by GF109203X (PKC inhibitor). Ca^{2+} influx was augmented in the LPS treated THP-1 cells, which was abolished by KCl-induced depolarization. LPS-induced cytokine secretion (TNF α and IL-8) was weakened by ML-133 (Kir2.x inhibitor). The spontaneous decay of $I_{Kir-LPS}$ at 24 h was reversed by PI3 kinase (PI3K) inhibitors (wartmannin and LY294002), while the $I_{Kir-LPS}$ was further suppressed by bpV(phen), a PTEN (PIP_3 phosphatase) inhibitor. $I_{Kir-LPS}$ was unaffected by Akt inhibitors, suggesting that $I_{Kir-LPS}$ decayed due to decreased PIP_2. Dynamic reciprocal changes between PIP_2 and PIP_3 by inhibitors of PI3K and PTEN were confirmed using confocal microscopy for PLC δ-PH-GFP and Akt-PH-GFP expressed THP-1 cells treated with 24h LPS. Taken together, we firstly report that Kir2.2 is translocated via PKC-dependent trafficking, and then spontaneously decayed by decreased PIP_2 in LPS-stimulated human monocytes. The concomitant augmentation of Ca^{2+} influx and cytokine release suggests physiological role of Kir2.2 in the innate immunity of monocytes.

Key Words: monocyte, LPS, Kir2.2, THP-1, PIP_2

P04-23

Effects of Hydrogen Peroxide on K^+ channels in Human Cardiac Fibroblasts

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Cardiac fibroblasts represent the most numerous cell types in heart and play an important role in cardiac function and diseases, including arrhythmogenesis. In addition, fibroblast ion channels may be involved in governing proliferation and differentiation. Hydrogen peroxide (H2O2), a membrane permeable and relatively stable reactive oxygen species (ROS), has emerged as an important signaling molecule in the regulation of physiological and pathophysiological processes in heart. The purpose of the present study was to define how an exposure to H2O2 alters the membrane currents and proliferation in human cardiac fibroblasts. We found various subtypes of voltage-dependent K^+ channels (Kv), transient outward K^+ channels (KTA), and Ca^{2+}-activated K^+ channels (KCa) in the cells by RT-PCR and Western blots. In whole cell mode patch-clamp recording, Kv, KTA, and KCa currents were recorded but only KCa currents were stimulated by H2O2 in the bath solution. The stimulating effect of H2O2 on KCa currents was inhibited by pretreated KT5720 (PKA inhibitor), bisindolylmaleimide Ⅱ (PKC inhibitor) and KT5823 (PKG inhibitor). On the other hand, H2O2 effect for apoptosis of the cells was not inhibited by various K^+ channels blockers. Our results suggest H2O2 may stimulate KCa currents through PKA, PKC and PKG pathway but K^+ currents may not involve in the apoptosis of human cardiac fibroblast.

Key Words: Hydrogen peroxide, Ca^{2+}-activated K^+ channel, Human Cardiac Fibroblast

P04-24

The Stimulating Effect of Nitric Oxide for Delayed Rectifier Potassium Channels in Human Cardiac Fibroblasts by PKG Pathway

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Cardiac fibroblasts are the major cell type involved in regulating the extracellular matrix in the heart and play an important role in cardiac remodeling after myocardial infarction or in response to hemodynamic overload. Voltage-dependent K^+ currents play a role in the proliferation of the fibroblast which may play a role in cardiac remodeling. Nitric oxide (NO) was shown to exert a variety of pharmacological effects including cardioprotective properties. However, its mechanisms of action are not completely understood. The aims of this study were to examine whether NO may alter cell proliferation through delayed rectifier potassium channels(Kv), the main component of voltage-dependent K^+ currents, and to identify the putative underlying signaling pathways in human cardiac fibroblasts. We investigated the presence the various subtypes of Kv channel by RT-PCR, Western blot, and immunocytochemistry in human cardiac fibroblasts and found the presence of the channels on RNA, protein, and membrane levels. In whole-cell mode patch clamp techniques, Kv current was found in most cells (60%) and S-nitroso-N-acetylpenicillamine (SNAP, a NO donor) significantly increased Kv current and this effect was abolished by pretreatment with KT5823 (PKG inhibitor), but not KT5720 (PKA inhibitor) and bisindolylmaleimide Ⅱ (PKC inhibitor). These data suggest that NO activates Kv channel through the PKG pathway, but not the PKA and PKC pathways in human cardiac fibroblasts

Key Words: Nitric Oxide, Delayed Rectifier Potassium Channel,Human Cardiac Fibroblast, PKG Pathway

P04-25

Homer2 Regulates PMCA-mediated Ca^{2+} Signaling in Mouse Parotid Gland Acinar Cells

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The Homer proteins are scaffold molecules with a domain structure consisting of an N-terminal Ena/VASP homology 1 (EVH) protein-binding domain and C-terminal leucine zipper/coiled-coil domain. The EVH domain recognizes proline-rich motifs and binds multiple Ca^{2+} signaling proteins, including G protein-coupled receptors, inositol 1,4,5-triphosphate receptors (IP3Rs), ryanodine receptors (RyRs), and transient receptor potential channels. However, their role in Ca^{2+} signaling in non-excitable cells is not well understood. In the present work, we investigated the role of Homer2 on Ca^{2+} signaling in parotid gland acinar cells using Homer2 deficient (Homer2-/-) mice. Homer2 showed increased polarized localization at the apical pole in wild-type acinar cells. Deletion of Homer2 did not affect IP3R localization or channel activity and did not affect the expression and activity of sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps. In contrast, Homer2 deletion markedly increased expression of plasma membrane Ca^{2+} ATPase (PMCA) pumps, in particular PMCA4, at the apical pole. Accordingly, Homer2 deficiency increased Ca^{2+} extrusion by acinar cells. These findings were supported by co-immunoprecipitation of Homer2 and PMCA in wild-type parotid cells and transfected human embryonic kidney 293 (HEK293) cells. We identified a Homer binding PPXXF-like motif in the N-terminus of PMCA that specifically interacts with Homer2. Mutation of the PPXXF-like motif did not affect the interaction of PMCA with Homer1, but inhibited its interaction with Homer2 and increased Ca^{2+} clearance by PMCA. These findings reveal an important regulation of PMCA by Homer2 that has a central role on PMCA-mediated Ca^{2+} signaling in parotid acinar cells.

Key Words: Protein-protein interaction, Calcium ATPase, Calcium transport, Scaffold protein, Cell signaling, Homer proteins, Plasma membrane Ca^{2+} ATPase, proline-rich motifs, parotid gland

P04-26

Activation of Ca^{2+}-activated K^+ channel (SK4) rescues squamous cancer cells from iononmycin-induced cell death

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Abstract The ion channels in cancer cells are drawing attention regarding cell proliferation and migration. Intracellular Ca^{2+} ($[Ca^{2+}]_i$)-dependent signaling has crucial influence to the fate of cancer. Here we investigate the role of Ca^{2+}-activated K^+ channels in head and neck squamous carcinoma cells (HNSCC); SNU-1076, OSC-19 and HN5. Treatment with 1 μM ionomycin (24-48 h) induced cell death in all the three cell lines. Whole-cell patch clamp study revealed the functional expression of Ca^{2+}-activated Cl^- channels (CaCC, Ano-1) and Ca^{2+}-activated non-selective cation channels (CAN). Ca^{2+}-activated K^+ channel (SK4) activity was variable between cell lines; at a raised $[Ca^{2+}]_i$ (0.6 μM) or with 1 μM ionomycin, only SNU-1076 showed prominent SK4 current (ISK4). However, an application of SK4 activator (1-EBIO) induced robust ISK4 with membrane hyperpolarization in OSC-19 as well as SNU-1076 while not in in HN5 cells. The EBIO-induced ISK4 was completely suppressed by TRAM-34, a selective SK4 blocker. Interestingly, the ionomycin-induced cell death was effectively prevented by 1-30 μM 1-EBIO in SNU-1076 and OSC-19. Consistently, the rescue effect was annihilated by combined treatment with TRAM-34. The rescue by 1-EBIO was not effective in HN5. Above results newly demonstrate the role of SK4 and membrane hyperpolarization in HNSCCs' proliferation and death. Pharmacological modulation of SK4 might provide an intriguing novel tool for the anti-cancer strategy in HNSCC.

Key Words: cancer, SK4 channel,1-EBIO, proliferation and cell death

P04-27

Modulation of Tonic AMPA Current by Synaptic and Extrasynaptic NMDA Receptors Activation in the Supraoptic Nucleus Neurons

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In several neuronal types of the CNS, glutamate and GABA receptors mediate a persistent current which reflects the presence of a low concentration of transmitters in the extracellular space. Glutamate is recognized as a prominent excitatory transmitter in the supraoptic nucleus (SON) and is involved in transmission of osmoregulatory information from the osmoreceptors to the vasopressin (VP) and oxytocin (OT) neurons. While the activation of extrasynaptic NMDA receptor has been known to induce tonic NMDA current (INMDA), the role of extrasynaptic AMPA receptor is not well known in the SON neurons. The purpose of this study was to explore the tonic activation of AMPA receptors, and the interaction between NMDA receptors and

AMPA receptors, using a whole-cell voltage-clamp method in the SON neurons. Glutamate (30 μM) caused small tonic AMPA current (IAMPA) and NMDA current (INMDA) in the presence of (DL)-2-amino-5-phophonovaleric acid (APV), and 6,7-dinitroquinoxaline-2,3- dione (DNQX), respectively. Interestingly, glutamate induced much larger tonic current (IGlutamate) than the sum of IAMPA and INMDA in the same neurons. We tested the hypothesis that the activation of extrasynaptic NMDA receptors altered IAMPA amplitudes in the SON neurons, for IGlutamate was mainly blocked by DNQX (~90% of total IGlutamate). For this purpose, we compared the effects of APV on IAMPA induced by 1 μM AMPA. While APV did not affect IAMPA at -70 mV, it inhibited ~25% of IAMPA at -40 mV, suggesting that IAMPA was enhanced by the activation of NMDA receptors. To further know the role of extrasynaptic NMDA receptors on IAMPA, we used low Mg^{2+} ACSF. In the low Mg^{2+} ACSF, INMDA amplitudes, shown by the outward shift in Iholding by NMDA, were ranged ~10 pA. APV blocked AMPA (1 μM) - induced current ~35% at -70 mV in the low Mg^{2+} ACSF. Furthermore, 10 μM glutamate induced tonic current (~90 pA), which was blocked by DNQX to ~ 60%, in the low-Mg^{2+} ACSF, while it failed to cause any changes in Iholding in normal ACSF. Taken together, the results suggested that glutamate activates tonic AMPA current as well as tonic NMDA currents, and that IAMPA is under control of the activation of synaptic and extrasynaptic NMDA receptors in the SON neurons.

Key Words: Glutamate, AMPA, NMDA

P04-28

Rhizochalin as a potential inhibitor of aquaglycerolporins

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Aquaporins (AQPs) are a family of integral membrane proteins that transport water and small solutes such as glycerol. AQP-based modulators may have broad potential for clinical utility including disorders of water balance and energy metabolism. Until now, no AQP modulators have yet been developed as a suitable candidate for clinical implication. We have screened extracts from marine natural products for potential modulators of AQP-mediated water and glycerol transport. Rhizochalin and its derivatives had no significant effects on the osmotic water permeability measured by stopped-flow light scattering using murine RBC suspensions subjected to a 300-mM inwardly directed gradient of sucrose. However, when RBC suspensions were exposed to a 300-mM inwardly directed gradient of glycerol, Rhizochalin and peracetyl aglycon Rhizochalin blocked the decrement rate of the late phase of light-scattering kinetics which is responsible for glycerol influx, whereas the other derivatives we tested did not. Cell volume kinetics measured by YFP fluorescence of AQP1, AQP3 and YFP triple-transfected HaCaT cells also showed that Rhizochalin and peracetyl aglycon Rhizochalin inhibited AQP3-mediated glycerol-induced changes of cell volume. Direct measurement of glycerol transport across AQP3 overexpressed HaCaT cell monolayers grown on permeable transwell supports confirmed the inhibitory effects of Rhizochalin and peracetyl aglycon Rhizochalin on glycerol permeability. These results suggest that Rhizochalin and peracetyl aglycon Rhizochalin might be suitable drug leads as potential modulators of aquaglyceroporins.

Key Words: Aquaporins 3, Rhizochalin, Peracetyl aglycon Rhizochalin, stopped-flow light scattering, YFP fluorescence

P04-29

Disappearance of hypoxic pulmonary vasoconstriction and oxygen sensitive nonselective cationic current in arterial myocytes of rats under chronic hypoxia

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Constriction of pulmonary artery (PA) in response to acute hypoxia is an crucial to protect ventilation/perfusion mismatch in lungs. As for the cellular mechanism of hypoxic pulmonary vasoconstriction (HPV), hypoxic inhibition of voltage-gated K(+) channel (Kv) in PA smooth muscle cell (PASMC) has been suggested. In addition, our recent study showed that thromboxane A_2 (TXA_2) and hypoxia-activated nonselective cation channel (INSC) is also essential for HPV. However, it is not well understood whether HPV is maintained in the animals exposed to ambient hypoxia for two days (2d-H). Specifically, the associated electrophysiological changes in PASMCs have not been studied. Here we investigate the effects of 2d-H on HPV in isolated ventilated/perfused lungs (V/P lungs) from rats. HPV was almost abolished without structural remodeling of PA in 2d-H rats, and the lost HPV was not recovered by Kv inhibitor, 4-aminopyridine. Patch clamp study showed that the hypoxic inhibition of Kv current in PASMC was similar between 2d-H and control. In contrast, hypoxia and TXA_2-activated INSC was not observed in PASMCs of 2d-H. From above results, it is suggested that the decreased INSC might be the primary functional cause of HPV disappearance in the relatively early period (2d) of hypoxia.

Key Words: hypoxic pulmoanary vasoconstriction, chronic hypoxia, nonselective cation channel, pulmonary artery

P04-30

Stichoposide C as a potential activator of aquaglycerolporins

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Marine natural products are a rich source of potential drugs due to their low toxicity, suitability for oral application and vast variety of mechanisms of their action. Stichoposide C (STC), a hexaoside which first isolated from the holothurian Stichopus chloronotus, is an active membrane-acting agent. STC contains quinovose as the second monosaccharide unit whereas STD has glucose. AQPs are important drug targets for a variety of diseases including aquaporinopathies. However, the progress in the discovery of AQP modulators is very slow despite the extensive high-throughput screening of diverse compound collections. STC (1 μM) and STD (1 μM) had no significant effects on the osmotic water permeability measured by stopped-flow light scattering using murine RBC suspensions subjected to a 300-mM inwardly directed gradient of sucrose. However, when RBC suspensions were exposed to a 300-mM inwardly directed gradient of glycerol, STC, but not STD, enhanced the decrement rate of the late phase of light-scattering kinetics which is responsible for glycerol influx. The stimulating effect of STC on glycerol permeability was dose-dependent (EC50=100 nM). Direct measurement of glycerol transport across AQP3 overexpressed HaCaT cell monolayers grown on permeable transwell supports also showed STC, but not STD, increased glycerol permeability. These results suggest that STC might be a suitable drug lead as a potential modulators of aquaglyceroporins.

Key Words: Aquaglyceroporins, AQP 3, Stichoposide C, Glycerol permeability, Stopped-flow light scattering

P04-31

Cortisone and Hydrocortisone Block Human Ether-a-go-go-related Gene (hERG) K^+ Channel Expressed in Xenopus Oocytes

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Glucocorticoids, inactive form cortisone and active form hydrocortisone, are important stress hormones, maintain organisms homeostasis from challenges via regulating various physiological activities, such as increasing blood sugar, suppressing the immune system and aiding in fat, protein and carbohydrate metabolism. GCs also exert effect on heart including increasing cardiac output, blood pressure as well as protect heart from myocardial infarct. The human ether-a-go-go-related gene encodes the pore-forming subunits of the channel carrying rapidly activating delayed outward rectifying current, which plays an important role in initiating the repolarization during cardiac action potential. We investigated the effect of cortisone and hydrocortisone on human ether-a-go-go-related gene (hERG) K^+ channels expressed in Xenopus oocytes by using two-microelectrode voltage-clamp. Both cortisone and hydrocortisone inhibited hERG K^+ channel currents with IC50 value of 234.0±29.2 μM and 197.8±10.7 μM, respectively. Also their block on hERG K^+ channels decreased progressively relative to the degree of depolarization. Cortisone and hydrocortisone inhibits the hERG K^+ channels in the open, closed and inactivated state, indicating a masking-block. These research indicated that cortisone and hydrocortisone blocked hERG channels with a similar mechanism.

Key Words: cortisone, glucocorticoid, hydrocortisone, hERG channel

P04-32

Cortisone and Hydrocortisone Decrease Human Kv1.5 Channel Currents Expressed in Xenopus Oocytes

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Glucocorticoids are the primary hormones that respond to stress and protect organisms from dangerous situations. The glucocorticoids hydrocortisone and its dormant form, cortisone, affect the cardiovascular system with changes such as increased blood pressure and cardioprotection. Kv1.5 channels play a critical role in the maintenance of cellular membrane potential and are widely expressed in pancreatic β-cells, neurons, myocytes, and smooth muscle cells of the pulmonary vasculature. We examined the electrophysiological effects of both cortisone and hydrocortisone on human Kv1.5 channels expressed in Xenopus oocytes using a two-microelectrode voltage clamp technique. Both cortisone and hydrocortisone rapidly and irreversibly suppressed the amplitude of Kv1.5 channel current with IC50 values of 50.2±4.2 μM and 33.4±3.2 μM, respectively. The inhibitory effect of cortisone on Kv1.5 decreased progressively from -10 mV to +30 mV, while hydrocortisone's inhibition of the channel did not change across the same voltage range. Both cortisone and hydrocortisone blocked Kv1.5 channel currents in a non-use-dependent manner and neither altered the channel's steady-state activation or inactivation curves. These results show that cortisone and hydrocortisone inhibited Kv1.5 channel currents differently, and that Kv1.5 channels were more sensitive to hydrocortisone than to cortisone.

Key Words: cortisone, glucocorticoid, hydrocortisone, Kv1.5 channel

P04-33

Mechanism of stretch-induced enhancement of spontaneous uterine contraction in pregnant rat uterus

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Spontaneous myometrial contraction (SMC) in pregnant uterus is greatly related with gestational age and growing

in frequency and amplitude toward the end of gestation to initiate labor. But, an accurate mechanism has not been elucidated. In human and rat uterus, all TRPCs except TRPC2 are expressed in pregnant myometrium and among them, TRPC4 are predominant throughout gestation, suggesting a possible role in regulation of SMC. Therefore, we investigated whether the TRP channel may be involved SMC evoked by mechanical stretch in pregnant myometrial strips of rat using isometric tension measurement and patch-clamp technique. In the present results, hypoosmotic cell swelling activated a potent outward rectifying current in G protein-dependent manner in rat pregnant myocyte. The current was significantly potentiated by 1 μM lanthanides (a potent TRPC4/5 stimulator) and suppressed by 10 μM 2-APB (TRPC4-7 inhibitor). In addition, in isometric tension experiment, SMC which was evoked by passive stretch was greatly potentiated by lanthanide (1 μM) and suppressed by 2-APB (10 μM), suggesting a possible involvement of TRPC4/5 channel in regulation of SMC in pregnant myometrium. These results provide a possible cellular mechanism for regulation of SMC during pregnancy and provide basic information for developing a new agent for treatment of premature labor.

Key Words: Transient receptor potential C4/5, spontaneous uterine contraction, osmotic stress, stretch-activated nonselective cation channel

P04-34

Imidazoline I_2 receptors activation inhibits N-type Ca currents in rat peripheral sympathetic neurons

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Agmatine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I2 receptors (IR2) at postganglionic sympathetic neurons. But, the cellular mechanism of IR2-induced inhibition of noradrenaline (NA) release is still unknown. To investigate this possibility, we investigated the effect of IR2 activation on N-type Ca^{2+} currents (ICa-N) in isolated neurons of the celiac ganglion (CG), which is involved in the sympathetic regulation of mesenteric artery vascular tone. In the present study, agmatine diminished voltage-gated Ca^{2+} currents (ICa), measured using the patch-clamp method, in an irreversible manner in rat CG neurons, while, thrombin had little effect on ICa. This agmatine-induced inhibition was nearly completely prevented by ω-CgTx, a potent N-type Ca^{2+} channel blocker, suggesting involvement of N-type Ca^{2+} channel in the PAR-2-induced inhibition. In addition, agmatine inhibited ICa-N in a voltage-independent manner in rat CG neurons. Moreover, agmatine reduced action potential firing frequency measured using the current-clamp method in rat CG neurons and this inhibition of AP firing induced by agmatine nearly completely prevented by ω-CgTx, indicating IR2 activation may regulate the membrane excitability of peripheral sympathetic neurons through modulation of N-type Ca^{2+} channels in rat CG neurons. In conclusion, the present findings demonstrate that the activation of IR2 suppresses peripheral sympathetic outflow by modulating N-type Ca^{2+} channel activity located in peripheral sympathetic nerve terminals, which appear to be involved in IR2-induced hypotension.

Acknowledgements: This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (2012-0009525)

Key Words: Imidazoline I2 receptors, Hypotension, Peripheral sympathetic output, N-type Ca^{2+} channel, agmatine

P04-35

Inhibition of the Extrinsic Aging-related Ion Channels TRPV1 and ORAI1 by Constituents of the Fruits of Fennel (Foeniculum vulgare)

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Ultraviolet (UV) exposure due to the solar radiation is the most important cause of extrinsic skin aging (photoaging), which is characterized clinically by deep skin wrinkling and pigmentation. These phenomena are due to the increasing of metalloproteinases-1 (MMP-1) expression in keratinocytes and tyrosinase activation in melanocytes. In a recent study, it was reported that two Ca^{2+} channels, a transient receptor potential vanilloid type-1 (TRPV1) and a calcium release-activated calcium channel protein 1 (ORAI1), are involved in UV-induced MMP-1 expression and tyrosinase activity, respectively. In the present study, we evaluated whether the fruits of fennel (Foeniculum vulgare) have inhibitory effects on TRPV1 and ORAI1 using the whole-cell patch-clamp technique and intracellular Ca^{2+} measurements. In our electrophysiological study of the extract and its fractions, the dichloromethane and hexane fractions were found to strongly block capsaicin-induced TRPV1 and ORAI1 currents. Furthermore, of the 18 compounds isolated from the hexane and dichloromethane fractions, trans-anethole in the hexane fraction had inhibitory effects on both ORAI1 and increases in cytoplasmic Ca^{2+} concentrations in response to ORAI1 activation (both by ~70% at 100 μM). Our findings suggest that the fruit extract of fennel provides possible novel ways of treating and preventing UV-induced skin aging

Key Words: TRPV1, Orai1, trans-anethole, Foeniculum vulgare

P04-36

Inhibitory Effect of Oleanolic acid from the Rhizomes of Cyperus rotundus on TRPV1 Channel

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Cyperus rotundus (Cyperaceae) is used as an analgesic and sedative in oriental medicine, and has been reported to exhibit anti-nociceptive and anti-inflammatory effects. On the other hand, transient receptor potential vanilloid channel 1 (TRPV1) is a nonselective cation channel that senses various noxious chemical and thermal stimuli. However, it has recently been reported that epidermally expressed TRPV1 is involved in heat- and UV-induced skin aging. The aim of this study was to evaluate whether C.rotundus extract and its constituents inhibit TRPV1. Ethylacetate and hexane fractions of the methanol extract were found to partially inhibit TRPV1 activity, and at a concentration of 90 μM oleanolic acid, which was one of three constituents isolated from the ethylacetate fraction, inhibited TRPV1 activity by 61.4±8.0%. This is first electrophysiological study to be conducted on the effects of C.rotundus extract and of their constituents on TRPV1. The results obtained provide insight of the potential therapeutic effects of C.rotundus in the contexts of analgesia and UV-induced photoaging.

Key Words: TRPV1, Cyperus rotundus, photo aging

P04-37

Open Collaborations in Ion Channel Drug Discovery

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Ion channels are membrane protein complexes that play an important roles in various physiological and pathophysiological processes by allowing the movement of selective ions across cell membranes. Ion channels are most promising targets for a wide range of applications, including hypertension, cardiac diseases, pain treatment, gastrointestinal disorders, cancer and other diseases. Ion channel modulators currently account for 17% of world pharmaceutical sales. Daewoong life science research institute focus on potentially first-in-class new chemical entities for pain, GERD, cystic fibrosis and dry eye/mouth with core ion channel technology. As a complementary approach, we have devised a structure-based, systematic analysis of the biological and chemical space exploited by the design of in-house compound libraries. Herein, we will introduce our strategies for scientific value, patient focus, and collaborative approach in ion channel drug discovery. We believe that ion channels will give the opportunity to make a significant difference for patients in first-in-class/best-in-class/best-in-disease.

Key Words: Ion Channel, Drug

P05-01

Resveratrol induced cell death of human ovarian cancer cells via caspase-dependent mechanism involving ROS/ Notch-1/Akt pathway

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In the present study, we investigated the effect of resveratrol on cell viability in human ovarian cancer cell lines A2780 and SKOV3. Resveratrol treatment induced apoptotic cell death of both cell lines in a dose- and time- dependent manner. It was accompanied by a transient increase of reactive oxygen species (ROS) generation. Resveratrol-induced cell death was attenuated by antioxidant, N-acetylcysteine (NAC). Western blot analysis showed that resveratrol induced decrease of Notch1 cleavage, activation of p-PTEN and de-phosphorylation of Akt. Overexpression of Notch1 or p-Akt by transfection with EF.hICN1.CMV.GFP (caNotch1) or a constitutively active form of Akt (caAkt) or pretreatment with p-PTEN inhibitor (SF1670) prevented the resveratrol-induced cell death. Resveratrol suppressed the expression level of X-linked inhibitor of apoptosis protein (XIAP) and it was accompanied by activation of caspase-3. Resveratrol-induced cell death was attenuated by caspase-3 inhibitor. Taken together, these results demonstrated that resveratrol induced cell death of human ovarian cancer cell lines via caspase-dependent mechanism involving ROS/ Notch1/PTEN/Akt pathway.

Key Words: Resveratrol, Ovarian caner, ROS, Notch, Caspase

P05-02

Role of miR-302 in proliferation and protective effect of oxidant-induced cell death of hADSCs

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Mesenchymal stem cells (MSCs) are a heterogeneous population of cells that proliferate in vitro as plastic-adherent cells, have a fibroblast-like morphology, form colonies in vitro and can differentiate into bone, cartilage and fat cells. The abundance, ease and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provide clear advantages for the use of human adipose tissue-derived mesenchymal stem cells (hASDCs) in clinical applications. We screened microRNAs that affected the proliferation and survival of hADSCs. Transfection of miR-302d mimic increased cell proliferation and protected cells from oxidant-induced cell death in hADSCs, which was supported by Flow cytometric analysis. miR-302d did not affect the expression of Bcl-2 family members or anti-oxidant molecules. The Nrf2-Keap1 system, which is one of the major mechanisms for the cellular defense against oxidative stress, was not altered by transfection of miR-302d mimic. To identify the target of the miR-302d actions on proliferation and survival of hADSCs, a microarray analysis was performed using miR-302d-overexpressing hADSCs. Real-time PCR analysis showed that transfection of miR-302d mimic inhibited the CDKN1A and CCL5 expression. Downregulation of CDKN1A with a specific siRNA blocked the effect of miR-302d on hADSCs proliferation, but did not affect miR-302d-induced cell survival. Downregulation of CCL5 protected oxidant-induced cell death as like miR-302d, inhibited oxidant-induced ROS generation and the addition of recombinant CCL5 inhibited the protective action of miR-302d on oxidant-induced cell death. This study indicates that miR-302 controls proliferation and cell survival of hADSCs through different targets and that this microRNA can be used to enhance the therapeutic efficacy of hADSCs transplantation in vivo.

Key Words: miR-302d, proliferation, hADSCs

P05-03

TAK1 plays an important role in the TNF-α-induced activation of NF-κB and osteogenic differentiation

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TNF-α has multiple effects on the proliferation and differentiation of hMSCs. In this study, we examined the role of TAK1 in TNF-α induced action in hADSCs. The downregulation of TAK1 mRNA affected neither the basal proliferation of hADSCs, nor the increase in proliferation induced by TNF-α. The inhibition of TAK1 expression by siRNA transfection inhibited NF-κB promoter activity, which was increased by TNF-α treatment. The inhibition of TAK1 expression did not affect the basal adipogenic differentiation, as well as its TNF-α-induced inhibition. In contrast, the inhibition of TAK1 expression inhibited both the TNF-α-induced increase of osteogenic differentiation, and the basal osteogenic differentiation. Western blot analysis showed that treatment with TNF-α induced IκB degradation, but that TAK1 siRNA transfection did not provide protection from this TNF-α-induced IκB degradation. The transfection of TAK1 siRNA also did not affect TNF-α induced IκB phosphorylation and ERK1/2 phosphorylation. However, TNF-α was found to increase p65 phosphorylation at 5 and 10 min after treatment, and the downregulation of TAK1 inhibited this TNF-α-induced S536 phosphorylation of the p65 subunit. TNF-α treatment induces p38 phosphorylation, which was inhibited by the transfection of TAK1 siRNA. The addition of p38 inhibitor inhibited TNF-α-induced p65 phosphorylation, NF-κB promoter activity and TNF-α-induced increase of osteogenic differentiation of hADSCs. These data indicate that TAK1 is involved in the TNF-α-induced activation of p38 kinase, which subsequently phosphorylates the p65 subunit of NF-κB, and increases the transactivation potential of p65 and osteogenic differentiation in hADSCs.

Key Words: TNF-a, TAK1, NF-κB, osteogenesis, hADSCs

P05-04(PO-5)

Plant homeodomain finger protein 2 promotes bone formation by demethylating and activating Runx2 for osteoblast differentiation

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Plant homeodomain finger protein 2 (PHF2), which possesses the plant homeodomain and Jumonji-C domain, is known as an epigenetic regulator that demethylates H3K9me2. On the other hand, Runt-related transcription factor 2 (Runx2) plays essential roles in bone development and regeneration. Given previous studies demonstrating that the PHF2 mutation can cause dwarfism in mice and that PHF2 is correlated with Runx2 in differentiating thymocytes, this study investigated whether PHF2 plays an essential role in Runx2-mediated bone formation. In *in vivo* experiments, the PHF2 transgene facilitated bone development in newborn mice, whereas PHF2 knock-down delayed bone regeneration in injured calvaria of adult rats. In primary osteoblasts and C2C12 precursor cells, osteoblast differentiation was positively regulated by PHF2. Mechanistically, Runx2-driven transcription was inhibited by the lysine methyltransferase SUV39H1 but was recovered by PHF2. PHF2 was associated with the Runt domain in Runx2 through its Jumonji-C domain and erased the dimethylation of Runx2 at Lys245. Unexpectedly, H3K9me2 levels in two Runx2-binding DNA elements were not affected by PHF2 knock-down. The results suggest that at least in differentiating osteoblasts, SUV39H1 and PHF2 are likely to serve as post-translational regulators of Runx2, not as epigenetic regulators. The conclusion that PHF2 promotes bone formation by activating Runx2 may provide a theoret-

ical basis for developing a new therapeutic modality for patients with impaired bone development or delayed fracture healing.
Key Words: PHF2, SUV39H1, Runx2, lysine methylation, osteoblast differentiation

P05-05

A protein arginine methyltransferase isoform controls the HIF-1-mediated adaptation to hypoxia by reducing de novo synthesis of HIF-1 α protein

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Hypoxia-inducible factor 1 α (HIF-1 α), which is regulated oxygen-dependently, transactivates numerous genes essential for cellular adaptation to hypoxia. HIF-1 α expression is regulated at multiple steps from transcription to protein degradation. Moreover, the stability of HIF-1 α protein has been known to be determined by posttranslational modifications such as ubiquitination, sumoylation, neddylation, and acetylation, but the HIF-1 α 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-1 α expression under hypoxia. When the PRMT was knocked-down in glioblastoma cells, HIF-1 α 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-1 α induction, and found that HIF-1a 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-1 α protein regardless of oxygen level. Given many literatures supporting the cancer promoting action of HIF-1 α, the PRMT could be a potential target for cancer therapy.
Key Words: HIF-1 α, PRMT, Transcription, Translation

P05-06

Glucosamine enhances body weight gain and reduces insulin response in mice fed chow diet but mitigates obesity, insulin resistance and impaired glucose tolerance in mice high-fat diet

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The hexosamine biosynthesis pathway (HBP) is known to influence pathological conditions associated with diabetes or diabetes-induced insulin resistance. However, there remains controversy regarding the effects of the HBP on obesity and obesity-induced insulin resistance. Here, we examined body weight gain, serum lipid levels, glucose tolerance, and insulin resistance in mice fed normal chow diet (CD) or high-fat diet (HFD) accompanied with oral administration of glucosamine (GlcN). The results show that GlcN administration stimulated body weight gain (6.58±0.82 g vs. 11.1±0.42 g), increased white adipose tissue (WAT) fat mass (percentage of bodyweight, 3.7±0.32 g vs. 5.61±0.34 g), and impaired the insulin response in livers of mice fed CD. However, GlcN treatment in mice fed HFD led to reduction of body weight gain (18.02±0.66 g vs. 16.22±0.96 g) and liver weight (2.27±0.1 vs. 1.85±0.12 g). Furthermore, obesity-induced insulin resistance and impaired Akt insulin signaling in the liver were alleviated by GlcN administration. To examine cellular responses to fluctuations in nutrient availability along with GlcN administration, Akt phosphorylation was assayed in response to insulin under two different (5 or 25 mM) glucose concentrations in HepG2 cells. GlcN administration mimicked the effects of glucose by inhibiting the insulin response under low (5 mM) glucose conditions, whereas it restored the insulin response for Akt phosphorylation under high (25 mM) glucose conditions. Uptake of 2-deoxyglucose (2-DG) increased upon GlcN treatment under 5 mM glucose compared to control, whereas insulin-stimulated 2-DG uptake decreased under 5 mM and increased under 25 mM glucose. The current data collectively support the integrative function of the HBP reflecting the nutrient status of lipids or glucose and further implicate the importance of the pathway in insulin signaling for the regulation of metabolism.
Key Words: glucosamine, hexosamine biosynthesis pathway, high fat diet, obesity, insulin resistance

P05-07

miR-210-3p controls proliferation and differentiation of human adipose tissue stromal cells

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The elucidation of the molecular mechanisms underlying the differentiation and proliferation of human adipose tissue-derived mesenchymal stem cells (hADSCs) represents a critical step in the development of hADSC-based

cellular therapies. To examine the role of the microRNA-210-3p (miR-210-3p) in hADSC functions, miR-210-3p mimics were transfected into hADSCs in order to overexpress miR-210-3p. Osteogenic differentiation was induced for 14 days in an osteogenic medium and assessed using an Alizarin red S stain. The expression of a predicted target of miR-210-3p, IGFBP3, was determined by western blot, real-time PCR and luciferase reporter assays. Overexpression of miR-210-3p inhibited the proliferation of hADSCs and increased their osteogenic differentiation. In addition, it downregulated protein and mRNA levels of IGFBP3. In contrast, inhibition of miR-210-3p with 2'O methyl antisense RNA increased the proliferation and inhibited osteogenic differentiation of hADSCs. The luciferase reporter activity of the construct containing the miR-210-3p target site within the IGFBP3 3' untranslated region was lower in miR-210-3p-transfected hADSCs than in control miRNA-transfected hADSCs. RNA interference-mediated downregulation of IGFBP3 expression in hADSCs inhibited their proliferation and increased osteogenic differentiation. Conclusion The results of the current study indicate that miR-210-3p regulates the osteogenic differentiation of hADSCs and proliferation of hADSCs by directly targeting IGFBP3. These findings further elucidate the molecular mechanisms governing the differentiation and proliferation of hADSCs.
Key Words: microRNA, hADSC, osteogenic differentiation, proliferation, miR-210-3p

P05-08

Melatonin receptor 2 stimulates cytoskeletal reorganization in promoting mesenchymal stem cells motility

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Melatonin (Mel), a circadian-rhythm-promoting molecule, has a variety of biological functions, but the functional role of Mel in the motility of mesenchymal stem cells (MSCs) has yet to be studied. In a mouse skin excisional wound model, we found that transplantation of umbilical cord blood (UCB)-MSCs pre-treated with Mel enhanced wound closure, granulation, and re-epithelialization at mouse skin wound sites, where relatively more UCB-MSCs which were engrafted onto the wound site were detected. Thus, we identified the signaling pathway of Mel, which affects the motility of UCB-MSCs. Mel (1 μM) significantly increased the motility of UCB-MSCs, which had been inhibited by the knockdown of melatonin receptor 2 (MT2). We found that Gαq coupled with MT2 and that the binding of Gαq to MT2 uniquely stimulated an atypical PKC isoform, PKCζ. Mel induced the phosphorylation of FAK and paxillin, which were concurrently down-regulated by the blocking of the PKC activity. Mel increased the levels of active Cdc42 and Arp2/3, and it has the ability to stimulate cytoskeletal reorganization-related proteins such as profilin-1, cofilin-1, and F-actin in UCB-MSCs. Finally, a lack of MT2 expression in UCB-MSCs during a mouse skin transplantation experiment resulted in impaired wound healing and less engraftment of stem cells at the wound site. These results demonstrate that MT2 signaling triggers FAK/paxillin phosphorylation to stimulate reorganization of the actin cytoskeleton, which is responsible for Cdc42/Arp2/3 activation to promote UCB-MSCs motility. It thereby plays an important role in the mobilization of UCB-MSCs for cutaneous wound repair.
Key Words: Melatonin, Melatonin receptor 2, Mouse skin wound, Umbilical cord blood derived mesenchymal stem cells

P05-09(PO-8)

Echinochrome A Increases Mitochondrial Mass and Function by Modulating Mitochondrial Biogenesis Regulatory Genes

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Echinochrome A (Ech A) is a natural pigment from sea urchins that has been reported to have antioxidant properties and a cardio protective effect against ischemia reperfusion injury. In this study, we ascertained whether Ech A enhances the mitochondrial biogenesis and oxidative phosphorylation in rat cardio myoblast H9c2 cells. To study the effects of Ech A on mitochondrial biogenesis, we measured mitochondrial mass, level of oxidative phosphorylation, and mitochondrial biogenesis regulatory gene expression. Ech A treatment did not induce cytotoxicity. However, Ech A treatment enhanced oxygen consumption rate and mitochondrial ATP level. Likewise, Ech A treatment increased mitochondrial contents in H9c2 cells. Furthermore, Ech A treatment up-regulated biogenesis of regulatory transcription genes, including proliferator-activated receptor gamma co-activator (PGC)-1α, estrogen-related receptor (ERR)-α, peroxisome proliferator-activator receptor (PPAR)-γ, and nuclear respiratory factor (NRF)-1 and such mitochondrial transcription regulatory genes as mitochondrial transcriptional factor A (TFAM), mitochondrial transcription factor B2 (TFB2M), mitochondrial DNA direct polymerase (POLMRT), single strand binding protein (SSBP) and Tu translation elongation factor (TUFM). In conclusion, these data suggest that Ech A is a potentiated marine drug which enhances mitochondrial biogenesis.
Key Words: Echinochrome A, mitochondrial biogenesis, oxygen consumption rate

P05-10

NecroX-5 suppresses sodium nitroprusside-induced cardiac cell death through inhibition of JNK and caspase-3 activation

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Sodium nitroprusside (SNP) is an effective hypotensive drug used in pediatric intensive care units and in treatment of heart failure. However, its clinical is limited due to its cardiotoxic nature. A newly-synthesized drug NecroX-5 (NX-5) is capable of inhibiting necrotic cell death, although knowledge about the drug is limited due to insufficient current literature addressing the suppression power of NX-5 in SNP-induced cell death or its mechanism of action. Therefore we investigated the protective role of NX-5 against SNP-induced cell death in cardiomyocyte-like H9c2 cells. Severe cell death was induced by SNP, which might occur through the phosphorylation of stress-activated protein kinase/c-JunNH2-terminal kinase (JNK) and the activation of the apoptotic signaling pathway, including Bcl-2 downregulation and caspase-3 cleavage. But SNP-induced cell death was suppressed by NX-5 through inhibition of JNK activation and suppression of both downregulation of Bcl-2 protein expression and caspase-3 cleavage. Taken altogether, these results will provide insights and aid in the development of antidotes to SNP toxicity in cardiac cells.
Key Words: NecroX-5, SNP, cell death, H9c2 cells, apoptosis, JNK, Bcl-2

P05-11

Echinochrome A protects mitochondrial function in cardiomyocytes against cardiotoxic drugs

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Echinochrome A (Ech A) is a naphthoquinoid pigment from sea urchins that possesses antioxidant, antimicrobial, anti-inflammatory and chelating abilities. Although Ech A is the active substance in the ophthalmic and cardiac drug Histochrome®, its underlying cardioprotective mechanisms are not well understood. In this study, we investigated the protective role of Ech A against toxic agents that induce death of rat cardiac myoblast H9c2 cells and isolated rat cardiomyocytes. We found that the cardiotoxic agents tert-Butyl hydroperoxide (tBHP, organic reactive oxygen species (ROS) inducer), sodium nitroprusside (SNP; anti-hypertension drug), and doxorubicin (anti-cancer drug) caused mitochondrial dysfunction such as increased ROS level and decreased mitochondrial membrane potential. Co-treatment with Ech A, however, prevented this decrease in membrane potential and increase in ROS level. Co-treatment of Ech A also reduced the effects of these cardiotoxic agents on mitochondrial oxidative phosphorylation and adenosine triphosphate level. These findings indicate the therapeutic potential of Ech A for reducing cardiotoxic agent-induced damage.
Key Words: echinochrome A, mitochondrial function, cardiotoxic drugs, SNP, tBHP, doxorubicin

P05-12

SB743921, a kinesin spindle protein inhibitor, induces cell death via the mitochondrial pathway in Multiple Myeloma cells

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Background: Multiple myeloma (MM) is the second most prevalent hematologic malignancy and it is uniformly fatal. Although survival rate has been increased over the last few decades, MM remains as a largely incurable disease with mortality rate. A second generation of kinesin spindle protein (KSP) inhibitor is a new anti-cancer treatment and its targets the mitotic motor kinesin. **Methods:** MM cell line-KMS20 exhibited cell death, cell cycle arrest and mitochondrial Ca^{2+} concentrations, mitochondrial ROS level, mitochondrial membrane potential ($\psi\triangle$m), oxygen consumption and ATP production, under non-treated and SB743921 treated conditions. **Results:** KSP inhibitor SB743921 suppressed KMS20 cell proliferation, cell morphology became abnormal with apoptotic bodies and cell membrane shrinkage were significantly increased than untreated group. SB743921 also actively blocked cell cycle such as G2/M phase. Treatment of KMS20 cells with SB743921 results in reduced the mitochondrial membrane potential ($\triangle\psi$m), increased the mitochondrial calcium and mitochondrial ROS were detected before significant activated caspase-3,8,9 and PARP cleavage. SB743921 induced mitochondria damage with a

subsequent release of cytochrome C into the cytoplasm. The mitochondrial functional differences were involved in the susceptibility of KMS20 cells to undergo SB743921-mediated cell death. **Conclusion:** Taken together, our results strongly suggested that KSP inhibition leads to myeloma cell apoptosis through mitochondria-dependent pathway. We elucidated that SB743921 may represent a novel treatment agent for Multiple Myeloma patients.
Key Words: multiple myeloma, kinesin spindle protein inihibitor, cell death, mitochondria

P05-13

B7-H4 downregulation induces mitochondrial dysfunction and enhances doxorubicin sensitivity via the cAMP/CREB/PGC1-α signaling pathway in HeLa cells

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B7-H4 is a B7 family coregulatory protein that inhibits T-cell-mediated immunity. B7-H4 is overexpressed in various cancers; however, the functional role of B7-H4 in cancer metabolism is poorly understood. Because mitochondria play pivotal roles in development, proliferation, and death of cancer cells we investigated molecular and functional alteration of mitochondria in B7-H4-depleted HeLa cells. In a human study, overexpression of B7-H4 was confirmed in the cervices of adenocarcinoma patients (n=3) compared to noncancer patients (n=3). In the cell line model, B7-H4 depletion was performed by transfection with small interfering RNA (siRNA). B7-H4 depletion suppressed oxygen consumption rate, ATP production, mitochondrial membrane potential and mass, and increased reactive oxygen species production. In particular, electron transport complex III activity was significantly impaired in siB7-H4-treated cells. Coincidently, depletion of B7-H4 suppressed a major mitochondrial regulators (peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PGC1-α] and mitochondrial transcription factor A), a component of oxidative phosphorylation (ubiquinol-cytochrome c reductase core protein 1), and an anti-apoptosis protein (Bcl-XL). Mitochondrial dysfunction in siRNA-treated cells significantly augmented oxidative stress, which strongly activated the JNK/P38/caspase axis in the presence of doxorubicin, resulting in increased apoptotic cell death. Investigating the mechanism of B7-H4-mediated mitochondrial modulation, we found that B7-H4 depletion significantly downregulated the cAMP/cAMP response element-binding protein/PGC1-α signaling pathway. Based on these findings, we conclude that B7-H4 has a role in the regulation of mitochondrial function, which is closely related to cancer cell physiology and drug sensitivity.
Key Words: B7-H4, mitochondria, PGC1-α, JNK, p38, adenocarcinoma

P05-14

Defective Mer signaling enhances local and systemic inflammation and down-regulates of LXR transcriptional activity

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Mer plays a central role in intrinsic inhibition of the inflammatory response by immune cells. We aimed to understand the function of Mer signaling on resolution of acute sterile inflammation and the molecular mechanisms involved. Utilizing Mer-/- mice in a model of sterile, zymosan-induced acute inflammation, we demonstrate that deficiency of Mer further enhanced the local and systemic inflammatory responses. In particular, expression of liver X receptor (LXR) α/β and its target molecules was reduced over the course of inflammation in peritoneal macrophages, spleen, and lung. In vitro exposure of macrophages to Gas6 was unable to inhibit zymosan-induced production of TNF-α or MIP-2 in siRNA LXRα-transfected macrophages, while these cytokines were inhibited in negative-control siRNA-transfected cells. Thus, we elucidated a novel pathway for the resolution of acute sterile inflammation that involves enhanced Mer signaling in the recovery and up-regulation of LXR expression and activity over the course of the inflammatory response.
Key Words: Mer, LXR, inflammation, Gas6, macrophages

P05-15

Differential expressions of bitter taste receptors in murine circumvallate papillae

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Purpose: Evolution makes the animal have bitter taste sense for detecting poisonous substances. Bitter taste is more sensitive in posterior part of tongue, where taste buds belong to circumvallate papillae. Bitter taste receptors are encoded in 35 kinds of tas2rs in mouse. Each receptor has different substrate specificity and sensitivity. However, expression pattern of 35 kinds of tas2rs in circumvallate papillae have not been studied. Expression patterns of tas2rs in circumvallate papillae would be important for elucidating physiological roles of taste senses in circumvallate papillae. **Materials and Methods:** Epithelial taste tissue including circumvallate papillae of male DBA2 mice (about 60 days old) were used. For expression analysis of bitter taste receptors, qPCRs were carried out. The relative expression levels of bitter taste receptors were compared to those of GAPDH. **Result:** All tas2rs were expressed in epithelial taste tissue including circumvallate papillae. However, the expression levels of bitter taste receptors were various.

Expression levels of tas2r108 and tas2r137 were relatively higher than those of other bitter taste receptors. Bitter taste receptors were not equally expressed in epithelial taste tissue including circumvallate papillae. **Conclusion:** All tas2rs expressed in circumvallate papillae. tas2r108 and tas2r137, the bitter receptors responding to most bitter substances, were expressed more than others.
Key Words: bitter taste, taste receptor, circumvallate papillae, qPCR

P05-16

Functional Expression of Bitter Taste Receptors in Murine Salivary Glands

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Purpose: Taste is essential for maintaining quality of life or survival of organisms. Bitter, sweet, and umami tastes are transduced by G protein coupled receptors in taste buds. Structures and distributions of taste receptors were recently investigated. Some of taste receptors were discovered in salivary glands. The purposes of this study are to confirm whether taste receptors in salivary glands are functional or not and to determine the relative expression levels of bitter taste receptors in salivary glands. **Materials and Methods:** Submandibular, sublingual, parotid glands of male DBA2 mice (about 60 days old) were used. For expression analysis of bitter taste receptors, qPCRs were carried out. The relative expression levels of 35 kinds of bitter taste receptors were compared to those of GAPDH. Fura-2 fluorescence imagings were used measuring intracellular Ca^{2+} activity changes. **Result:** Almost tas2rs were expressed in salivary glands, however, the expression levels of taste receptors were various. Expression pattern of tas2rs in salivary glands were not much different from those of taste tissue. Surprisingly, a few tas2rs were expressed more in salivary glands than in taste tissue. The bitter tastants including cycloheximide, denatonium and phenylthiourea elicited changes in Ca^{2+} activity. The time course, dose-response relationships, and/or to amplitudes of changes in Ca^{2+} activity elicited by bitter tastants showed characteristic features. The ratios of bitter tastants responding cells were different among salivary glands. **Conclusion:** Functional tas2rs were expressed in every major salivary gland. The responses to bitter tastants were dependent on bitter substances.
Key Words: bitter taste, taste receptor, salivary gland, qPCR, Ca^{2+} imaging

P05-17

Statin inhibits lipopolysaccharide induced epithelial-mesenchymal transition via downregulation of TLR4 in SV40-tramsformed normal human cholangiocyte cell line, H69

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Aims: Epithelial-mesenchymal transition (EMT) of biliary epithelial cells (BECs) has a role for biliary fibrosis. Lipopolysaccharide (LPS) promotes EMT in BECs. We studied the effects of simvastatin on the EMT of BECs induced by LPS and the molecular mechanism was investigated. **Methods:** Transformed human BECs (H69) were exposure with LPS (1 μg/mL) or transforming growth factor-β1 (TGF-β1, 5 ng/ml) for 48 hour. The expression of E-cadherin, vimentin and toll-like receptor 4 (TLR4) were determined by quantitative real-time PCR (qRT-PCR), western blotting and confocal microscopy. The effect of simvastatin (1 μM) on the EMT induced by LPS or TGF-β1 was determined by the expression of E-cadherin, vimentin and TLR4. **Results:** BECs stimulated with TGF-β1 or LPS showed the EMT like changes via TLR4 upregulation; increased vimentin and decreased E-cadherin in the expression of the mRNA and proteins. Simvastatin inhibits LPS induced EMT via TLR4 downregulation, manifested as increased E-cadherin and decreased vimentin. These findings indicated that LPS or TGF-β1 promoted EMT, and the treatment of the BECs with simvastatin blocked these changes by inhibition of TLR4. **Conclusion:** Simvastatin inhibits the LPS induced EMT of cultured human BECs. This result suggests that simvastatin can be considered as a new agent to prevent biliary fibrosis associated with EMT of BECs.
Key Words: epithelial-to-mesenchymal transition, biliary epithelial cells, lipopolysaccharide, simvastatin, Toll-like receptor- 4

P05-18

Genome-wide transcriptome analysis reveals that down-regulation of AURKA/B by resveratrol inhibits proliferation of colon cancer cells

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Colorectal cancer is the third most common cancer worldwide and its incidence in South Korea has dramatically increased as a result of changes in the economy and lifestyle. Although genetic predisposition is an important contributing factor for the occurrence of colorectal cancer, the main mechanisms underlying the pathogenesis of colorectal cancer remain largely unclear. Resveratrol is naturally occurring polyphenol that provides a number of anti-aging health benefits including cancer prevention. We utilized genome wide transcriptome analysis to identify important gene expression patterns following treatment with resveratrol on colon cancer cells (HCT116). Statistical analyses of gene expression data from resveratrol treated cells revealed that 1573 genes were significantly upregulated, while 1284 genes were down- regulated. Putative

gene networks showed that several cell cycle regulators (AURKA, AURKB, Myc, and CCNB1) were significantly suppressed by resveratrol treatment. Reseveratrol inhibited cell proliferation of HCT116 cells in a dose-dependent manner and resulted in a significant inhibition of colony formation in HCT116 cells. Cleaved-PARP, cleaved-caspase-3 and -9 protein levels were significantly increased in a dose dependent manner by resveratrol treatment. Therefore, system level characterization of our findings suggests for the first time that AUKRKA, AURKB, Myc, and CCNB1, which are major genes involved in cell cycle regulators, were significantly down-regulated by resveratrol treatment in colon cancer cells.
Key Words: resveratrol, colon cancer cells, cell cycle regulators, gene expression profiling

P05-19

Activation of the β-catenin/c-Myc signaling pathway by CTHRC1 stimulates invasion and metastasis of esophageal adenocarcinoma cells

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Esophageal adenocarcinoma (EAC) is one of high mortality cancers in the West and its occurrence has increased recently worldwide. Despite many attempts to understand the clinical biology of EAC, the biological mechanisms of EAC progression remain elusive. In the present study, we investigated the underlying molecular mechanisms by which CTHRC1 regulates metastasis in EAC cells. Knockdown of CTHRC1 significantly diminished the invasion of EAC cell lines BE3 and OE33 by Matrigel invasion assay. The mRNA and protein levels of vimentin, twist, MMP9, and uPA were significantly decreased in CTHRC1 knockdown EAC cells. In addition, knockdown of CTHRC1 in EAC cells significantly reduced levels of β-catenin and c-Myc, but increased p-β-catenin level. Therefore, CTHRC1 regulates the migration and invasion of EAC cells through activation of the β-catenin/c-Myc pathway. Our results suggest that targeting CTHRC1 may constitute a potential therapeutic strategy for EAC.
Key Words: esophageal adnocarcinoma cells, CTHRC1, β-catenin/c-Myc, metastasis, invasion

P05-20

HN1 stimulates invasion and metastasis of Hepatocellular carcinoma cells through activation of β-catenin

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Hepatocelluar carcinoma (HCC) is one of the most commonly occurring malignancies worldwide, having high morbidity and mortality. Every year, more than one million cases of late stages hepatocelluar carcinoma are reported and incidences are increasing continuously. Our previous study demonstrated HN1 is strongly associated with survival of patients with hepatocellular carcinoma. However, the biological functional roles of HN1 on adhesion and invasion in HCC cells (hepG2 cells) remain largely unknown. In this study, we investigate the underlying molecular mechanisms by which HN1 regulates metastasis in HCC cells. Knockdown of HN1 significantly diminished the invasion of hepG2 cells. The mRNA levels of vimentin, β-catenin and uPA were significantly decreased in HN1 shRNA hepG2 cells. In addition, knockdown of HN1 in HepG2 cells significantly reduced protein levels of uPA and P-β-catenin. Therefore, our results suggest that HN1 regulates the migration and invasion of HCC cells and targeting HN1 may constitute a therapeutic strategy for HCC.
Key Words: HN1, hepatocellular arcinoma, metastasis, invasion, β-catenin

P05-21

DNA microarray profiling of genes differentially regulated by the histone deacetylase inhibitor panobinostat in gastric cancer cells

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

DNA damage regulation.
Key Words: panobinostat, gastric cancer cells, apoptosis, gene expression profiling

P05-22

Regulation of airway inflammation by G-protein regulatory motif peptides of activator of G-protein signaling 3

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Respiratory diseases such as asthma, COPD, and lung infections have critical consequences on mortality and morbidity in humans. The aims of the present study were to examine the mechanism by which CXCL12 affects MUC1 transcription and airway inflammation, which depend on activator of G-protein signaling (AGS) 3 and to identify specific molecules that suppress CXCL12-induced airway inflammation by acting on G-protein-coupled receptors. Herein, AGS3 suppresses CXCL12-mediated upregulation of MUC1 and TNF α by regulating G αi. We found that the G-protein regulatory (GPR) motif in AGS3 binds to G αi and downregulates MUC1 expression; in contrast, this motif upregulates TNF α expression. Mutated GPR peptide increased the expression of MUC1 and TGF β but decreased the expression of TNF α and IL-6. In addition, CXCR4-induced dendritic extensions in 2D and 3D matrix cultures were inhibited by the GPR Q34A peptide compared with a consensus GPR peptide. The GPR Q34A peptide also inhibited CXCL12-induced morphological changes and inflammatory cell infiltration in the mouse lung. In addition, the GPR Q34A peptide inhibited the production of inflammatory cytokines in bronchoalveolar lavage (BAL) fluid and the lungs. Our data indicate that the AGS3 GPR motif is critical for regulating MUC1/Muc1 expression and cytokine production in the inflammatory microenvironment.
Key Words: airway Inflammation, CXCL12/CXCR4, MUC1/Muc1, AGS3, GPR

P05-23 (PO-14)

Gas6/Mer signaling enhances LXR expression and activity in macrophages.

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Growth arrest-specific protein 6 (Gas6)/Mer signaling modulates the inflammatory response in macrophages. Liver X receptor (LXR) α/β also have a role for the maintenance of immune tolerance. In our previous study, it was demonstrated that the Mer signaling enhances transcriptional LXR α/β activity and suppresses local and systemic inflammation. We demonstrate here a mechanism how Mer activation regulates LXR signaling in macrophages. The Mer ligand Gas6 enhanced LXR mRNA and protein expression in bone marrow-derived macrophages (BMDM), peritoneal macrophages and RAW 264.7 cells. Gas6 treatment also enhanced LXRE activity and mRNA or protein expression of its target genes, including ABCA1, ABCG1, and APOE. Moreover, the induction of markers of alternative macrophage activation AIM, Arg2, and VGEF were enhanced by Gas6 treatment. Specific small interfering RNA (siRNA) of Mer inhibited Gas6-induced LXR expression. The specific inhibitor of PI3K, Akt, p38 MAP kinase, JNK, JAK2, or JAK3, but not ERK, inhibited the induction of LXR. Our data suggest that Gas6/Mer signaling promotes the upregulation of transcriptional LXR activity and the involvement of PI3K/Akt, p38 MAP kinase, JNK, JAK2, or JNK3 pathway in Mer-mediating LXR activation.
Key Words: Gas6, Mer, LXR, macrophage

P05-24

P66shc-mediated oxidative stress & endothelial activation in CRIF1 deficient endothelial cells

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Mitochondrial dysfunction has been implicated in the pathophysiology of various cardiovascular diseases. CRIF1 is a protein present in the mitochondria associated with large mitoribosomal subunits, and CRIF1 knockdown induces mitochondrial dysfunction and promotes ROS production. p66shc is a redox enzyme implicated in mitochondrial ROS generation and translation of oxidative signals and, therefore, is a key factor for oxidative stress in endothelial cells. In this study, we investigated whether mitochondrial dysfunction induced by CRIF1 knockdown induces p66shc stimulation and plays any role in mitochondrial dysfunction-induced endothelial activation. Knockdown of CRIF1 decreased the expression of mitochondrial oxidative phosphorylation (OXPHOS) complexes I, III and IV, leading to increased mitochondrial ROS (mtROS) and hyperpolarization of the mitochondrial membrane potential. Knockdown of CRIF1 also stimulated phosphorylation of p66shc and increased cytosolic ROS in endothelial cells. Furthermore, the expression of vascular cell adhesion molecule-1 and endoplasmic reticulum stress proteins were increased upon CRIF1 knockdown in endothelial cells. However, p66shc knockdown blunted the alteration in mitochondrial dynamics and ROS production in CRIF1 knockdown endothelial cells. In addition, p66shc knock-

down reduced the CRIF1 knockdown-induced increases in adhesion between monocytes and endothelial cells. Taken together, these results suggest that CRIF1 knockdown partially induces endothelial activation via increased ROS production and phosphorylation of p66shc.
Key Words: CRIF1, p66shc, OXPHOS complex, oxidative stress

P05-25

Vascular function changes of spontaneously hypertensive rats by Rg3-enriched Korean Red ginseng

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Abstract Panax ginseng has distinct and impressive health benefits, such as improved blood pressure and immune system functioning. Rg3-enriched Korean red ginseng (REKRG) isolated from Korean red ginseng contains a high percentage of Rg3. In this study, we examined the effects of REKRG on eNOS activation and adhesion molecules in endothelial cells and vascular function in rats. REKRG dose-dependently increased endothelial nitric oxide synthase (eNOS) phosphorylation and nitric oxide (NO) production in endothelial cells. In addition, REKRG markedly inhibited the TNF-α-mediated induction of intercellular adhesion molecule (ICAM)-1 and cyclooxygenase (COX)-2 expressions in endothelial cells. REKRG improved endothelium-dependent vasorelaxation in Wistar-Kyoto rat (WKY) and spontaneously hypertensive rats (SHRs) compared with controls. Furthermore, REKRG treatment for 6 weeks increased serum NO levels and reduced the mean aortic intima-media thickness compared with controls. Taken together, these results suggest that REKRG increased vascular function and improved immune system functioning. Therefore, REKRG is a very useful food for preventing or improving various cardiovascular diseases.
Key Words: panax ginseng, REKRG, SHR, eNOS, NO

P05-26

TRB3 is associated with Glucose Enhancing Effect on LPS-induced NO production

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Tribbles homolog 3 (TRB3), a human homolog of Drosophila tribble, is a pseudokinase, of which expression is regulated by stress response and change in the nutritional status. It has been reported that TRB3 participates not only in the regulation of normal cellular functions such as energy metabolism but also pathological processes including inflammation and cell death. In the previous reports we showed high glucose (25mM) enhanced LPS-induced NO production in macrophages. However the specific mechanism of glucose enhancing effect has not been defined yet. In this study, we investigated the role of the nutrient dependent protein TRB3 in the LPS- induced TLR4 activation, adopting MALP-2, a TLR2 specific agonist, poly(I:C), a TLR3 specific agonist, and LPS. TRB3 expression was downregulated in high glucose condition. High glucose enhanced iNOS expression and NO production in Raw264.1 macrophages treated with LPS or MALP-2. LPS or poly(I:C)-induced IFN-β expression was heightened under high glucose condition. These results suggest that TRB3 could be a negative modulator of MyD88 and TRIF pathways, and thus could be associated with glucose enhancing effect. This study was supported by a grant from Ajou University School of Medicine and Gyunggi-do through CCRB-GRRC
Key Words: TRB3,LPS,iNOS

P05-27

17β-estradiol promotes the odontogenesis mediated by estrogen receptor-α activation of c-Src/MAPK pathway in human dental pulp cells

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Estrogen can regulate the differentiation of periodontal ligament stem cells and bone marrow-derived mesenchymal stem cells. However, the biological effects of estrogen in human dental pulp cells (HDPCs) have not been well studied. This study investigated the effects of the endogenous estrogen 17β-estradiol (E2) on odontoblastic differentiation in HDPCs and their molecular mechanism with involvement of estrogen receptor (ER). E2 significantly promoted the HDPC proliferation in a dose dependent manner, which was mediated by extracellular signal-related kinase 1/2. E2 upregulated the odontogenic differentiation markers dentin sialophosphoprotein (DSPP), dentin sialoprotein (DSP) and dentin matrix protein1 (DMP1), and enhanced alkaline phosphatase activity, which was indicative of early odontoblastic differentiation, in a differentiation inductive medium including 100 μM/L ascorbic acid and 10 mM/L β-glycerophosphate. In addition, HDPC nodule formation was enhanced by exposure to estradiol for 7 days. E2 increased ER-alpha (ER-α) mRNA expression and phosphorylated ER-α, but not ER-beta (ER-β). The addition of the ER down-regulator fulvestrant attenuated the expression of DSPP, DMP1 and DSP, which were up-regu-

lated during estrogen-induced odontoblastic differentiation. Moreover, c-Src and mitogen-activated protein kinases (MAPKs) were activated with E2 treatment upon odontogenic induction, whereas chemical inhibition of c-Src and MAPK blocked upregulation of DSPP, DMP1 and DSP, and mineralization enhanced by E2. In addition, pretreatment with fulvestrant reduced E2-induced phosphorylation of c-Src and MAPK, and inhibition of c-Src attenuated activation of MAPKs during estrogen-induced odontoblastic differentiation. These results indicate that E2 stimulates odontoblastic differentiation of HDPCs via ER-α, c-Src and MAPK signaling pathways, which may play a key role in the regeneration of dentin.
Key Words: Estrogen, Odontogenesis, Src, MAPK, Human Dental Pulp Cell

P05-28

High glucose-induced E-cadherin repression regulates migration of human mesenchymal stem cells via Notch, Snail, and Polycomb complex signaling pathway

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Glucose is an essential source of cellular energy and is an important substrate for protein and lipid synthesis. High glucose mediates cell migration in various cell types including fibroblasts/myofibroblasts, keratinocytes, and endothelial cells. We investigated the effect of high glucose (25 mM) on regulation of human mesenchymal stem cell migration and related signaling pathways. High glucose increased reactive oxygen species (ROS) generation that promoted the activation of c-Jun N-terminal kinase (JNK). ROS-dependent activation of JNK regulated γ-secretase which can cleave Notch proteins and Notch intracellular domain (NICD) translocates into the nucleus. PI3K-Akt pathway was regulated by the activated NICD. Phosphorylated Akt inhibited GSK3β which involved in phosphorylation and stabilization of β-catenin and expression of Snail. These activities were blocked by N-acetyl-L-cysteine (ROS inhibitor), SP 600125 (JNK inhibitor), L-685,458 (γ-secretase inhibitor), LY 294002 (PI3K inhibitor), or an Akt inhibitor. β-catenin level was increased by treatment with LiCl (GSK-3β inhibitor) and stabilized β-catenin bound with EZH2, then EZH2 and β-catenin translocated to the nucleus. High glucose induced Snail-dependent Polycomb complex formation which is required for E-cadherin repression. Our results show that Snail interacted with components of the PRC2 complex. These results indicated that Snail recruited PRC2 to the E-cadherin promoter in the nucleus and the expression of E-cadherin was repressed. Collectively, these studies implicate high glucose-induced Notch mediated E-cadherin repression that resulted in hMSC migration via interplay between PRC2 and Snail.
Key Words: high glucose, human mesenchymal stem cells, migration

P05-29

APE1/Ref-1 as a serological biomarker for the detection of bladder cancer

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To determine whether elevated serum levels of apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) are associated with bladder cancer. We used tumor tissues and serum from patients with bladder cancer who had received neither chemotherapy nor radiotherapy (n=51), and serum from healthy controls (n=55). The expression levels of APE1/Ref-1 and its cellular distribution were analyzed by immunoblotting and immunohistochemistry, respectively. Serum APE1/Ref-1 levels were quantified using an enzyme-linked immunosorbent assay. The area under the receiver operating characteristic curve (ROC-AUC) was applied to determine the association between various clinical factors and serum APE1/Ref-1. Immunohistochemistry and immunoblotting revealed that the APE1/Ref-1 protein level in bladder tissues was elevated in patients with bladder cancer. Furthermore, the mean serum APE1/Ref-1 level was significantly elevated in bladder cancer patients compared to healthy controls, and its levels correlated with tumor stage and grade. The ROC-AUC for APE1/Ref-1 serum levels in bladder cancer was 0.824. In this study, the optimal combination of sensitivity and specificity were determined as 93% and 59%, respectively, for a cutoff value of serum APE1/Ref-1 of 2.83 ng/100 μl. Serum APE1/Ref-1 levels were also elevated in patients with recurrent bladder cancer. Serum APE1/Ref-1 may help to diagnose patients with bladder cancer.
Key Words: APE1/Ref-1, Bladder cancer

P05-30

The hexane fraction of Naematoloma sublateritium extract suppresses the TNF-α-induced metastatic potential of MDA-MB-231 breast cancer cells through modulation of the JNK and p38 pathways

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Naematoloma sublateritium (Fr.) P. Karst is a basidiomy-

cete that has been used as traditional medicine. N. sublateritium produces a triterpenoid antitumor compound, clavaric acid, but, in general, the effects of N. sublateritium constituents against tumor invasion and metastasis have been poorly studied. To investigate the inhibitory effect of N. sublateritium constituents on highly invasive and metastatic tumor cells, the TNF-α-stimulated human breast cancer cell line, MDA-MB 231 was treated with the hexane fraction of an N. sublateritium extract (HFNS). Non-cytotoxic concentrations of HFNS markedly inhibited the invasion and migration of the MDA-MB 231 cells in the Matrigel invasion assay and wound-healing analysis, respectively. Gelatin zymography showed that HFNS suppressed the activity of MMP-9, but not of MMP-2. Immunoblotting demonstrated that treatment with HFNS had decreased the level of MMP-9 and urokinase plas¬minogen activator-1 (uPA-1), but had upregulated expression of the endogenous inhibitor proteins, including TIMP-1,-2, and PAI-1, in a dose-dependent manner. Furthermore, HFNS suppressed the phosphorylation of p38 and JNK1/2, but not that of ERK1/2. This was confirmed by pretreatment of cells with specific inhibitors prior to stimulation with TNF-α. HFNS treatment also led to a dose-dependent inhibition of the DNA-binding activities of AP-1 and NF-κB, which are downstream targets of JNK and p38. These data suggested that HFNS inhibits the metastatic potential of MDA-MB 231 cells by inhibiting the phosphorylation of JNK/p38 and reducing AP-1 and NF-κB DNA-binding activities. Therefore, HFNS may be a potential therapeutic agent against metastasis of breast cancer.
Key Words: anti-metastatic effect, breast cancer cell, Hexane fraction, MAPK pathway

P05-31

Mitochondrial APE1/Ref-1 suppressed protein kinase C-induced mitochondrial dysfunction in mouse endothelial cells

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Protein kinase C induces mitochondrial dysfunction, which is an important pathological factor in cardiovascular diseases. The role of mitochondrial human apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) has not been variously investigated. In this study, phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, induced mitochondrial hyperpolarization and reactive oxygen species generation and also increased mitochondrial translocation of APE1/Ref-1. APE1/Ref-1 overexpression suppressed PMA-induced mitochondrial dysfunction; however, gene silencing of APE1/Ref-1 increased the sensitivity of mitochondrial dysfunction. Moreover, mitochondrial targeting sequence-fused APE1/Ref-1 potently suppressed PMA-induced mitochondrial dysfunctions. These results suggest that mitochondrial APE1/Ref-1 is contributed to the protective role to protein kinase C-induced mitochondrial dysfunction in endothelial cells.
Key Words: APE1/Ref-1, endothelial cell, mitochondria, phorbol 12-myristate 13-acetate

P05-32

Autophagy is induced for odontogenic differentiation of human dental pulp stem cells via PI3K/Akt/mTOR

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Autophagy plays an important role in homeostasis, development, and disease through a survival or cell death pathway. However, there is little information about the role of autophagy in odontogenic differentiation of human dental pulp stem cells (HDPSC). The present study is aimed to establish whether autophagy is involved in differentiation of HDPSC isolated from human dental pulp tissues. For differentiation experiments, cells were cultured in differentiation inductive medium (DM) including 50 μM ascorbic acid and 5 mM β-glycerophosphate. DM increased expression of dentin matrix protein-1 (DMP-1) and dentin sialoprotein (DSP), markers of odontoblastic differentiation. Expression of LC3-II, a marker of autophagy, was increased, whereas p62 protein level, another indicator of autophagy, was decreased during differentiation of HDPSC. 3-Methyladenine (3MA) and Bafilomycin A1 (Baf-A1), autophagy inhibitors, decreased alkaline phosphatase (ALP) and Alizarin red S staining enhanced with DM in a dose-dependent manner. In addition, acidic autophagolysosomal vacuoles by Acridine orange staining, indicating the possible induction of autophagy, were increased during differentiation. 3MA decreased acidic organelles and downregulated DMP-1 in HDPSC. Moreover, 3MA or Baf-A1 restored expression of Oct4 and Nanog, key pluripotency factors, declined in DM. HDPSC were induced odontogenic differentiation by enhancement of autophagy via PI3K/Akt/mTOR inhibition. In DM, expression of Wnt5a, which act as antagonists of the Wnt/β-catenin pathway, was gradually reduced for 7 days. However, 3MA, Baf-A1 or shLC3 increased their expression. These findings suggest that autophagy stimulates odontogenic differentiation of HDPSC, which is regulated by inhibition of mTOR and Wnt5a.
Key Words: autophagy, odontogenesis, differentiation, HDPSC

P05-33

Gas6 or apoptotic cells/Mer signaling promotes epithelial cell growth and wound repair via up-regulation of HGF in macrophages

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Growth arrest-specific protein 6 (Gas6)/Mer signaling modulates cytokine secretion and helps to regulate the immune response and apoptotic cell clearance. We report that Gas6 or apoptotic cells/Mer/RhoA signaling can induce the production of epithelial growth factor HGF in macrophages which ultimately promotes epithelial cell proliferation and wound repair. Conditioned medium from RAW 264.7 cells that had been exposed to Gas6 or apoptotic cells enhanced LA-4 epithelial cell proliferation and wound closure. Co-treatment with an HGF receptor-blocking antibody or c-Met antagonist was found to down-regulate this enhancement. Inhibition of Mer with siRNA or the RhoA/Rho kinase pathway by RhoA siRNA or Rho kinase pharmacological inhibitor suppressed Gas6 or apoptotic cell-induced HGF mRNA and protein expression in macrophages and blocked epithelial cell proliferation and wound closure induced by the conditioned medium. Our data provide evidence that macrophages can be re-programmed by Gas6 or apoptotic cells to promote epithelial proliferation and wound repair via HGF, which is induced by Mer/RhoA-dependent pathway. Thus, defects in Gas6/Mer/RhoA signaling in macrophages may delay tissue repair after injury to the alveolar epithelium.

Key Words: Gas6/Mer/RhoA, epithelial cell, wound repair, HGF

P05-34

PPAR γ activation following apoptotic cell instillation promotes resolution of bleomycin-induced lung inflammation and fibrosis

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Changes in macrophage phenotype have been implicated in apoptotic cell-mediated immune modulation via induction of peroxisome proliferator-activated receptor gamma (PPAR γ). In this study, we characterized PPAR γ induction by apoptotic cell instillation over the course of bleomycin-induced lung injury in C57BL/6 mice. Next, the role of PPAR γ activation in resolving lung inflammation and fibrosis was investigated. Our data demonstrate that apoptotic cell instillation after bleomycin results in immediate and prolonged enhancement of PPAR γ mRNA and protein in alveolar macrophages and lung. Moreover, PPAR γ activity and expression of its target molecules, including CD36, macrophage mannose receptor, and arginase 1, were persistently enhanced following apoptotic cell instillation. Co-administration of the PPAR γ antagonist, GW9662, reversed the enhanced efferocytosis, and the reduced pro-inflammatory cytokine expression, neutrophil recruitment, myeloperoxidase (MPO) activity, apoptotic activity, fibrogenic markers and hydroxyproline contents in the lung by apoptotic cell instillation. In addition, inhibition of PPAR γ activity reversed the expression of transforming growth factor beta (TGF-β), interleukin (IL)-10, and hepatocyte growth factor (HGF). These findings indicate that one-time apoptotic cell instillation contributes to anti-inflammatory and anti-fibrotic responses via upregulation of PPAR γ expression and subsequent activation, leading to regulation of efferocytosis and production of pro-resolving cytokines.

Key Words: PPAR γ, apoptotic cell, bleomycin

P05-35

Regulation of lipocalin-2 gene by pro-inflammatory cytokines in islet β-cells

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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 pro-inflammatory 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

P06-01

Transient receptor potential canonical type 3 channels control the vascular contractility of mouse mesenteric arteries

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Aims: Transient receptor potential canonical type 3 (TRPC3) channels are non-selective cation channels and regulate intracellular Ca^{2+} concentration. We examined the role of TRPC3 channels in agonist-, membrane depolarization (high K^+)-, and mechanical (pressure)-induced vasoconstriction and vasorelaxation in mouse mesenteric arteries. Methods and Results: Vasoconstriction and vasorelaxation of endothelial cells intact mesenteric arteries were measured in TRPC3 wild-type (WT) and knockout (KO) mice. Calcium concentration ([Ca^{2+}]) was measured in isolated arteries from TRPC3 WT and KO mice as well as in the mouse endothelial cell line bEnd.3. Nitric oxide (NO) production and nitrate/nitrite concentrations were also measured in TRPC3 WT and KO mice. Phenylephrine-induced vasoconstriction was reduced in TRPC3 KO mice when compared to that of WT mice, but neither high K^+- nor pressure-induced vasoconstriction was altered in TRPC3 KO mice. Acetylcholine-induced vasorelaxation was inhibited in TRPC3 KO mice and by the selective TRPC3 blocker pyrazole-3. Acetylcholine blocked the phenylephrine-induced increase in Ca^{2+} ratio and then relaxation in TRPC3 WT mice but had little effect on those outcomes in KO mice. Acetylcholine evoked a Ca^{2+} increase in endothelial cells, which was inhibited by pyrazole-3. Acetylcholine induced increased NO release in TRPC3 WT mice, but not in KO mice. Acetylcholine also increased the nitrate/nitrite concentration in TRPC3 WT mice, but not in KO mice. Conclusions: The present study directly demonstrated that the TRPC3 channel is involved in agonist-induced vasoconstriction and plays important role in NO-mediated vasorelaxation of intact mesenteric arteries.

Key Words: TRPC3, endothelium, nitric oxide, vasoconstriction, vasorelaxation

P06-02

Endoplasmic Reticulum Stress Contributes Coronary Artery Dysfunction in Type 2 Diabetic Mice

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Background: Vascular dysfunction is a major complication in type 2 diabetes. Although endoplasmic reticulum (ER) stress has been suggested to be a contributing factor in cardiovascular diseases, the relationship between ER stress and type 2 diabetic vascular dysfunction remains unclear. Thus, in the present study, we examined whether ER stress contributes coronary artery dysfunction and ER stress inhibition ameliorates vascular function in type 2 diabetes. **Methods and Results:** Type 2 diabetic and their control mice were treated with or without ER stress inhibitor (taurine-conjugated ursodeoxycholic acid, TUDCA) for 2 weeks. In type 2 diabetic mice, blood glucose, body weight, and insulin level are elevated compared to control mice. The myogenic response is potentiated and endothelium-dependent relaxation is impaired in type 2 diabetic mice. Interestingly, treatment of ER stress inhibitor normalized myogenic responses and endothelium-dependent relaxation. These data were associated with an increase in ER stress marker expression (CHOP, ATF6, XBP-1, and phosphorylated-eIF2α) in type 2 diabetic mice, which were reduced by treatment with ER stress inhibitor. **Conclusion:** ER stress inhibition normalizes myogenic response and improves vascular function in type 2 diabetes. Therefore, ER stress could be a potential target for cardiovascular diseases in diabetes mellitus.

Key Words: ER stress, Coronary artery, Type 2 diabetes, Myogenic response

P06-03

Palmitic acid regulation of cardiac inotropy in healthy and hypertensive rat heart

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Background and aim: Palmitic acid (PA) is a predominant metabolic substrate that plays important roles in cardiac metabolism and signaling. So far, the effect of PA on cardiac contraction and underlying mechanisms in healthy and diseased heart remain unclear. Here, we analyzed PA-regulation of myocyte contraction in left ventricular (LV) myocytes from sham and angiotensin II (Ang II)-induced hypertensive rats. Results Our results showed that PA (100 μM) increased the amplitude of sarcomere shortening in LV myocytes (field stimulation, 2Hz) from shams but not in that from hypertension. Intracellular ATP levels are increased by PA only in shams. Etomoxir, a selective carnitine palmitoyltransferase I inhibitor, which is critical for beta-oxidation and ATP production in mitochondria) blunted the positive inotropic effect of PA in shams, suggesting that increased metabolism following PA treatment is fundamentally important in cardiac inotropy. Mechanistically, PA did not increase the amplitude of Ca^{2+} transients or the density of peak L-type Ca^{2+} current (ICa) in either group (Na+-Ca^{2+} exchanger activity, INCX, was tended to be increased by PA). Further experiments suggest that PA increased myocyte contraction via increased myofilament Ca^{2+} sensitivity (Myo-Ca-Sen) in shams. In hypertension, Myo-Ca-Sen was lower compared to shams and it maintained at this level with PA. PA reduced nitric oxide (NO) production in shams but increased NO from neuronal nitric oxide synthase (nNOS) in hypertension. Importantly, nNOS inhibition with S-methyl-l-thiocitrulline (SMTC) restored Myo-Ca-Sen and PA-induced cardiac inotropy in hypertension without affecting contraction in shams. Conclusion: PA increases myocardial metabolism and potentiates LV myocyte contraction via myofilament Ca^{2+} sensitization in healthy heart. nNOS-dependent myofilament Ca^{2+}-desensitization restricts PA-dependent cardiac inotropy in hypertension.

Key Words: cardiac myocyte contraction, myofilament Ca^{2+} sensitivity, hypertension, nNOS, palmitic acid

P06-04(PO-9)

Structural and functional significances of T-tubules in rat atrial cardiomyocytes

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The transverse tubules (T-tubules) are invaginations of the cell membrane that include important membrane proteins (ion channels and transporters) devoted to the excitation-contraction (EC) coupling of the heart muscle cells. In ventricular myocytes, an important role of regularly organized T-tubules on EC coupling has been well established. However, the presence of T-tubules in atrial myocytes has been a matter of debate and was traditionally believed to be absent or very sparse. Therefore, the present study aimed to validate the presence of T-tubules in rat atrial myocytes. With a noninvasive live cell surface nano-imaging technique (scanning ion conductance microscopy, SICM), we found that the atrial myocytes have surface T-tubule openings and unique membrane nano-structures. We categorized rat atrial surface structures into five types based on the presence of T-tubule openings and the surface nanostructures. Using SICM imaging, structural differences of the atrial T-tubule opening size and Z-groove index were compared with the ventricular myocytes. Intracellular T-tubule membranes were visualized by a confocal imaging of di-8-ANEPPS-stained atrial myocytes that reveals degree of intracellular T-tubule network was positively correlated with width and volume of the cells. Structural and functional consequences of the loss of T-tubules were evaluated by formamide-induced detubulaton of atrial myocytes. Detubulation of atrial myocytes resulted in a decrease of cell membrane capacitance and L-type Ca^{2+} channel density, and altered kinetics of cell shortening upon electric field stimulation. Taken together, our results validate that rat atrial myocytes have an appreciable T-tubule system and the loss of T-tubules can alter atrial EC-coupling by refined loss of T-tubular ion channels.

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Key Words: T-tubules, artial myocyte, SICM, EC-coupling

P06-05

Comparison of the calcium kinetics and cellular contraction between the left and right ventricular myocytes from adult rat heart

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The heterogeneity in intraventricular characteristics of the heart go beyond its structural differences, as the left and right ventricles have also been found to come from different progenitor cells and have distinct electrophysiological properties. However, whether or not these characteristics can influence the excitation-contraction coupling in the cardiac myocytes has not yet been established. In this study, left (LV) and right ventricular (RV) myocytes were isolated from male Wistar rats via Langendorff perfusion. Myocyte shortening, $[Ca^{2+}]i$ transients and L-type calcium currents were evaluated to compare the calcium kinetics and contractility between the LV and RV myocytes. Percentage of cell length change during cell shortening was insignificant between the LV and RV myocytes, as well as the time to peak and relaxation time. However, the calcium transients of the LV and RV myocytes showed that RV myocytes have a faster time to peak (RV 102.27 ms; LV 121.07 ms) but takes a longer time to decay at 50% of the baseline (RV 343.37 ms; LV 166.27 ms). Interestingly, the LV myocyte has been observed to have a greater influx of calcium through the L-type calcium channel ($p < 0.05$). Moreover, the LV myocytes showed a greater response to isoproterenol (200 nM), as reflected by a greater calcium transient and cell shortening amplitudes. LC/MS with non-labeling peptide count was performed to detect proteome change in the LV and RV myocytes. Of the 728 identified proteins, only 49 proteins were found significantly different between the ventricles ($p < 0.05$). 34 were detected uniquely or predominantly on the LV, while the remaining 15 were detected on the RV. Interestingly, among these proteins, the calcium handling protein RyR2 was found to be more prominent in the right ventricle. Collectively, these results show that the left ventricle of the adult rat heart is more significant in terms of excitation-contraction coupling. LV myocytes were also more sensitive to a beta-adrenergic agonist in the adult rat heart.

Key Words: calcium kinetics, contraction, heart ventricles

P06-06

Regional differences of Structure and Contractility of Aorta

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Background: The aortic aneurysm is common disease with dysfunction of vascular contraction and relaxation. The aortic aneurysm is mainly found in abdominal and thoracic aorta but less found in arch and ascending aortic region. However, the path physiological cause of the spatial specificity of aortic aneurysm was unclear. To understand this spatial specificity, we tested whether different aortic regions differently response to contracting and relaxing stimulations. Furthermore, we tested whether there are expressional differences in contractile protein components between four dif-

ferent regions of aorta including ascending, arch, thoracic and abdominal regions. **Methods:** Aortic isometric tension and effect of Phenylephrine (PE) and Acetylcholine (Ach) were measured for the four different aortic regions in Wistar rats. Histological studies of the four different aortic tissues were performed by using Hematoxylin-Eosin and Masson's Trichrome stains for elastin and collagen, respectively. **Results:** Phenylephrine dose-dependent contraction in aortic strips was significantly different ($P<0.001$).The ascending aorta showed significantly higher contraction than the three other aortic regions. The Ach induced relaxation in thoracic aortic regions significantly lower than three other aortic regions ($p<0.001$). In the histological study, we observed that the shape of ascending and arch aortic lumens were elliptic, but abdominal and thoracic are circular shape. The elliptic areas were divided into thin and thick tunica media.The thickness between thin and thick tunica media area were significantly differences in ascending and arch aorta ($p<0.001$). The amounts of collagen in the ascending and arch aortic segments are less than in the thoracic and abdominal aortic wall. **Conclusion:** These data suggest that thoracic aorta has lower relaxibility with higher intraceullar collagen contents which may related with higher occurrence of aneurysm in this aortic regions.
Key Words: aorta, collagen fiber, elastic fiber, contraction, relaxation.

P06-07

Schisandrin B suppresses TGF β1 signaling by inhibiting Smad2/3 and MLCK

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Transforming growth factor β1(TGF β1) plays a key role in the pathogenesis of vascular fibrotic diseases. Actin stress fiber formation is a crucial cellular process for vascular smooth muscle cell (VSMC) migration in response to vascular injury, which plays an important role in the pathogenesis of vascular fibrotic diseases. Schisandra chinensis fruit extract (SCE) has been used as a traditional oriental medicine for treating vascular diseases. However, the pharmacologic mechanisms of SCE on vascular fibrosis are largely unknown. In this study, we investigated the effect of SCE and its active ingredients on TGF β1 signaling. We found that SCE and its active ingredient schisandrin B (SchB) potently inhibited cell migration through inhibition of TGF β1 signaling in A7r5 VSMCs. SchB reduced TGF β1-mediated phosphorylation of SMAD2/3 and myosin light chain (MLC). Radiometric enzyme assays confirmed that SchB directly inhibited myosin light chain kinase (MLCK) activity. We also showed that SchB decreased TGF β1-mediated induction of α-smooth muscle actin and stress fiber formation. Our results demonstrate that SCE and SchB effectively inhibit TGF β1-induced fibrotic changes. Our findings may help future investigations to develop multi-targeted therapeutic strategies that attenuate vascular fibrotic diseases.
Key Words: TGF β1, Vascular smooth muscle cell, Schisandrin B, Fibrosis

P06-08

Physiological stretch and thromboxane A2 concertedly activate NOS3 in pulmonary arterial smooth muscle: a mechanism for the low resistance of pulmonary circulation

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Nitric oxide (NO), a potent vasodilator, is conventionally thought to be released from endothelial cells via NOS3 (eNOS) activation. In endothelium-denuded rat pulmonary arteries (PAs), nonspecific NOS inhibitor (L-NAME, 100 uM) induces a weak contraction in the presence of 5 nM U46619 with low pretone (0.1 g, S(-)). With increased pretone (0.3 g, S(+)), however, L-NAME induced robust contraction of PAs (110 % of 80 mM KCl contraction). In endothelium-denuded systemic arteries (e.g. renal and mesenteric arteries), the L-NAME/U46619-induced contraction was feeble even with high pretone. Neither NOS1 inhibitor (SMTC) nor NOS2 inhibitor (1400W) mimicked the L-NAME effect in PAs. Muscular expression of NOS3 in PA was confirmed by RT-PCR and immunohistochemistry. Interestingly, a partial depolarizing condition (20 mM KCl) combined with 2 nM U46619 induced similar sensitivity to L-NAME. Pretreatment with GsMTx4 or with DIDS (inhibitor of stretch-activated non-selective cation channels (SACs)) effectively abolished the the L-NAME/U46619 contraction in the PAs with higher pretone. The contraction was also diminished by ROS scavengers (Tiron and PEG-Catalase). Vice versa, exogenous H2O2 (> 0.1 μM) effectively mimicked the pretone effect in the L-NAME/U46619 contraction. Patch clamp study demonstrates the activation of SACs by U46619 with H2O2 or with membrane stretch. Taken together, we suggest that PA myocytes express NOS3 that is concertedly activated by TXA2 and mechanical stretch via H2O2 and/or SACs. The consequential decrease in the contractility might contribute to the relatively low peripheral resistance of pulmonary circulation with fluctuating PA pressure.
Key Words: Pulmonary smooth muscle, NOS3 (eNOS), Mechanical stretch, Thromboxane A2

P06-09

Oscillation of Na^+/Ca^{2+} exchanger current contributes to the pacemaker activity

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The mechanism for cardiac pacemaker potential has been intensively investigated. Recent studies newly suggest that coupling of membrane clock (ion channels) and Ca^{2+} clock (Ca^{2+} release from SR) may regulates and ignites a spontaneous action potential in SA node cells. Nonetheless, the mechanism in human heart was never verified yet. Human embryonic stem cell derived cardiomyocytes (hES-CMs) under current-clamp conditions generally show spontaneous action potentials with pacemaker activity. On converting to voltage-clamp mode (holding voltages ranging from -70 to +20 mV) under nystatin-perforated patch conditions, regular activation of Spontaneous Inward Currents (SICs) were observed in ca. 35 % of hES-CMs without spontaneous decay. SICs could be eliminated by lowering $[Ca^{2+}]_{ext}$, Ca^{2+} channel blocker, or caffeine treatment (SR Ca^{2+} depletion). In addition, NCX inhibitor SN-6 treatment effectively abolished SICs. Under the conditions inhibiting SICs, switch to current clamp mode revealed decreased slope of diastolic depolarization (DD), i.e. slowed pacemaker potential. It was notable that the upward peak level of SIC was commonly lowered by Ca^{2+} removal, caffeine, and SN-6. Thus reverse mode NCX outward current might occur following the transient inward current via forward NCX. Putative SR Ca^{2+} releases would underlie the large transient inward SICs (forward NCX current) reflecting the Ca^{2+} removal process. During the later decay period as well as upstroke par of SICs, owing to the acccumulation of Na^{+} and Ca^{2+} efflux, a rebound switching to the reverse NCX might occur. Putatively, the reverse NCX along with Ca^{2+}-permeable channels would concertedly provide Ca^{2+} influx refilling SR that is critical for the subsequent Ca^{2+} release. In summary, the alternative activation of forward/reverse NCX might explain the robust SICs observed in hES-CMs.

Key Words: hES-CMs, Ca^{2+} clock, pacemaker current, Na+/Ca^{2+} exchanger

P06-10

Distinct roles of neuronal nitric oxide synthase in metabolic substrate-induced arrhythmias in healthy and hypertensive rat heart

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Backgroud & Aim: Excessive fat or fatty acids (metabolic syndrome) are the precuresors of cardiovascular complications, such as ventricular arrhythmias (sudden cardiac death), heart failure and stroke. Neuronal nitric oxide synthase (nNOS) protects the myocardium from stress-induced arrhythmias and contractile dysfunction. Here, we tested the roles of nNOS in cardiac contraction and arrhythmias in sham and angiotensin (Ang II)-induced hypertensive rat heart following metabolic substrates treatment in vitro. **Result:** Our results demonstrated that metabolic substrates (oleic acid 200 μM, palmitic acid 100 μM, linoleic acid 100 μM, lactate 1 mM, pyruvate 100 μM and carnitine 50 μM, termed NF) significantly increased the amplitude of LV myocyte shortening in both sham and hypertension. NF induced spontaneous contraction (arrhythmias) in LV myocytes in the presence of isoprenaline (ISO) and the rates of arrhythmias were significantly higher in hypertension than sham group. Insulin, which abolished ISO-induced increase in myocyre contraction in NT, did not affect basal or ISO-induced myocyte contraction in NF, suggesting impaired insulin-dependent signaling. Inhibition of eNOS with L-NG-nitroarginine methy ester (L-NAME, mM) in sham, or nNOS with SMTC in hypertension, reversed the anti-adrenergic effect of insulin in NT but did not affect NF-increase of LV myocyte contraction. However, both L-NAME and SMTC significantly increased arryhthmias in sham but reduced it in hypertension. Futher results showed that the peak $[Ca^{2+}]$ transient were not elevated in NF, ISO increased $[Cas^{2+}]$ transients and diastolic $[Ca^{2+}]$ and induced spontaneous $[Ca^{2+}]$ in NF. KB-R7943 (10 μM), an inhibitor of Na+-Ca^{2+} exchanger, did not affect the amplitude of myocyte contraction but significantly reduced arrhythmias in NF+ISO. The role of nNOS in cardiac handling and arrhythmias needs further investigation. **Conclusion:** Our results demonstrate that nNOS plays distinctive roles in metabolic substrate supplimentation-induced cardiac arrhythmias following beta-adrenergic stimulation in normal and hypertension rat heart.

Key Words: metabolic substrate, insulin response, nitric oxide synthase, arrhythmias

P06-11

Altering the sphingolipid acyl chain length decreases gastric smooth muscle contractility by upregulating KCa1.1

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We explored the effect of the ceramide acyl chain length on KCa1.1 expression in gastric smooth muscle cells (GSMC) by using ceramide synthase 2 (CerS2) null mice. In CerS2 null gastric smooth muscle and GSMC, levels of CerS4, CerS5 and CerS6 were increased, and the sphingolipid profile was altered. mRNA and protein levels of KCa1.1 were elevated, and KCa1.1 currents were increased without a change in the conductance or Ca^{2+} sensitivity of single KCa1.1 channel. CerS5 overexpression or CerS2 knockdown induced KCa1.1 upregulation, whereas CerS4 or CerS6 overpression did not. Phospho-phosphoinositide 3-kinase p85, phospho-protein kinase C ζ (p-PKC ζ) and phospho-JNK were involved in KCa1.1 upregulation. KCa1.1 upregulation was reversed by PKC ζ or NF- κB inhibitors in CerS2 null SMCs. KCa1.1 upregulation inhibited intracellular Ca^{2+} increase induced by agonist and decreased the levels of phosphorylated myosin light chain (p-MLC). Contractile dysfunction was found in gastric smooth muscle from CerS2 null mice. Interestingly, KCa1.1

and CerS5 upregulation and changes in sphingolipid composition were also found in SMCs from aged wild type mice. Exogenously added sphingosine and sphingosine 1-phosphate upregulated KCa1.1. Our results, suggest that altering the sphingolipid acyl chain length regulates KCa1.1 expression via a PI3K/PKC ζ /JNK/NF- κ B-mediated pathway and hence contractility of gastric smooth muscle.
Key Words: sphingolipids, smooth muscle, Ca^{2+}-activated K^+ channel, contractile dysfunction

P06-12

DJ-1 is associated with vascular neointima formation mediated by shingosine-1 phosphate receptor pathway

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Activation of sphingosine-1-phosphate (S1P) receptor regulates diverse responses including differentiation, migration and proliferation in vascular smooth muscle cells (VSMCs). S1P receptor 1 (S1PR1) and S1P receptor 2 (S1PR2) in major subtypes of S1P receptor induced positive and negative regulation in vascular neointima formation, respectively. DJ-1 was involved in vascular neointimal formation. In the present study, we explored correlation between DJ-1 and S1PrR1/2 in vascular neointimal formation linked to the developments of atherosclerosis. S1PR1 mRNA level in VSMCs was increased in DJ-1 knockout (DJKO) mice, but S1PR2 level was decreased in DJKO, compared with compared with type (DJWT) mice. Expression level of S1PR1 and S1PR2 protein also showed a similar tendency to their mRNA expression levels in VSMCs. DJ-1 overexpression decreased S1PR1 mRNA level and increased S1PR2 mRNA level in DJKO cells. Exposure of DJWT cells to H2O2 caused enhancement of the mRNA levels of S1PR1 and diminution of that of S1PR2. Histone deacetylase-1 recruitment and H3 histone acetylation at S1PR1 promoter were elevated and diminished, respectively, in DJKO compared with DJWT controls. S1P-induced migration was greater in DJKO VSMCs than DJWT controls. S1PR1 inhibitor decreased S1P-stimulated migration in DJKO cells but S1PR2 inhibitor increased S1P-stimulated migration in both DJKO and DJWT cells compared with each control. The level of vascular neointima formation was predominant in DJKO compared with DJWT) mice. Immunohistochemistry analysis showed that S1PR1 expression on vascular neointima was higher and S1PR2 expression was lower in DJKO than DJWT mice. From these results, we suggest that DJ-1 may participate in vascular neointimal formation linked to S1P receptor-mediated pathway.
Key Words: DJ-1/Park7, Sphingosine 1-phosphate, S1P receptor 1/2, vascular smooth muscle cells, Neointima

P06-13

Ketoconazole-induced apoptosis is involved in Parkin overexpression via reactive oxygen species in neonatal rat cardiomyocytes

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The azole antifungal ketoconazole has been reported to stimulate reactive oxygen species (ROS) generation and apoptosis in various cell types. However, function of ketoconazole in heart cells has not been investigated. In the present study, we explored the correlation between the induction of ROS and apoptosis and the alteration of Parkin gene in neonatal rat cardiomyocytes in response to ketoconazole. Cardiomyocytes was decreased by treatment with ketoconazole. Ketoconazole increased H_2O_2 generation and TUNEL- positive apoptosis in cardiomyocytes in a dose-dependent manner. ketoconazole induced up-regulations of mRNA and protein expression level of Parkin. H_2O_2 up-regulated the expression levels of Parkin mRNA and protein and treatment with antioxidants, tiron and tempol, decreased ketoconzole-increased apoptosis and expression of Parkin in cardiomyocytes. Moreover, ketoconazole-induced cell viability and apoptosis was inhibited in cardiomyocytes subjected to Parkin knockdown. Based on the present study, we concluded that ketoconazole may up-regulate Parkin expression via ROS, especially H_2O_2, pathway, resulting in apoptosis in cardiomyocytes. This possible new mechanism for ketoconazole-induced responses may be useful information for the understanding of antifungal agent function in cardiomyocytes.
Key Words: Ketoconazole, Cardiomyocytes, Apoptosis, Reactive oxygen species, Parkin

P06-14

Fetuin-B contributes to the development of acute myocardial infarction through monocyte activation

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The rupture of an atherosclerotic plaque is one of the main causes of coronary artery thrombotic occlusion, leading to myocardial infarction. However, the exact mechanism and causable risk factor for plaque rupture remain unclear. In

this study, to identify the new molecule influencing atherosclerotic plaque rupture, we investigated proteins expressed differentially in serum from patients with acute myocardial infarction (AMI) and stable angina (SA), using a proteomic analysis. The expressions of six proteins, including fibrinogen, fetuin-B, keratin 9, proapolipoprotein, and fibrinogen gamma-B chain precursors, were altered in serum from patients with AMI compared with SA. Of these, fetuin-B, proapolipoprtein, fibrinogen gamma-B chain precursors and fibrinogen expression were greater and keratin 9 expression was lesser in serum from AMI than from SA patients. Increased fetuin-B expression in serum from AMI patients was also confirmed by Western blot analysis. However, the expression of fetuin-A, another form of fetuin protein, did not differ between the two groups. Treatment with recombinant human fetuin-B significantly increased the migration in U937 monocytes in a concentration- dependent manner. Fetuin-B also enhanced the activation of matrix metalloproteinase-2 in the monocytes. These findings indicate that fetuin-B may be a potential modulator involved in the development of AMI. This study may provide a therapeutic advantage for patients at increased risk of AMI.
Key Words: Acute myocardial infarction, Fetuin-B, Migration, Monocytes, Proteomics

P06-15

Expression and Functional Role of WNK1 in Diabetic Skeletal Muscle

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WNK (with-no-lysine [K]) kinases are a family of serine/threonine protein kinases with an atypical placement of the catalytic lysine. Mammalian WNK kinases consist of four members (WNK1-4) with tissue-specific expression. WNK1 is ubiquitously expressed including skeletal muscle. WNK1 has been implicated in vesicle trafficking via regulating SNARE protein complex whose defects in skeletal muscle are highly correlated with insulin resistance and diabetes. However, expression pattern and functional role of WNK1 in skeletal muscle have not been examined. Moreover, linkage between WNK1 and SNARE complex involving glucose transport in skeletal muscle with type II diabetes remains elusive. In the present study, WNK1 and WNK2, not WNK4 are expressed skeletal muscle in mice. The expression levels of both WNK1 and WNK2 are significantly decreased in db/db mice, a hyperinsulinemic type II diabetic model compared to that of wild type mice. Phosphorylated Akt in skeletal muscle is decreased in db/db mice supporting insulin resistance in skeletal muscle. Insulin stimulates Akt phosphorylation followed by WNK1 phosphorylation. Insulin-mediated activation of WNK1 is blunted by phosphoinositide-3-kinase inhibitors. In addition, an expression of GLUT4, a downstream effector of Akt and/or WNK1, is markedly reduced in db/db mice, whereas GLUT1 expression level is not altered suggesting that GLUT1 and GLUT4 are differently regulated in type II diabetic skeletal muscle. Thus, these results may provide new insights for increased susceptibility to insulin resistance and diabetes in skeletal muscle.
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Key Words: WNK1, WNK2, Skeletal muscle, Type II diabetes, GLUT4

P06-16

Dual Modulation of the Mitochondrial Permeability Transition Pore and Redox Signaling Synergistically Promotes Cardiomyocyte Differentiation From Pluripotent Stem Cells

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Background: Cardiomyocytes that differentiate from pluripotent stem cells (PSCs) provide a crucial cellular resource for cardiac regeneration. The mechanisms of mitochondrial metabolic and redox regulation for efficient cardiomyocyte differentiation are, however, still poorly understood. Here, we show that inhibition of the mitochondrial permeability transition pore (mPTP) by Cyclosporin A (CsA) promotes cardiomyocyte differentiation from PSCs.
Methods and Results: We induced cardiomyocyte differentiation from mouse and human PSCs and examined the effect of CsA on the differentiation process. The cardiomyogenic effect of CsA mainly resulted from mPTP inhibition rather than from calcineurin inhibition. The mPTP inhibitor NIM811, which does not have an inhibitory effect on calcineurin, promoted cardiomyocyte differentiation as much as CsA did, but calcineurin inhibitor FK506 only slightly increased cardiomyocyte differentiation. CsAtreated cells showed an increase in mitochondrial calcium, mitochondrial membrane potential, oxygen consumption rate, ATP level, and expression of genes related to mitochondrial function. Furthermore, inhibition of mitochondrial oxidative metabolism reduced the cardiomyogenic effect of CsA while antioxidant treatment augmented the cardiomyogenic effect of CsA. **Conclusions:** Our data show that mPTP inhibition by CsA alters mitochondrial oxidative metabolism and redox signaling, which leads to differentiation of functional cardiomyocytes from PSCs.
Key Words: metabolism, mitochondria, myocytes, redox, stem cells

P06-17

The influence of lengthened immobilization on apoptotic changes in skeletal muscles of rats

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Muscle immobilization is often implemented when it is required to fix a part of the skeletal structure with the bone or muscle. The atrophic changes of skeletal muscles during short-positioned immobilization have been previously reported; however, it is less identified whether lengthened immobilization can negatively affect skeletal muscles. Therefore, the aim of this study was to investigate the pathophysiological changes associated with lengthened immobilization. The experimental group (SD male rats, weighing 200-250 g) was designed to cast right ankle in different condition of neutral-positioned immobilization (0^o ankle angle) or full-lengthened immobilization (55^o ankle angle); the naive group did not receive this casting. For behavioral test, the foot pressure was measured for 3 weeks. The expression of apoptosis and myosin heavy chain protein in the soleus were measured using western blot; TUNEL assay was used to observe apoptotic nuclei changes expressed by the 55^o-lengthened immobilization at 7, 14, and 21 days. We found that both the neutral-positioned and 55^o-lengthened immobilization groups decreased the threshold of foot pressures on the ipsilateral side. In particular, the 55^o-lengthened immobilization group had a significant increased expression in Bax (pro-apoptotic Bcl-2 family member) and MyoD (myogenic differentiation factor D), and produced a significant reduced expression of MYH (myosin heavy chain) in the soleus compared to the other groups. In addition, there was a significant increase in apoptotic nuclei of the 55^o-lengthened immobilization group compared to the neutral positioned immobilization group. Therefore, our results implicate that full-lengthened immobilization can increases apoptotic changes and decreases motor protein expression.

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

P06-18

Angiotensin IV Protects Heart against Ischemia and Reperfusion Injury in Rats

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In this study, we investigated whether angiotensin IV (Ang IV) protects the heart against myocardial ischemia/reperfusion (I/R) injury in rats. Left anterior descending (LAD) coronary artery in rats was occluded for 45 min followed by varying periods of reperfusion. Ang IV (1 mg/kg) was injected intraperitoneally for 3 days before ischemia. Plasma levels of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) were significantly increased in rats subjected to I/R injury compared with sham-operated rats. Rats receiving Ang IV displayed significantly reduced CPK and LDH levels. In addition, the infarct size of Ang IV-treated rats was significantly decreased when compared with I/R-operated rats. Pretreatment with insulin-regulated aminopeptidase (IRAP) antagonist or with inhibitors of downstream signaling pathway which phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (mTOR) attenuated the improvement of CPK, LDH and infarct size induced by Ang IV. Compared with sham, I/R group increased Bax, caspase-3 and caspase-9 protein leves, and decreased Bcl-2 protein level, which were attenuated by treatment with Ang IV. These protein patterns were not observed in pretreatment with IRAP antagonist or with inhibitors of PI3K, Akt and mTOR. These results suggest that Ang IV attenuated myocardial I/R injury via inhibiton of apoptotic injury through IRAP-PI3K-Akt-mTOR pathway.

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Key Words: Angiotensin IV, ischemia/reperfusion, insulin-regulated aminopeptidase, apoptosis

P06-19

Higher frequency of spontaneous transient outward currents in mesenteric arterial myocytes might underlie the weak myogenic tone in comparison to cerebral artery

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Myogenic tone (MT) of resistance artery is induced by increased luminal pressure, thereby stabilizing regional blood flow albeit the fluctuation of perfusion pressure. Mechanosensitive depolarization of membrane potential and activation of voltage-gated Ca^{2+} channels are major cellular mechanisms for MT in smooth muscle cells (SMCs). Large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) in SMCs are activated by both depolarization and increased $[Ca^{2+}]_i$, antagonizing the contractile signals and participating the determination of MT. Using video-analysis of pressurized (60 mmHg) artery, we found that the MT in mesenteric artery (MA) is absent or significantly smaller than cerebral artery (CA). Since the minute MT of MA was effectively recovered by iberiotoxin, we hypothesize that differential activity of BK_{Ca} might underlie the MT variance. The iberiotoxin-induced significant contraction in MA while not in CA. Interestingly, CA pretreated with 50 μM Ba^{2+}, an inwardly rectifying K^+ channel (Kir) inhibitor, showed strong contraction in response to iberiotoxin. Whole-cell patch clamp recording of SMCs showed that the current density of BK_{Ca} was similar between the CA and MA. However, the frequency of spontaneous transient outward currents

(STOCs) reflecting the BK_{Ca} activity in responses to Ca^{2+} sparks was higher in MA than CA. In addition, the unitary slope conductance of BK_{Ca} was larger in MA than CA. The Ba^{2+}-sensitive Kir current was prominent in CASMCs while negligible in MASMCs. Taken together, it is suggested that more frequent STOCs and larger average outward current via BK_{Ca} could explain the weak MT in MA.
Key Words: STOCs, BK channel, Arterial smooth muscle, Myogenic tone

P06-20(PO-15)

Heteromeric TRPC3 with TRPC1 formed via its ankyrin repeats regulates the resting cytosolic Ca^{2+} levels in skeletal muscle

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The main tasks of skeletal muscle are muscle contraction and relaxation, which are mediated by changes in cytosolic Ca^{2+} levels. Canonical-type transient receptor potential 3 (TRPC3) contains an ankyrin repeat (AR) region at the N-terminus (38-188 amino acids) and forms extracellular Ca^{2+}-entry channels by homo or heteromerization with other TRP subtypes in various cells including skeletal myotubes. However, previous research has not determined which region(s) of TRPC3 is responsible for the heteromerization, whether the AR region participates in the heteromerizations, or what is the role of heteromeric TRPC3s in skeletal muscle. In the present study, the heteromerization of TRPC3 with TRPC1 was first examined by GST pull-down assays of TRPC3 portions with TRPC1. The portion containing the AR region of TRPC3 was bound to the TRPC1, but the binding was inhibited by the very end sub-region of the TRPC3 (1-37 amino acids). In-silico studies have suggested that the very end sub-region possibly induces a structural change in the AR region. Second, the very end sub-region of TRPC3 was expressed in mouse primary skeletal myotubes, resulting in a dominant-negative inhibition of heteromeric TRPC3/1 formation. In addition, the skeletal myotubes expressing the very end sub-region showed a decrease in resting cytosolic Ca^{2+} levels. These results suggest that the AR region of TRPC3 could mediate the heteromeric TRPC3/1 formation, and the heteromeric TRPC3/1 could participate in regulating the resting cytosolic Ca^{2+} levels in skeletal muscle.
Key Words: Canonical-type transient receptor potential 3 (TRPC3), Canonical-type transient receptor potential 1 (TRPC1), Skeletal muscle, Ankyrin repeat, and Heteromerization

P06-21

Mitochondrial pyruvate dehydrogenase phosphatase 1 overexpression suppresses early differentiation of cardiomyocytes from mouse embryonic stem cells

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Background: Mitochondria are crucial to maintain the properties of stem cells and their subsequent differentiation into diverse cell lineages, including cardiomyocytes. This study aimed to identify mitochondrial regulators that modulate mouse embryonic stem cell (mESC) differentiation into cardiomyocytes. **Methods and Results:** We performed expression microarray to assess mRNA expression changes of embryoid bodies (EBs) at differentiation day 8 (D8) compared with undifferentiated mESCs (D0). Among the differentially expressed genes, we identified 140 mitochondria-related genes and their functional clusters. Several genes associated with the pyruvate dehydrogenase complex were significantly decreased at D8. Particularly, pyruvate dehydrogenase phosphatase catalytic subunit 1 (PDP1) expression was 27-fold decreased at D8 compared to D0. To determine whether PDP1 expression is essential for regulating mitochondrial energy metabolism and cardiomyocyte differentiation, we performed a gain-of-function study in a PDP1 overexpression cell line. PDP1 overexpression increased both mitochondrial ATP production and oxygen consumption. Additionally, D8 PDP1 cells showed higher mitochondrial membrane potentials and greater reactive oxygen species generation. However, PDP1 overexpression significantly suppressed cardiomyocyte differentiation at D8. **Conclusions:** This study screened differentially expressed genes important in energy metabolism during EB differentiation. Our findings demonstrate that at an early stage, mitochondrial PDP1 may be a crucial modulator of energy metabolism during mESC differentiation into cardiomyocytes.
Key Words: Stem cells, Cardiomyocytesm Differentiation, Energy Metabolism, PDP1

P07-01

Reactive oxygen species involve in the downstream signaling of mGluR1 in the cerebellar Purkinje cells

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Metabotropic glutamate receptors (mGluRs) are members of G protein-coupled receptors that have seven transmembrane domains. At the parallel fiber to Purkinje cell synapses, particularly mGluR1 is known to mediate slow excitatory postsynaptic current (sEPSC) but the activation mechanism of this slow current remains poorly understood. Based on the previous study that showed protein tyrosine phosphatase (PTP) regulates sEPSC of cerebellar Purkinje cells and the report that hydrogen peroxide (H_2O_2), one of the reactive oxygen species, modulates the activity of PTP, we surmised that H_2O_2 may involve in the activation of mGluR1-mediated sEPSC. Bath application of H_2O_2 reduced sEPSC in the cerebellar Purkinje cells. In addition, H_2O_2 induced an inward current and this inward current was completely inhibited by SKF96365, inhibitor of TRPC channel which is known as the molecular identity of mGluR1-mediated sEPSC. We interpreted that H_2O_2 induced slow current via the activation of TRPC channel and reduction of sEPSC by the treatment of H_2O_2 was an occlusion effect. For the further investigation, NADPH oxidase inhibitor (DPI) and reducing agent (DTT) were treated to Purkinje cells. Both DPI and DTT successfully abolished the activation of sEPSC induced by repetitive stimulation of parallel fibers. The inhibitory effect of DPI was reversible. In this study, we explored the role of H_2O_2 in mGluR1-mediated sEPSC and suggest that H_2O_2 is required for the induction of sEPSC in the cerebellar Purkinje cells.

Key Words: metabotropic glutamate receptor, Purkinje cell, ROS, TRPC

P07-02

GABA-mediated responses on the substantia gelatinosa neurons of the medullary dorsal horn in streptozotocin injected mice

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Peripheral neuropathy is a frequent complication of diabetes mellitus and a common symptoms of neuropathic pain whose mechanism has been proved to be complex and involve both peripheral and central components of sensory system. The lamina II called substantia gelatinosa (SG) of the medullary dorsal horn is well known be a critical site for processing of orofacial nociceptive information. In this study, we used the whole-cell patch clamp technique to investigate the influence of diabetic neuropathy on the excitatory and inhibitory receptors on the SG neurons of mice. In adult, streptozotocin (200 mg/kg) (STZ)-induced diabetes indicated a small decrease of body weight and a significant increase of blood glucose level when compared with respective control group. However, the application of glycine (100 μM), GABA (100 μM) and glutamate (30 μM) on SG neurons from diabetic mice did not show any significant difference when compared with their control counterparts. On the other hand, in juvenile pups by the multiple injections of STZ (40 mg/kg) for 5 consecutive days to the pregnant mice showed a significant decrease of body weight and hypoglycemia in pups compared to the control. Glycine and glutamate responses in the SG neurons in pups from diabetic mother were similar to those of control pups. However, GABA response on SG neurons in pups from diabetic mother was larger than that of control pups. Further, GABA-mediated responses between pups from diabetic mother and control were examined at different concentrations ranging from 3-1,000 μM. At every point of GABA concentration, the mean inward current induced by the SG neurons from pups from diabetic mother was larger than that from control mice. These results put forth that SG neurons in pups from diabetic mice are more sensitive to GABA compared to control. These results suggest that orofacial pain processing in the pups from diabetic mother can be altered by increasing GABA sensitivity.

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Key Words: whole-cell patch clamp, substantia gelatinosa neuron, STZ-induced diabetes, GABA receptor

P07-03

Neuroactive steroids mediated tonic conductance decreases with advancing postnatal age of GnRH neurons

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Neurosteroids play important role in the regulation of gonadotropin releasing hormone (GnRH) neuronal activities. Although facilitation of conventional phasic postsynaptic currents (PSCs, Iphasic) has been considered as the main mechanism by which neurosteroids influence neuronal activities, type A gamma-amino butyric acid (GABAA) receptors mediate a sustained tonic current (Itonic) as well as Iphasic on GnRH neurons. In addition, steroidal modulation of Itonic on GnRH neuron is still unknown. So, in this study, we examined the influence of neurosteroids on GnRH neurons over postnatal development and tried to figure out the type of GABAA receptors (GABAAR) mediating neurosteroid sensitivity of Itonic on GnRH neurons. 3α,5α-THDOC increased inward currents on GnRH neurons and the inward currents were persisted in the presence of gabazine, which blocks the synaptically mediated PSCs. In addition, 3α,5α-THDOC-mediated currents were persisted in the presence of amino acid blocking cocktail (AP-5, CNQX, strychnine: and TTX) suggesting that the tonic currents were mainly postsynaptic events. Further, 3α,5α-THP also induced gabazine-insensitive inward currents on GnRH neurons which got partially blocked by L-655708 (10 μM), a GABAA-α5 inverse agonist. 3α,5α-THP-induced inward currents were age dependent and gradually decreased with advancing postnatal age. Further, blockage of neuronal and glial GAT induced inward current which decreased with advancing postnatal age. These results suggest that there exists an α5 and/or ㅿ GABAAR-mediated tonic conductance via

prognane neurosteroids on GnRH neurons and the magnitude of these tonic currents depend on postnatal age.
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Key Words: Gonadotropin releasing hormone neuron, tonic GABAA current. Whole cell recording

P07-04

Age dependent effect of Bisphinol-A an Endocrine Disruptor on GnRH Neurons

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Endocrine disrupting chemicals (EDCs) are group of exogenous substances interfering with the endocrine system imitating or antagonizing the action of endogenous hormones. The sources of EDCs are natural compounds like phytoestrogen genistein or man-made substances like widespread chemical Bisphenol-A (BPA). In this study, we investigated the effects of the BPA on gonadotropin-releasing hormone (GnRH) neurons using single cell electrophysiology on GnRH-green fluorescent protein (GnRH-GFP) transgenic mice over postnatal development. Whole cell patch-clamp recordings from GnRH-GFP neurons showed that 100% of GnRH neurons responded to 300 μM of BPA with a markedly prolonged inward current. This effect not only persisted in the presence of tetrodotoxin, but also in the presence of amino acid receptor antagonists, indicating the direct site of action is on postsynaptic GnRH neurons. We found that 300 μM of BPA increased the post synaptic current on GnRH neurons in intact and in the presence of TTX along with EPSC blockers (AP5-20 μM and CNQX-10 μM). Further, BPA induced inward currents were concentration dependent. In addition, these inward currents were not blocked by gabazine, a synaptic GABAA receptor blocker but were completely blocked by bicuculline, a broad GABAA antagonist. Interestingly, BPA induced inward currents were gradually decreased with advancing postnatal age and the effect of BPA was significantly lower in adults than in juvenile GnRH neurons. These results demonstrate that BPA acts directly on GnRH neurons to induce excitation via extrasynaptic GABAA receptors and gradually decreases with advancing postnatal age.
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Key Words: Endocrine disrupting chemicals, Bisphenol-A, GnRH neurons, patch-clamp, GABAergic.

P07-05

Involvement of PKC and CaMKII in NR2B ser1303 phosphorylation in the spinal cord of rats after peripheral nerve injury

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NMDA receptor is known to be involved in central sensitization, which is one of the mechanisms to understand neuropathic pain after nerve injury until now. However, intrathecal treatment of NMDAR related drugs cause severe side effects such as hallucination because NR1 is ubiquitously located in the whole central nervous system. In contrast, NR2B is more restrictively located than NR1. They appear in the superficial lamina of the spinal cord, forebrain and so on. For this reason, many evidences are accumulated that spinal NMDAR containing NR2B contributes to neuropathic pain in several animal models. Therefore, our study explored which kinases phosphorylate NR2B subunit, especially NR2B ser1303, after peripheral nerve injury. Spinal nerve ligation model in Sprague-Dawley rats (180-200 gram) were used in this study. Quadrants of L4 and L5 spinal cord were obtained from SNL operated rats and normal rats. Temporal expression of NR2B, NR2B Tyr1472, NR2B Ser1303, tCaMKII, pCaMKII and PKC were quantified by western blotting. To evaluate the analgesic efficacy of a NR2B antagonist (Ro 25-6981), a CaMKII inhibitor (AIP) or a PKC inhibitor (Chelerythrine) were intrathecally injected after nerve injury. Co-immunoprecipitation was performed to analyze change of the interaction between Ser1303 and CaMKII, and Ser1303 and PKC. Protein expression of NR2B was increased until 4 days after nerve injury. Phosphorylation of Ser1303 was increased and maintained until 14 days after nerve injury. Interaction between Ser1303 and CaMKII was increased in the early phase, and interaction between Ser1303 and PKC was existed in the late phase. Increased interaction between Ser1303 and CaMKII was reduced by AIP and Ro 25-6981, but not chelerythrine. Reduced paw withdrawal threshold after nerve injury was reversed by AIP and Ro 25-6981 which effect was more effective in the early phase than the late phase. These results demonstrated that NR2B ser1303 phosphorylation may play a key role in neuropathic pain, and CaMKII can be a candidate kinase for manageable target in the early phase after nerve injury.
Key Words: NR2B, neuropathic pain, peripheral nerve injury, CaMKII, PKC

P07-06

Hypotaurine induces glycine-receptor mediated inhibitory currents in substantia gelatinosa neurons of trigeminal subnucleus caudalis

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The substantia gelatinosa (SG) neurons of the trigeminal subnucleus caudalis (Vc), which is also called the medullary dorsal horn, play an important role in orofacial nociceptive transmission. Hypotaurine, the immediate precursor of taurine is biosynthesized from cysteine in astrocytes, and the physiological role of hypotaurine in the central nervous system remains unclear. So, to better understand the effect and action mechanism of hypotaurine on orofacial pain processing, we used a patch clamp technique to examine the direct effects and receptor types involved in hypotaurine-induced membrane currents in the SG neurons. Under the condition of high chloride pipette solution, hypotaurine (300 μM) induced repeatable inward currents. The hypotaurine-induced inward currents showed clear concentration dependency at range of 30-3000 μM on SG neurons. Hypotaurine-mediated responses were not affected by gabazine (3 μM), a synaptic GABAA receptor blocker, but almost blocked by strychnine (2 μM), a glycine receptor antagonist, suggesting that hypotaurine-induced inward currents were mediated by glycine receptor activation. The responses to 300 μM hypotaurine and a maximal concentration of glycine (3mM) were not additive indicating that hypotaurine and glycine act on the same glycine receptors. These results indicate that hypotaurine affects SG neuronal activities by glycine receptor activation on the SG neurons and hypotaurine can be a target molecule for orofacial pain modulation.

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Key Words: Hypotaurine, orofacial pain, patch clamp technique, SG neurons, glycine receptors

P07-07

The role of activated peripheral delta opioid receptors(DOR) on the alleviation of arthritis pain

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It has been well known that the systemic treatment of opioids can give rise to the strong analgesic effects. However, it was not completely identified whether the targeting restrictedly into peripheral opioid receptor contribute to this antinociceptive effects. In particular, the inhibitory effect of intra-articular injection of delta opioid receptor (DOR) agonist on inflamed knee joint is not clear. To answer this question, we investigated whether the application of SNC80 (DOR agonist) into inflamed knee joint of rats could have inhibitory effects on nociceptive behavior, the production of pro-inflammatory cytokines and the activity of afferent fibers. For behavior test, carrageenan (1%, 50 μl/200 g) was injected into the knee joint space of SD rats (male, 7 weeks) to induce acute arthritis. At 4 hour after the induction of arthritis, we injected SNC80 (70 μl) and saline (70 μl) in knee joint cavity, and measured consecutively the peak value of weight load into affected hindlimb at before (baseline) and 4, 6, 8 hour and 1 day after carrageenan. We found that the activation of peripheral DOR through SNC80(1 μM and 100 μM) significantly recovered the reduced weight load onto the inflamed joint compared to saline treatment at 6 and 8 hour post-carrageenan injection time. For in vivo single nerve recording, we anesthetized rats with urethane (250 mg/kg, i.p.) at about 3 hours after carrageenan injection, and placed teased fine afferent fibers over a platinum bipolar electrode to record the neuronal activity. SNC80 (10 nM, 1 μM and 100 μM; 70 μl) and saline (70 μl) were injected to knee joint cavity through tubing connected with needle. We measured conduction velocity and counted the peak spikes per second of afferents evoked by the application of von Frey filaments (6 and 26g) at base and 10, 20, 30 and 60 minutes after drugs injection. We found that SNC80 at dose of 1 μM, 100 μM significantly inhibited the neuronal response to mechanical stimulation into inflamed joint compared to saline treatment. For western blotting, we measured the expression of pro-inflammatory cytokines and cyclooxygenase (COX)-2 in both the synovial membrane and meniscus. Arthritic animals treated with DOR showd a reduction of interleukin (IL)-1β, COX-2 in the synovial membrane and in the meniscus, compared with the saline group. Taken together, these results showed that the activation of peripheral DOR in inflamed joint contributes to the decrease of nociceptive behavior and the neuronal excitation of afferent fibers stimulated by mechanical force, anti inflammatory effects.

Key Words: peripheral delta opioid receptor, acute arthritis, in vivo single nerve recording, intra-articular injection, weight load

P07-08

Enhancement of AMPA receptor phosphorylation through PI3-kinase activation is involved in neuropathic pain

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Reactive oxygen species (ROS) in the spinal cord, which plays a crucial role in sensitization of dorsal horn neurons, has been implicated in neuropathic pain. ROS is an important mediator for regulating spinal AMPA receptor phosphorylation to induce persistent pain. ROS is involved in inducing neuropathic pain via PI3-kinase activation in spinal dorsal horn. In this work, we investigate whether PI3-kinase activation modulates the phosphorylation of spinal AMPA receptors in the neuropathic state. Mechanical hyperalgesia of hind paw, evaluated by measuring paw withdrawal threshold upon the application of von Frey hairs, was induced by L5 spinal nerve ligation (SNL). PI3-kinase activity was analyzed by Western blotting, and PIP3 protein level by ELISA. L5 SNL-induced mechanical hyperalgesia was attenuated by pretreatment with either

ROS scavenger alpha-phenyl-N-tert-butyl nitron (PBN) or PI3-kinase inhibitor wortmannin. Both PIP3 level and PI3-kinase activity in the lumbar spinal dorsal horn were increased in rats with L5 SNL, and such increases were attenuated by pretreatment with either PBN or wortmannin. In rats with L5 SNL, the phosphorylation of spinal AMPA receptors at GluA1 (S831) and GluA2 (S880) subunits was increased. The increased AMPA receptor phosphorylation was reversed by inhibition of PI3-kinase. The results suggest that PI3-kinase-dependent changes in the phosphorylation of spinal AMPA receptors, through the action of ROS, are crucial for the development of neuropathic pain. The research was supported by a grant from the Korea Health technology R & D Project, Ministry of Health & Welfare, Republic of Korea (A120254).
Key Words: glutamate receptor, reactive oxygen species, phosphatidylinositol-4,5-bisphosphate 3-kinase, neuropathic pain, central sensitization

P07-09(PO-6)

Motor cortex stimulation activates the incertothalamic pathway in an animal model of spinal cord injury

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Electrical stimulation of the motor cortex reduces spontaneous pain-like behaviors in animals with spinal cord injury (SCI). Because SCI-produced pain behaviors are associated with abnormal inhibition in the inhibitory nucleus zona incerta (ZI) and because inactivation of the ZI blocks motor cortex stimulation (MCS) effects, we hypothesized that the antinociceptive effects of MCS are due to enhanced inhibitory inputs from ZI to the posterior thalamus (Po), an area heavily implicated in nociceptive processing. To test this hypothesis, we used a rodent model of SCI pain and performed in vivo extracellular electrophysiological recordings in single well-isolated neurons in anesthetized rats. We recorded spontaneous activity in ZI and Po from 48 rats before, during, and after MCS (50 μA, 50 Hz; 300 μs pulses). We found that MCS enhanced spontaneous activity in 35% of ZI neurons and suppressed spontaneous activity in 58% of Po neurons. The majority of MCS-enhanced ZI neurons (81%) were located in the ventrorateral subdivision of ZI, the area containing Po-projecting ZI neurons. In addition, we found that inactivation of ZI using muscimol (GABAA receptor agonist) blocked the effects of MCS in 73% of Po neurons. Although we cannot eliminate the possibility that muscimol spreads to areas adjacent to ZI, these findings support our hypothesis and suggest that MCS produces antinociception by activating the incertothalamic pathway.
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Key Words: Analgesia, neuropathic pain, zona incerta, posterior thalamus, central pain

P07-10

Activation of TRPV1 by D1R agonist SKF-38393 in mouse dorsal root ganglia neuron

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Dopamine (DA) is a neurotransmitter that plays the important roles such as motor control, cognition, and pain modulation in the CNS. Dopamine receptor is a class of GPCR that has 5 subtypes, D1R to D5R. Dopamine receptors are categorized as D1-like/D2-like receptor by activation/inhibition of AC or PLC intracellular signaling pathway. Previous studies have shown dopamine receptor expressed in DRG neuron. However, very little is studied on the effect of dopamine receptor in PNS. TRPV1 is a ligand-gated ion channel expressed on nociceptive sensory neuron, which transduces stimuli such as, noxious heat, proton, and chemical (Capsaicin) to neuronal signal. It is a well-known TRPV1 activity is modulated by intracellular signaling pathway. In this study, the effect of a D1R activation on TRPV1 in mouse DRG neuron using Ca^{2+} imaging. D1 R agonist SKF-38393 induces Ca^{2+} responses in DRG neuron through an activation of TRPV1 rather than Ca^{2+} released from intracellular Ca^{2+} stores. Further, diacylglycerol (DAG) is produced by the activation of D1 R through PLC signaling pathway, and it seems that DAG directly activates TRPV1. Thus, these results suggest that activation of D1R leads to the activation of TRPV1 through PLC/DAG pathway in nociceptive sensory neurons, and may also be contribute to modulation of pain signaling in nociceptive sensory neurons.
Acknowledgements: This research was supported by Nano-Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012M3A7B4035199).
Key Words: Dopamine, D1R agonist, TRPV1, Itch

P07-11

Facilitation of hyperpolarization-activated cyclic nucleotide-gated channel by Prostaglandin E1 in dorsal root ganglion neurons in mice

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels and their current, I_h have been suggested to play an important role in neuropathic pain by involvement in spontaneous ectopic discharges after peripheral nerve injury. Prostaglandin E1 (PGE_1) has been well known as a therapeutic agent for lumbar spinal stenosis in clinical fields. The present study investigated the cellular action of PGE_1 on HCN channel in primary dissociated neurons of dorsal root ganglion (DRG) in mice and pain behavioral responses. Amplitude of Ih increased about 130% by PGE_1 in a dose-dependent manner in the medium-sized (30-40 μm) DRG neurons. Adenylyl cyclase inhibitor and 8-Br-cAMP inhibited the facilitation of Ih current by PGE_1. EP2 receptor antagonist inhibited the facilitation of Ih induced by PGE_1. Exposure of HCN-expressing DRG neurons to PGE_1 increase in action potential frequency. In behavioral test, hind paw injection of PGE_1 to hindpaw reduced the threshold to mechanical stimuli, indicating allodynia. In addition, PGE_1-induced allodynia was blocked by CsCl and EP2 antagonist. In conclusion, PGE_1 facilitated Ih current in medium-sized DRG neurons in mice, which was mediated by EP2 receptors that transmit a signal to adenylyl cyclase and increase the intracellular concentration of cAMP. Exposure to PGE_1 would control action potential firing by enhancing their excitability in HCN-expressing DRG neurons. This molecular mechanism might be associated with PGE_1-induced allodynia.
Acknowledgements: This research was supported by Nano-Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012M3A7B4035199).
Key Words: HCN, PGE_1, Pain

P07-12

Comparison of electrophysiological properties of Magnocellular Neurosecretory & Parvocellular Preautonomic neurons in Rat Hypothalamic Paraventricular Nucleus

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The hypothalamic paraventricular nucleus (PVN) contains magnocellular neurosecretory neurons projecting to posterior pituitary, and parvocellular neurons projecting to anterior pituitary, medulla and/or spinal cord. The PVN neurons are different in their roles and projections; however, it is not well known whether their electrophysiological properties are also different. Here, we compared the basic electrophysiological properties of these neuron groups. The PVN neurons projecting to the posterior pituitary (PVN-MC), the intermediolateral cell column of spinal cord (PVN-IML) and/or the rostral ventrolateral medulla (PVN-RVLM) were identified by electrophysiological property and retrograde dye. The firing, EPSC and action potential (AP) were recorded in the cell-attached or whole-cell mode. Data were sampled at 20 KHz using pClamp 8 data-acquisition and analysis software. In the voltage clamp cell-attached mode, the frequency of spontaneous firing in the PVN-IML neurons was higher than those in the other two groups (PVN-IML, PVN-RVLM, PVN-MC: 2.84 vs. 1.89 vs. 1.11Hz, n=14, 27, 15; $p<0.05$). The input resistance (876 MΩ, n=17) and time constant (52 ms, n=17) of PVN-MC were significantly larger than the input resistances (380 vs. 367 MΩ, n=13, 22; $p<0.05$) and the time constants of two presympathetic neurons (23 vs. 26 ms, n=13, 22; $p<0.05$). However, there were no significant differences in resting membrane potential and cell capacitance. The amplitude of EPSCs in PVN-MC cells (19.4 mV, n=22) was larger than those of the PVN-RVLM and PVN-IML neurons (18.7 vs. 20 mV, n=11, 8; $p<0.05$), but mean frequencies of EPSCs were similar. The 37～90% decay time (5.8 ms, n=22) of the PVN-MC cells was also larger than those of PVN-RVLM and PVN-IML neurons (3.6 vs. 3.9 ms, n=11, 8; $p<0.05$). The properties of AP were determined in whole cell current clamp, decay time (PVN-RVLM, PVN-IML, PVN-MC: 0.79 vs. 0.74 vs. 1.27 mV n=10, 17, 20; $p<0.05$) and the half-width duration (PVN-RVLM, PVN-IML, PVN- MC: 1.63 vs. 1.48 vs. 1.98 ms n=10, 17, 20; $p<0.05$) in PVN-MC cells were larger than those of PVN-RVLM and PVN-IML neurons. The amplitude in PVN-MC was definitely the largest (78 vs. 65 vs. 69 mV, n=20, 10, 17; $p<0.05$). The results indicated that electrophysiological properties of three neuron groups were different. The PVN-IML neurons were more excitable than PVN-RVLM neurons, and PVN-MC neurons had larger input resistance and AP amplitude, longer time constant and half duration of AP. Functional significance of these differences remains to be studied later.
Key Words: Paraventricular Nucleus, whole cell, firing rate

P07-13

Assessment of chronic trigeminal neuropathic pain by the orofacial operant test in rats

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Classical behavioral tests in animal models of trigeminal neuropathic pain measure reflexive responses that are not necessarily measures of pain. To overcome the problem, we created a chronic constrictive nerve injury (CCI) rat model of pain by ligation of the infraorbital nerve (ION), and applied the orofacial operant test to assess behavioral responses to mechanical and cold stimulation in these rats. Animals were trained to voluntarily contact their facial region to a mechanical or a cold stimulation module in or-

der to access sweetened milk as a positive reward. ION-CCI rats displayed aversive behaviors to innocuous mechanical stimuli, as indicated by a significant decrease in both contact time and the numbers of long contact events in comparison with sham group. For cold stimulation, ION-CCI rats displayed aversive behaviors to both innocuous (17°C) and noxious cold temperatures (12°C and 5°C), as indicated by a significant decrease in both contact time and the numbers of long contact events at the cooling temperatures. The decreases of the contact time and numbers in ION-CCI rats were partially abolished by morphine. Our orofacial operant test demonstrates mechanical allodynia, cold allodynia, and hyperalgesia in rats with chronic trigeminal nerve injury. The neuropathic pain in ION-CCI rats was partially alleviated by morphine. Thus, orofacial operant test provides a desirable behavioral assessment method for preclinical studies of chronic trigeminal neuropathic pain.

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Key Words: orofacial pain; intraorbital nerve ligation; chronic construction injury; behavioral test

P07-14(PO-10)

RalBP1 hypomorphic mice display antidepressant-like behaviors

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Balanced excitatory and inhibitory synaptic transmission in neurons is critical for mood regulation as well as cognitive function in animals. RalBP1/RLIP76, a multifunctional protein that interacts with the small GTPase RalA and RalB, has been implicated in the inhibitory synaptic transmission and long-term depression of excitatory synaptic transmission. However, behavioral role of RalBP1 is still poorly understood. Here we present that reduced expression of RalBP1 leads to antidepressant-like behaviors. Mice hypomorphic for RalBP1 (RalBP1-/-) display reduced immobility/despair behaviors both in the tail suspension test and in the Porsolt forced swim test. However, anxiety-related behaviors and mnemonic functions were not changed by RalBP1 mutation. Antidepressant-like behaviors in RalBP1-/- mice were associated with abnormal synaptic inhibition in the hippocampus. In addition, pharmacological manipulation of inhibitory synaptic transmission restored antidepressant-like behaviors in RalBP1-/- mice. These results suggest that normal inhibitory synaptic transmission in the hippocampus is important for emotion and RalBP1 could be a potential therapeutic target for depressive disorders.

Key Words: RalBP1, Depression, Despair, Tail suspension test, Forced swimming test

P07-15

The effect of brief social isolation on nociception in routine pain test

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Herding together is known to be comfortable to rodents, so that social isolation provokes a stressful condition. Currently, the effect of social isolation on pain responses or drug efficacy remain elusive. Here, we examined whether brief social isolation, a common condition during behavioral tests, could affect pain behaviors and analgesic efficacy of morphine in mice. Mechanical and thermal pain thresholds of hindpaw were determined by von Frey filaments and Hargreaves' testings, respectively. The isolated mice showed higher pain threshold than the grouped animals, even with strangers. Such effects were reversed by pretreatments of naloxone (opioid receptor antagonist), atosiban (oxytocin and vasopressin receptor antagonist), and ketanserin (5-HT receptor antagonist), suggesting the isolated mice under the descending inhibitory control. All these drugs at the same dose did not show any significant effect on the grouped mice. In addition to normal pain behaviors, the analgesic effect of morphine on relieving thermal pain was decreased in the isolated mice compared to the control grouped animals. These data indicate that even brief social isolation during pain behavioral tests results in stress-induced analgesia, thereby alters normal pain sensitivities and the efficacy of analgesics.

Key Words: Isolation Stress Pain Spinal Analgesia

P07-16

Ionic mechanisms underlying the cardiac autonomic imbalance contributing to impairment of baroreflexin rat with liver cirrhosis

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Patients with liver cirrhosis frequently experience cardiac autonomic dysfunctions such as augmented cardiac sympathetic and reduced cardiac vagal functions which are highly associated with motility. However, the cirrhosis- induced autonomic imbalance has not been studied at cellular and molecular levels. In the present study, we examined alterations in excitability of cardiac sympathetic and parasympathetic neurons and ionic mechanisms underlying the cardiac autonomic imbalance in rats with liver cirrhosis. In this regard, cirrhotic rats were produced by bile duct ligation (BDL) or intraperitoneal injection of thioacetamide (TAA, 200 mg/kg). Three week after BDL or chemical injection, development of liver cirrhosis and blunted arterial baroreflex were evaluated. Action potentials (AP) were recorded in single neurons isolated from the stellate ganglia (STG) and the intracardiac ganglia

(ICG) using the gramicidin-perforated patch-clamp technique. Both types of neurons show tonic firing. In response to current injection (1X, 2X, and 3X), frequency of action potentials (AP) was significantly increased in the STG neurons, while decreased in the ICG neurons of cirrhotic rats when compared with sham control. Liver cirrhosis altered rheobase, AP duration, and afterhyperpolarization duration in the opposite direction in the STG and the ICG without affecting other parameters such as input impedance, resting membrane potentials, and AP amplitude. Real-time PCR analysis and voltage-clamp recording revealed that A (KV3.3, KV3.4, KV4.1 and KV4.3) and M (KV7.2)-type K^+ and N (α1B)-type Ca^{2+}channels were downregulated in the STG neurons, while M-type K^+ channels were significantly upregulated with downregulation of N- and L- (α1C and α1D) type Ca^{2+}channels in the ICG neurons of cirrhotic rats. Taken together, our data suggest that BDL- and TAA-induced liver cirrhosis causes an imbalance between cardiac sympathetic and parasympathetic neuronal activities through differential regulation of K^+ and Ca^{2+} channels. Therefore, liver cirrhosis-blunted baroreflex may arise from impaired functions of the autonomic efferent limbs in the reflex arc in addition to the afferent limb (baroreceptor neuron) dysfunction.

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Key Words: Sympathetic, Parasympathetic, Excitability

P07-17

Traumatic brain injury impairs baroreceptor reflex through cardiac autonomic imbalance

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The arterial baroreflex is one of the homeostatic mechanisms for maintaining blood pressure. In human patients, traumatic brain injury (TBI) is known to lower baroreflex sensitivity (BRS) which is associated with a high mortality rate. To date, cellular mechanisms underlying the TBI- blunted BRS remain unknown. With a hypothesis that the baroreflex dysfunction arises from autonomic imbalance, we tested whether TBI differentially modulates excitability of cardiac sympathetic and parasympathetic neurons. In this regard, TBI was induced in 8-week-old male Sprague-Dawley rats using a head impactor (TBI-0310, PSI, USA). Four week after TBI, the BRS was assessed by the phenylephrine pressor test. Compared with control rats (1.1±0.09, n=4), BRS was significantly reduced in TBI group (0.57±0.08, n=4). Under the gramicidin-perforated configuration of the current clamp, action potentials (AP) were recorded in sympathetic stellate ganglion (STG) and parasympathetic intracardiac ganglion (ICG) neurons. In response to current injection (1X, 2X, and 3X), the frequency of action potentials (AP) was significantly increased in the STG neurons, while decreased in the ICG neurons of TBI group. TBI altered rheobase and AP duration in the opposite direction in the STG and the ICG without affecting other passive and active properties. Real-time PCR analysis revealed that expression of Kv 3.3, Kv 3.4, Kv 4.1, Kv 4.3 and Kv 7.2 was down-regulated in the STG of TBI group. Consistent with this finding, A-and M-type K^+ currents were increased. Interestingly, high voltage-activated Ca^{2+} channels (α1A, α1B, α1C, αD, α1E) were down-regulated in the ICG neurons which may cause hypoexcitability. Taken together, these data suggest that TBI-blunted BRS arises from the imbalanced cardiac autonomic activities which may be caused by differential regulation of K^+ and Ca^{2+} channels.

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Key Words: traumatic brain injury (TBI), Autonomic, Baroreflex dysfunction

P07-18

AMINOPEPTIDASE P1 DEFICIENCY LEADS TO SEVERE COGINITIVE AND SYNAPTIC DYSFUNCTION

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Many biologically active peptides in mammals have a highly conserved proline residue in the penultimate N-terminal position. Thus far, 23 such peptides have been identified, including cytokines, growth hormones, and neuropeptides. Metabolic degradation of these peptides is mediated by a family of proline-dependent proteases known as aminopeptidase Ps (designated APP1?3). The deficiency of APP1 activity in humans and mice leads to excretion of undigested imino-oligopeptides in the urine. In addition to this peptiduria, developmental retardation and microcephaly were observed in the APP1-deficient mice. However, behavioral and cognitive consequences of APP1-deficiency in the brain remain unknown. In the present study, we investigated behavioral role of APP1 using Xpnpep1 knockout (KO) mice that lack APP1. Xpnpep1 KO mice exhibit enhanced seizure susceptibility, behavioral hyperactivity, and impaired learning and memory. These neurological and behavioral dysfunctions were associated with synaptic dysfunctions. These results suggest critical role for APP1-mediated peptide metabolism in normal brain function. In addition, our results provide synaptic mechanisms that may underlie intellectual disabilities in inborn errors of metabolism.

Key Words: Neurodegeneration, inborn errors of metabolic, NMDA receptor, developmental retardation

P07-19

The effect of propofol on dopaminergic neuronal firing in ventral tegmental area

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Propofol is the most widely used intravenous (i.v.) drug for induction of general anesthesia. Many evidences indicate that propofol is one of the choices of drugs being abused. Dopaminergic neurons in ventral tegmental area (VTA) have been implicated in the mediation of the rewarding effects of many addictive drugs. The rewarding properties of some drugs of abuse, for example ethanol and cocaine, are related to their ability to alter the firing of dopaminergic neurons in the VTA. Using whole cell patch-clamp technique, we investigated the propofol effect on excitability of dopamine neurons in VTA. We found that propofol administration decreased the neuronal firing and change hyperpolarization-activated cation current (Ih) and A-type potassium current (IA). We suggest that propofol in the VTA disturbs the status of intrinsic excitability, eventually leading to addictive behavior.

Key Words: Propofol, VTA, dopaminergic neuron, excitability

P07-20

Optogenetic stimulation of layer 2/3 pyramidal neurons in the primary visual cortex

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In the primary sensory cortex, feedforward sensory inputs from the thalamus arrive at layer 4 and lower layer 2/3, whereas feedback associative inputs from higher brain areas terminate in layer 1. Layer 2/3 pyramidal neurons integrate the two inputs and the polarized dendritic structure of the neurons may be well suited to receive these two inputs. In our previous studies, neuromodulators such as acetylcholine and serotonin involved in brain states exhibited layer-specific modulation of long-term synaptic transmission in layer 2/3 pyramidal neurons of visual cortical slices. Thus, neuromodulators may control the information flow between sensory and associative pathways. Although simple extracellular electrical stimulation located in layer 4 or 1 is used to activate synaptic inputs onto distal apical and basal dendrities, respectively, local electrical stimulation may activates other components than those specific inputs due to the structural complexity of the neocortex. Here we used optogenetic approach to investigate the pathway-specific synaptic activation. Adeno-associated viruses carrying channelrhodopsin-2 (ChR2) and fluorescent proteins (mCherry or eYFP) were injected in the LGN or the visual cortex of postnatal 2-week-old rat. Visual cortical slices were obtained after 2 weeks of viral injection. CaMKII promoter was used to express the ChR2 selectively in excitatory neurons. Whereas ChR2-expressing neurons in LGN densely project their axons to layer 4 and lower layer 2/3 of primary visual cortex, ChR2-expressing neurons in the visual cortex project their axons to layer 1 of the contralateral visual cortex. Optical stimulation (480 nm, 5-50 ms) to the middle of the ipsilateral visual cortex in LGN-positive rats evoked EPSPs and action potentials in layer 2/3 pyramidal neurons. The amplitude of EPSP evoked by optical stimulation was dependent of specific layer which was similar to the expression pattern of ChR2. The EPSPs were inhibited by the application of DNQX. Moreover, photostimulation also activated the functional GABAergic synapse. Thus, these methods will be used to selectively activate both sensory and associative inputs, providing a strong tool to investigation of local synaptic circuits in primary visual cortex. Supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013- 056534).

Key Words: Optogenetics, primary visual cortex, pathway-specific

P07-21

Evidence for the role of TLR3 in alcohol consumption

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Toll-like receptors (TLRs) are pattern recognition receptors which mediate the induction of the innate immune system. Recent evidences indicate that TLRs are involved in the regulation of behaviors such as learning, memory and anxiety in the absence of a pathogen-derived ligand. However, the relation between TLR3 and alcohol consumption pattern has not been investigated yet. We tested the voluntary alcohol consumption and the alcohol preference. The range of alcohol concentrations was varied from 5% to 20%. Alcohol consumption and preference were increased in TLR3 mutant mice in the 24-hour two-bottle choice test. This result suggests a novel role for neuroimmune signaling in regulation of alcohol consumption.

Key Words: TLR3, Alcohol consumption

P07-22

Analgesic effects of TENS on central neuropathic pain: mediation by opioid receptor in spinal cord injured rats.

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Transcutaneous electrical nerve stimulation (TENS) is commonly used to treat neuropathic pain and is applied with surface electrodes to the skin. TENS is beneficial providing it is administered at a sufficiently strong intensity, and close to the site of pain, but the stimulation parameters that would best achieve the therapeutic effect and its underlying mechanism are still unclear. The aim of this study was to test the effects of TENS on neuropathic pain and to compare the analgesic effects depending on different frequency on mechanical allodynia after spinal cord injury (SCI). Spinal cord injury was made at the level of the T12 by using aneurysm clip exerting a closing force of 30 g extradurally around the spinal cord for 30 seconds under isoflurane anesthesia. Two groups received either high-frequency (HF) or low-frequency (LF) TENS on the back at the level of the lesion or both hindlimbs. To test analgesic effect, paw withdrawal threshold (PWT) was measured. Opioid receptor inhibitors (naloxone, naltrindole, nor-BNI) were injected intraperitoneally or intrathecally 5 min before TENS application. High frequency (HF) TENS application at lesion area maintained analgesic effect for an hour. Intraperitoneal injected 3mg/kg naloxone partially inhibited the anti-allodynic effect of HF TENS, naloxone at dose 6 mg/kg completely inhibited the anti-allodynic effect of HF TENS. In case of TENS application on hindlimb, HF TENS increased PWT for 45 min but these anti-allodynic effect was completely blocked by naloxone with 3 mg/kg. To identify more suitable TENS application area, area under the curve (AUC) of the percentage of maximal possible effect (%MPE) was compared. Analgesic effect of TENS application at leision area was larger than at hindlimb but not significant. Low frequency TENS application didn't show any pain relief. Intrathecal injected 1 and 3ug naloxone, 1 and 5 ug naltrindole, 1 and 5 ug nor-BNI slightly inhibited analgesic effect of HF TENS, respectively. But analgesic effect of HF TENS was completely blocked by administration of 5 and 10 ug naloxone, 10 and 20 ug naltrindole and nor-BNI individually. These results suggest that HF TENS application can contribute to alleviate neuropathic pain by activation opioid receptor.

Key Words: TENS, spinal cord injury, neuropathic pain, mechanical allodynia, opioid

P07-23

The potential role of TLR2 on alcohol-induced behaviors

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Alcohol abuse and alcoholism cause brain damages. In some cases, alcohol consumption leads to neurodegeneration and behavior impairment. Recent studies demonstrate that the inflammatory response is associated with the activation of Toll-like receptors (TLRs) during brain infections, neurodegeneration and neural damage. This study was designed to evaluate the role of TLRs in behavioral consequence induced by alcohol consumption using TLR knockout (KO) mice. The behavior test was performed at 15 days of 10% alcohol consumption followed by 24h withdrawal. In two bottle test, the alcohol consumption and preference were increased in TLR2 KO mice. In dark and light box test, withdrawal anxiety was reduced in TLR2 KO mice. The locomotor activity was increased in TLR2 KO mice. In CPP, reward property was also increased in TLR2 KO mice. These results suggest that the role of TLR2 could be related to the alcohol-induced behaviors and TLRs might be a potential drug target for alcohol-induced disorders in the future.

Key Words: alcohol, TLR2, behavior

P07-24

Spontaneous firing system of the midbrain dopamine neuron: proximal dendrites as a leading pacemaker and the 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 pacemaker-counteract balancer model in which proximal dendrites act as a leading pacemaker 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 postfiring 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. The tonic rises in $[Ca^{2+}]c$ in the slow and large soma compartment leads to postfiring pause, thereby acting as a counteract balancer to the fast-responding proximal dendrites. This generator-counteract balancer model can explain dynamic aspects of glutamate-induced firing diversity in the dopamine neurons.

Key Words: dopamine neuron, spontaneous firing, pacemaker-counteract balancer model, dendritic excitability

P07-25(PO-16)

Functional organization of glutamatergic synapses in the dopamine neurons of the substantia nigra pars compacta

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In the midbrain dopamine neurons, glutamatergic synapses play critical roles in motivation and reward-based learning and drug addiction. Dopamine neurons are well known to generate burst firing in response to reward predicting cues or unexpected rewards via activation of glutamate receptors. In addition, the glutamatergic synapses of dopamine neurons are one of the main candidate sites responsible for drug addiction. However, it is completely unknown about the morphological and functional properties of glutamatergic synapses in the dopamine neurons yet. Here, in the dopamine neurons of the mouse substantia nigra pars compacta (SNc) using two-photon confocal microscopy, we demonstrate morphological and functional features of glutamatergic synapses in the dendrities of the dopamine neurons of the mouse SNc. The SNc dopamine neurons in brain slices show simple and low-branching dendritic arborizations by contrast with those of CA1 pyramidal neurons. By high resolution two photon confocal microscopy, contrary to the previous view, we found that there were common three types of dendritic spines in the SNc dopamine neurons; the thin, mushroom, and stubby spines, whose dimensions were very similar to those of CA1 pyramidal neurons. However, the SNc dopamine neurons had dendritic spines ~5 times less than CA1 pyramidal neurons. By analysis of PSD-95, GluR1 and GluN1 expressions with immunostaining, we identified that dendritic spine glutamatergic synapses expressed AMPA and NMDA receptors together. Surprisingly, we also found that more glutamatergic synapses were also present on the dendritic shafts in the SNc dopamine neurons which were also studded with AMPA and NMDA receptors. By separate recording of AMPA and NMDA receptor currents with two-photon confocal glutamate uncaing, we found functional dendritic spines for glutamatergic synapses in dopamine neurons which had a different AMPAR/ NMDAR ratio to those in dendritic shafts. Therefore, we first show morphologically and functionally distinct glutamatergic substrates for glutamatergic afferents in the SNc dopamine neurons.

Key Words: Dopamine neurons, Glutamatergic synapses, Dendritic spines, Dendritic shafts, EPSCs

P07-26

A new mouse model for trigeminal neuropathic pain; possible effect of mTOR inhibitor rapamycin in mechanical allodynia

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Many patients suffer from trigeminal neuralgia and other types of orofacial pain that are poorly treated, necessitating preclinical animal models for development of mechanisms-based therapies. The mTOR, mammalian target of rapamycin, is a serine-threonine kinase known to regulate cell proliferation and growth. Especially, mTOR is a negative regulator of autophagy, which is a major intracellular degradation pathway implicated in several pathological conditions and brain diseases. The present study introduces a new trigeminal neuropathic pain model caused by infraorbital nerve partial ligation (ION-PL) in mice. In addition, we show the effect of rapamycin, a widely used inhibitor of mTOR which induces autophagy in a variety of cell types, in the ION-PL mouse model. Mechanical and cold allodynia were measured in right and left vibrissal pads using von Frey filament and acetone for 28 days after surgery. Mechanical allodynia was observed in ipsilateral side from day 1 to day 28, whereas cold allodynia was developed bilaterally at day 1, and restored at day 4 after surgery. Clonidine, an α2-adrenoceptor agonist as positive analgesic compound (0.1 mg/kg), or rapamycin (1 mg/kg) was intraperitoneally administered at day 14 after surgery. ION-PL-induced decrease of 50% head withdrawal threshold significantly increased 30-60 min after clonidine injection. Rapamycin also increased head withdrawal threshold 30 min after injection. Moreover, clonidine or rapamycin was applied for consecutive 5 days to examine the effect of repetitive treatment of each drug. Unfortunately, neither daily treatment of clonidine nor rapamycin affected basal mechanical threshold and temporary anti-allodynic effect of each drug. These results demonstrate that our ION-PL model can be a new trigeminal neuropathic pain model in mice. Furthermore we suggest preliminary data implying that rapamycin as an inducer of autophagy can temporally relieve orofacial mechanical allodynia in the trigeminal neuropathic condition.

Key Words: Trigeminal neuralgia, autophagy, rapamycin, mechanical allodynia, clonidine

P07-27

Independent regulation of phasic and tonic inhibition in the rat visual cortex

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GABAA receptor-mediated inhibition is divided into phasic and tonic inhibition. GABAA receptors mediating the two inhibitions show differences in the subcellular localization and the subunit composition. Since phosphorylation of GABAA receptors differ depending on the sub-

unit composition, phasic and tonic inhibition might be differentially regulated by neuromodulators. However, the regulation of phasic and tonic inhibition has seldom been investigated concurrently. In the present study, we investigated how the two inhibitory modalities are differentially maintained and regulated in layer 2/3 pyramidal neurons of the rat visual cortex. Phasic inhibition was measure as inhibitory postsynaptic potential and current (IPSP and IPSC) with K-gluconate-based pipette solution at a 0 mV holding potential and with CsCl-based pipette solution at a -75 mV holding potential, respectively. Tonic currents were measured as changes in holding currents by the application of the GABAA receptor inhibitor bicuculline (10 μM). Depolarization (5 min of 0 mV holding) enhanced IPSP and IPSC via Ca^{2+} and CaMKII but tonic currents were not affected. Tonic currents were regulated by PKA. Serotonin [5-hydroxytryptamine (5-HT)] enhanced phasic inhibition via 5-HT_2 receptor and CaMKII. In contrast, 5-HT suppressed tonic inhibition via 5-HT_{1A} receptor and PKA. These results suggest that phasic and tonic inhibition might be independently regulated by different kinases in response to the changes in neuronal activity and neuromodulators. This independent regulation might confer the neural network more flexibility for the inhibitory control of information processing. Supported by the National Research Foundation of Korea (NRF-2014R1A1A1003382).
Key Words: CaMKII, PKA, 5-HT, GABAA receptor

P07-28

Neuroprotective effects of minocycline by mudulating the expression of heat shock protein 70 in rats with contusive spinal cord injury

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70-Kda Heat shock protein (Hsp70) is induced in central nervous system injuries and has neuroprotective effects. Investigating changes of Hsp70 expression following spinal cord injury (SCI) can be a first step to modulate Hsp70 as a therapeutic method for SCI. Minocycline also has neuroprotective effects. Although Hsp70 can involve in neuroprotection after SCI, the association between minocycline and Hsp70 is unclear. The aim of this study is to investigate changes of Hsp70 expression in early phase of SCI and its changes by minocycline. SCI was induced at T12 by a New York University impactor. Segments of the spinal cord at the epicenter were removed after SCI. Western blots for Hsp70 and cyclooxygenase-2 (COX-2) and 2,3,5-triphenyltetrazolium chloride staining were performed. Basso, Beattie, and Bresnahan locomotor rating scale and combined behavioral score were used to investigate motor function. Hsp70 and COX-2 expression significantly increased until 3 days after SCI. Changes of Hsp70 and COX-2 for 7 days after SCI were co-related. There were no significant improvements in motor function after SCI between groups, except the minocycline group at 3 days after SCI. The cell death area decreased after minocycline administration compared that of saline injection. Hsp70 and COX-2 significantly decreased after minocycline treatments after SCI. These results show changes of Hsp70 expression in early phase after SCI and Hsp70 decreased by minocycline. These changes of Hsp70 after SCI or by minocycline may be involved in changes of COX-2 expression.
Key Words: cyclooxygenase-2 (COX-2), heat shock protein 70 (Hsp70), minocycline, neuroprotection, spinal cord injury

P07-29

Peripherally and centrally administered botulinum toxin type A produces anti-nociceptive effects in orofacial pain of rats

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Botulinum toxin type A (BoNT-A), produced by the anaerobic bacterium Clostridium botulinum, inhibits acetylcholine release from the peripheral nerve terminal. Recently, BoNT-A has been used as an analgesics for myofascial pain syndrome, migraine, and other types of headaches. In the present study, we examined the effects of peripheral or central administration of BoNT-A on orofacial pain. For this purpose, BoNT-A was pre-treated subcutaneously (1 or 3 U/kg) or intracisternally (0.3 or 1 U/kg). Formalin (3%) and Complete Freund's Adjuvant (CFA) was injected subcutaneously to the vibrissa pad and NMDA was injected intracisternally 3days after BoNT-A. In addition to behavior test, we investigated the expression of GFAP (astrocyte marker), Iba-1 (microglia marker) using immunohistochemical analyses after peripheral or central administration of BoNT-A in CFA-treated rats. Both subcutaneous and intracisternal administration of BoNT-A attenuated formalin-induced scratching behavior in the second phase. In addition, both peripheral and central administration of BoNT-A blocked the decreases in latency time of head withdrawal response in CFA-treated rats. However, intracisternal injection of BoNT-A attenuated the number of scratches produced by NMDA injection, but subcutaneous injection of BoNT-A did not. Subcutaneous injection of CFA which produces thermal hyperalgesia increased GFAP and Iba-1 expression in the medullary dorsal horn compared to naive at 5 days after injection. Both peripheral and central injection of BoNT-A down-regulated the expression of

GFAP and Iba-1. These results indicate that central BoNT-A attenuate orofacial inflammatory pain as well. Moreover, anti-nociceptive effects of central BoNT-A may be involved in modulation of glial cells or neuronal activity in the medullary dorsal horn (supported by 2008-0062282, 2012M3A9B6055414 and Hugel Inc).
Key Words: botulinum toxin, orofacial pain, antinociception, NMDA

P07-30

A blockade of spinal glutamate recycling produces paradoxical antinociception in rats with orofacial inflammatory pain

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In our current study, we investigated the role of spinal glutamate recycling in the development of orofacial inflammatory pain. DL-threo-β-benzyloxyaspartate (TBOA) or methionine sulfoximine (MSO) was administered intracisternally to block spinal glutamate transporter and glutamine synthetase activity in the astroglia. The intracisternal administration of high dose TBOA (10 μg) produced thermal hyperalgesia in naïve rats but significantly attenuated the thermal hyperalgesia in rats that had been pretreated with interleukin (IL)-1β or Complete Freund's Adjuvant (CFA). An intracisternal injection of MSO produced anti-hyperalgesic effects against thermal stimuli in the CFA-treated rats only. To confirm the paradoxical antinociceptive effects of TBOA and MSO, we examined changes in c-Fos expression in the medullary dorsal horn produced by thermal stimulation in naïve, IL-1β or CFA-treated rats, after intracisternal injections of TBOA and MSO. The intracisternal administration of TBOA significantly increased the c-Fos immunoreactivity level in naïve rats. In contrast, the intracisternal administration of TBOA significantly decreased the up-regulation of c-Fos immunoreactivity in the medullary dorsal horn of IL-1β and CFA-treated rats. The intracisternal injection of MSO blocked the up-regulation of c-Fos immunoreactivity in the CFA-treated rats only. We also investigated the effects of botulinum toxin type A (BoNT-A) on TBOA-induced paradoxical antinociception in the CFA-treated rats, as BoNT-A inhibits the release of neurotransmitters, including glutamate. BoNT-A treatment reversed behavioral responses produced by intracisternal administration of TBOA in the CFA-treated rats. These results suggest that the paradoxical responses produced by blocking of glutamate transporter under inflammatory pain conditions are mediated by modulation of glutamate release from presynaptic terminals. Moreover, a blockade of glutamate reuptake indicates new therapeutic targets for the treatment of chronic inflammatory pain conditions (supported by 2008-0062282, 2012M3A9B6055414 and Hugel Inc.).
Key Words: glutamate, glutamate transporter, glutamine synthetase inhibitor, paradoxical antinociception, orofacial pain

P07-31

FM1-43 dye unloading reveals pathway-specific activation of synaptic inputs onto layer 2/3 pyramidal neurons in the primary visual cortex

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In layer 2/3 pyramidal neurons (L2/3 PyNs) of the primary sensory cortex, perisomatic dendritic area including basal dendrites receives sensory feedforward inputs from the thalamus and layer 4 whereas the distal apical dendrite in layer 1 receives feedback associative inputs from higher brain areas. Thus L2/3 PyNs is well suited for the integration of feedback and feedforward synaptic inputs. Although the properties of the information flow in these two pathways are critical for understanding of cortical information processing, almost nothing is known yet due to the structural complexity of the neocortex. Because simple extracellular electrical stimulation is widely used to activate synaptic inputs, it is a prerequisite to know whether local electrical stimulation activates specific inputs. Here, we investigated the layer-specific synaptic activation using FM1-43 dye unloading in the primary visual cortex. To load FM1-43 dye into synaptic vesicles, visual cortical slices were exposed to high KCl (40 mM) with FM1-43 dye (8 μM) and washed with ADVASEP-7 (chelator of FM dye). Loaded FM1-43 was stable more than 1.5 hrs. For the unloading of FM1-43 dye, electric stimulation (2 Hz) was delivered to either layer 1 or layer 4 with extracellular stimulus electrodes. Unloading of FM1-43 in layer 1 was occurred only by electrical stimulation of layer 1 but not by layer 4. Likewise, unloading of FM1-43 at the layer 2/3 was detected by electric stimulation of layer 4 but not of layer 1. The decay kinetics for fluorescence intensity was fitted well with single exponential function and decay time constants were 97.7±9.7 s and 125.9±21.7 s in layer 1 and layer 2/3, respectively (n=6). Layer-specific spatial profile of FM dye unloading showed that the unloading of FM-43 dye was very restricted in the layers where the local electrical stimulation was applied. Thus, these results indicate that stimulation of layer 1 and layer 4 specifically activates inputs in distal apical dendritic area and perisomatic basal dendritic areas, respectively. Moreover, FM1-43 dye unloading experiments revealed segregation of sensory and associative inputs in L2/3 PyN of the primary visual cortex. Supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013-056534).
Key Words: FM1-43, dendrite, visual cortex, pathway- specific

P07-32

Neuronal excitation in afferents fibers stimulated by the intradiscal pressure into normal or punctured lumbar intervertebral discs (IVDs) of rats

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Intervertebral disc (IVD) comprised of nucleus pulposus, annulus fibrosus and endplates is responsible for stabilizing the spine by resisting against mechanical load with a swelling pressure. The biochemical and mechanical failure of IVD cause a spinal stenosis accompanied by a herniated or bulging IVD, which can irritate adjacent spinal nerves or spinal cord. In addition, to understand the underlying mechanism of discogenic LBP, it is important to consider whether/how mechanical stimulus can affect mechanoreceptor in peripheral nerve terminal innervating IVDs themselves. It has been revealed that nerve fibers reach the IVDs through the sinuvertebral nerves or from branches of the paravertebral sympathetic trunks; however, to date there has been few study to characterize the responsiveness of afferent fibers in IVDs stimulated by intradiscal pressure using in vivo electrophysiology. Male SD rats (300-350 g; Orient Bio, Korea) were used for in vivo single nerve recording. Neuronal activities of afferent fibers in normal or punctured lumbar IVD were measured and analyzed by counting peak spikes per second while intradiscal pressure (100-3,000 mmHg, at interval of 70 seconds) were being applied. Afferents in punctured lumbar IVD, compared to normal and sham lumbar IVD, displayed significantly increased activities in pressure-response curve as well as significantly decreased threshold of pressure stimulus. The present study implicate that the sensory information from IVDs can be transmitted to spinal cord through afferent fibers, and furthermore excessive mechanical load or pressure can give rise to discogenic low back pain.

Key Words: lumbar intervertebral discs (IVDs), punctured IVD, intradiscal pressure, in vivo single nerve recording

P07-33

Development of a new mouse model for chronic migraine using repetitive nitroglycerin administration

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Despite the relatively high prevalence of migraine or headache, the pathophysiological mechanisms triggering headache, especially in relation to chronic migraine are unknown. Since nitric oxide (NO) is well known as a causative factor in the pathogenesis of migraine, we were to establish a new mouse model of chronic migraine using nitroglycerin (NTG), a donor of NO. NTG (10 mg/kg) was repetitively administrated every other day for 9 days. In the facial region and hind paws, the mechanical and cold allodynia were evaluated using von Frey filament and acetone, and the thermal hyperalgesia to radiant heat stimulus was also examined in the hind paws. Repetitive administration of NTG produced acute mechanical allodynia and thermal hyperalgesia in the hind paws 2 hours after each injection from the second injection day (day 3) of NTG (Post-treatment responses). In contrast, the cold allodynia significantly occurred in the facial region with similar time course. In addition, the NTG-treated mice appeared a progressive and long-lasting decrease of basal thresholds in the identical pain behavior tests (Basal responses). Interestingly these chronic basal pain responses also persisted for 10 days after cessation of NTG administration. The current findings demonstrated that repetitive NTG administration resulted in acute and long-lasting pain responses in both hind paws and facial region. Moreover these pain responses were dependent to stimulus site and modality. We suggest that this repetitive NTG-induced pain in different regions can be a useful mouse model for the study of underlying mechanisms and therapeutic approach in chronic migraine patients.

Key Words: migraine, animal model, nitroglycerin, mechanical allodynia, cold allodynia, thermal hyperalgesia

P07-34

Clonidine, an alpha-2 adrenoceptor agonist relieves mechanical allodynia in oxaliplatin-induced neuropathic mice; potentiation by p38 MAPK inhibition without motor dysfunction

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The chemotherapeutic agent, oxaliplatin produces robust painful neuropathy, which results in neuropathic pain such as mechanical allodynia. Although the administration of clonidine, an alpha-2 adrenoceptor agonist, significantly attenuates nociception and hyperalgesia in several pain models, its effect was not determined in oxliplatin-induced neuropathic pain. In addition, the clinical trial of clonidine is limited by its side effects such as drowsiness, hypotension and sedation. Here we were to examine whether intraperitoneal injection of clonidine reduces mechanical allodynia in oxaliplatin-induced neuropathy model, and whether this effect was associated with the change of MAPK sig-

naling such as p38 or ERK phosphorylation. Clonidine injection significantly reduced oxaliplatin-induced mechanical allodynia in a dose-dependent manner. In addition, oxaliplatin-induced increase of p-p38 MAPK expression, but not ERK phosphorylation in spinal dorsal horn, decreased in clonidine-treated mice. Subsequently we investigated whether SB203580, a p38 MAPK inhibitor also reduced mechanical allodynia in oxaliplatin-induced neuropathic mice, and whether the co-administration of SB203580 enhanced lower-dose clonidine-induced effect without the disruption of motor coordination. Intrathecal SB203580 injection dose-dependently relieved mechanical allodynia, and the sub-effective of dose SB203580 (3 nmol) significantly potentiated the anti-allodynic effect of lower-dose clonidine (0.01 or 0.03 mg/kg). Especially, a middle dose of clonidine (0.03 mg/kg) in combination with SB203580 produced an effect similar to that of high-dose clonidine, but without a significant motor dysfunction in rota-rod test. On the other hand, high-dose of clonidine injection resulted in severe impairment in motor coordination. These findings demonstrate that clonidine treatment reduces oxaliplatin-induced mechanical allodynia, and p38 MAPK inhibitor, SB203580 can potentiate lower-dose clonidine-induced anti-allodynic effect without motor dysfunction.
Key Words: oxaliplatin, clonidine, neuropathic pain, mechanical allodynia, p38 MAPK

P07-35

The Effects of Noisy Galvanic Vestibular Stimulation on Neuronal Activities of the Pedunculopontine nucleus in Hemiparkinonian Rats

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Noisy galvanic vestibular stimulation (noisy GVS) has been thought to be a novel therapy for Parkinson's disease (PD). However, the mechanism of alleviating symptoms of PD is unclear. Because PPN and motor cortex are considered essential areas linked to abnormal behavior of PD patients, 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 PPN 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 6-OHDA lesioning slightly increased not only the rate and irregularity of neuronal firing in PPN but also the correlation between PPN and motor cortex. Noisy GVS induced to the mild regularization of neuronal irregularity pattern in PPN considered as abnormal neuronal firing pattern of PD. On the other hand, the firing rate of PPN neurons as well as correlation between PPN and motor cortex did not significantly change after noisy GVS. These findings demonstrate that noisy GVS can regularize neuronal firing irregular pattern in PPN, indicating the pathophysiology of PD. Therefore, the modulation of neuronal firing pattern of PPN after noisy GVS may be a potential mechanism for relieving symptoms of PD.
Key Words: vestibular stimulation, pedunculopontine nucleus, Parkinson's disease

P07-36

Role of WNK-SPAK/OSR1 signaling in the activity of serotonergic dorsal raphe nucleus neurons 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 and nitrogen species (ROS and RNS) and oxidative stress developed during wakefulness. The dorsal raphe nucleus (DRN) is known to induce wake state in sleep-wake cycle and is related to rapid-eye movement (REM) sleep. Most DRN neurons are serotonergic which projects to various regions of the brain. The WNK-SPAK/OSR1 kinase complex is composed of "with no lysine kinase" (WNK) and Ste20-related proline alanine rich kinase (SPAK)/oxidative stress response kinase (OSR1). The WNK-SPAK/OSR1 kinase signaling regulates intracellular Cl- concentration, extracellular osmolarity, and cell volume. Chloride homeostasis in the brain neurons is regulated by cation-chloride co-transporters such as Na-K-Cl co-transporter (NKCC1) and K-Cl co-transporter (KCC2). WNK-SPAK/OSR1 signaling is regarded as an up-stream regulator of NKCC1 and KCC2. Previous our study showed that the expression of NKCC1 and KCC2 in DRN neurons is changed during sleep-wake cycle. In this study, we examined the role of WNK and SPAK/OSR1 signaling in DRN neurons during sleep-wake cycle and the effect of ROS and RNS on the WNK-SPAK/ORS1 signaling. The results indicate that ROS and/or RNS alter WNK-SPAK/OSR1 pathway, which might regulate the activity of the serotonergic DRN neurons through the modulation of NKCC1 and KCC2 activities during sleep-wake cycle.
Key Words: Dorsal raphe nucleus, Sleep-wake cycle, Oxidative stress, WNK kinase, SPAK/OSR1 kinase

P07-37

Role of NKCC1 and KCC2 in the activity of serotonergic dorsal raphe nucleus neurons during sleep-wake cycle

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Sleep is a typical subject that most species go through during lifetime. It is a vital component to perform fundamental and healthy lives. Sleep is a highly organized and regulated homeostatic mechanism of human and other mammals. Various neuronal networks and neurotransmitters in the brain are involved in the control of sleep-wake cycle. Neurons releasing GABA or galanin are known to induce sleep in sleep-wake cycle, while neurons releasing serotonin, norepinephrine, acetylcholine, histamine, and orexin induce wake state. Serotonin is well known as a regulator of sleep-wake cycle and is mainly synthesized and released in the dorsal raphe nucleus (DRN). DRN neurons receive abundant input signals from GABAergic neurons. GABAergic synaptic activity induces activation of GABA-activated chloride channels and inhibits the target neuronal activity. Intracellular chloride concentration of the neurons is regulated by cation-chloride co-transporter including Na-K-Cl co-transporter (NKCC1) and K-Cl co-transporter (KCC2). Intracellular chloride concentration is increased by NKCC1 but decreased by KCC2. In this study, we examined the role of NKCC1 and KCC2 activity in the activity of serotonergic DRN neurons during sleep-wake cycle. The results suggest that changes of NKCC1 and KCC2 expression during sleep-wake cycle might play an important role in the wake-inducing function of DRN neurons.

Key Words: Dorsal raphe nucleus, Sleep-wake cycle, GABA, NKCC1, KCC2

P07-38

Sigma-1 receptors activated spinal astrocytes release D-serine leading to the development of mechanical allodynia in a mouse model of neuropathic pain

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We have reported that the activation of spinal sigma-1 receptors (Sig-1Rs) play an important role in the development of mechanical allodynia via phosphorylation of the NMDA receptor GluN1 subunit (pGluN1). It is also demonstrated that Sig-1R are localized in astrocytes, and that blockade of Sig-1Rs inhibits the pathologic activation of astrocytes in neuropathic mice. However, it is unknown that how Sig-1R in astrocytes modulate pGluN1 in neuron. D-serine is an endogenous co-agonist for NMDA receptor glycine site and can control NMDA receptor activity. D-serine is synthesized from L-serine by enzyme serine racemase (SRR) in astrocyte. Therefore, the present study was designed to investigate: (1) whether the inhibition of SRR could suppress MA or TH development in chronic constriction injury (CCI) mice; (2) whether the inhibition of Sig-1R could modulate CCI-induced increase in D-serine release or SRR expression; (3) the precise cellular location of D-serine and SRR in spinal cord dorsal horn; (4) whether the inhibition of SRR could suppress CCI-induced pGluN1. Sustained intrathecal treatment with the SRR inhibitor reduced the development of MA, but not TH. The expression of D-serine was significantly increased in the spinal cord after CCI surgery. Sustained intrathecal treatment with Sig-1R antagonist, BD-1047, attenuated CCI-induced increase in D-serine release. D-serine was increased in astrocyte and neuron. In addition, the expression of SRR also increased in the spinal cord after CCI surgery, which was reduced by BD-1047 treatment. SRR was also found in astrocyte. Moreover, SRR inhibitor treatment significantly reduced CCI-induced increase in pGluN1. Finally, D-serine treatment reversed the antinociceptive effect of BD-1047 on development of MA. These findings demonstrate that the blockade of Sig-1Rs inhibits D-serine release from activated astrocytes, which ultimately prevents the development of MA in neuropathic mice.

Key Words: Sigma 1 receptor, D-serine, Astrocyte activation, pGluN1, Mechanical allodynia

P07-39

Blockage of spinal interleukin-1 β lead to development of mirror-image mechanical allodynia via upregulation of connexin43 expression and astrocyte activation in a rat model of inflammatory pain

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Damage on one side of the body can also result in pain from the contralateral unaffected side of the body, called Mirror-image pain (MIP). Currently the cell types, the cell mediators and the exact mechanisms responsible for the development of MIP are unknown. Interleukin-1 β (IL-1 β) is one of early proinflammatory cytokines which not only initiate inflammatory cascades, but also regulate nociceptive processing in the CNS. On the other hand, astrocytes are frequently connected to one another via gap junctions to form a syncytium through which intercellular signaling may propagate. Thus, local nociceptive input can diffuse to other parts of the CNS through this astrocytic network. In this study, we investigated the role of relationship between spinal IL-1 β and astrocytic gap junction in the development of MIP using a unilateral inflammation model. After 2% carrageenan (CA) was injected into hind-paw of rats, mechanical allodynia (MA) was evaluated at each time point. Western blot assay were used to determine the changes of IL-1 β, GFAP (marker for astrocyte), and connexin 43(astrocyte gap junction protein) expression in the spinal dorsal horn. Contralateral MA was

developed day 5 post-CA injection in intrathecally (i.t.) vehicle-treated control rats. i.t. injection of carbenoxolone (CBX; a gap junction decoupler) at days 0-3 post-CA injection blocked the development of contralateral MA. Furthermore, i.t. CBX reversed the expression level of Cx43 and GFAP which were upregulated at 10 day post-CA injection. On the other hand, spinal IL-1β expression was increased at 3 hour post-CA injection. i.t. injection of interleukin-1 receptor antagonist (IL-1ra) at days 0-3 post-CA injection, interestingly, significantly advanced the appearance of contralateral MA. Moreover i.t. IL-1ra increased Cx43 and GFAP expression at 3 hour post-CA injection as compared to that of control rats. These results demonstrated that spinal astrocyte and its gap junction plays a major role for development of contralateral MA during the early phase of peripheral inflammation. Also, blocking of spinal IL-1β induced the Cx43 expression and astrocyte activation which was ultimately facilitated development of contralateral MA in peripheral inflammatory pain model, suggesting that the relationship between spinal IL-1β and astrocyte gap junction plays an important role in the regulation of induction time of MIP.

Key Words: Mirror-image pain, Astrocyte, Gap junction, connexin 43, Interleukin-1β

P07-40

Change of GABAergic synaptic activity in serotonergic dorsal raphe nucleus neurons during sleep-wake cycle

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The dorsal raphe nucleus (DRN) contains a largest number of serotonin (5-hydroxytryptamine, 5-HT)-containing neurons which project to various region of the brain. DRN neurons have been implicated in a wide variety of physiological functions, including mood control, feeding, aggression, anxiety, motor control and control of behavioral states. Another important function of DRN neurons is a wake-induction and/or is related to the rapid eye movement (REM) sleep during sleep-wake cycle. DRN neurons receive abundant input signals from GABAergic neurons such as ventrolateral preoptic area (VLPO) neurons known as sleep-inducing neurons. GABAergic synaptic activity induces activation of GABA-activated chloride channels and inhibits the target neuronal activity. Chloride homeostasis in the brain neurons is regulated by cation-chloride co-transporters such as Na-K-Cl co-transporter (NKCC1) and K-Cl co-transporter (KCC2). Intracellular chloride concentration is increased by NKCC1 but decreased by KCC2. Previous our study showed that the expression of NKCC1 and KCC2 in DRN neurons is changed during sleep-wake cycle. In this study, we examined the change of GABAergic synaptic activity in the DRN neurons during sleep-wake cycle by using gramicidin-perforated patch-clamp recordings in the slice preparation of the rat DRN. The results suggest that alteration of GABAergic synaptic activity is important to the physiologic function of serotonergic DRN neurons during sleep-wake cycle.

Key Words: Dorsal raphe nucleus, Sleep-wake cycle, GABA, Chloride channel, Patch-clamp

P07-41

Peripheral Sigma-1 receptors contribute to the ischemic mechanical allodynia in thrombus induced ischemic pain (TIIP) rats: potential interaction with ASIC and P2X

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Sigma-1 receptors (Sig-1Rs) are ligand gated molecular chaperone which play an important role in various neuronal functions. Owing to their neuromodulatory actions, Sig-1Rs have been examined for the pain control target especially in spinal cord. Although Sig-1Rs are located in peripheral nervous system, their roles in peripheral nociception under normal and pathological state have not been thoroughly investigated. Since there is a large amount of evidence that Sig-1Rs exert their action under ischemic condition, we investigate the possible role of peripheral Sig-1Rs on ischemia induced pain hypersensitivity using thrombus induced ischemic pain (TIIP) model. Western blot was performed to observe changes of Sig-1R expression in peripheral tissues including skin, DRG and sciatic nerve. The Sig-1R antagonist BD1047 was administered intraplantarly from postoperative days 0 to 3 (induction phase of ischemic pain) or from days 3 to 6 (maintenance phase). Peripheral Sig-1R expression significantly increased in the skin, DRG and sciatic nerve from days 1 to 3 after TIIP surgery. BD1047 administrated on induction phase significantly attenuated TIIP induced mechanical allodynia in the treatment period. In contrast, BD1047 treatment during the maintenance phase had no effect on mechanical allodynia. Interestingly, analgesic effects of BD1047 was synergistically enhanced by co-administration of sub-effective dose of amiloride (ASICs blocker) or TNP-ATP (P2X antagonist) during the induction phase, but these facilitatory effects were not seen in the maintenance phase-treated group. In naive rats, direct activation of peripheral Sig-1R by intraplantar injection of PRE084 did not produce mechanical allodynia; however, co-administration with acidic pH or ATP, by activating ASICs or P2X respectively, produced significant mechanical allodynia but not thermal hyperalgesia. In this study, we demonstrated that Sig-1Rs play critical role in the early induction of ischemic mechanical allodynia, and we also had shown that the action of peripheral sig-1R was enhanced by modulating ASIC and P2X receptors. These findings implicated that peripheral Sig-1R can be important factor in ischemia induced mechanical allodynia and potential therapeutic target for the use of Sig-1R antagonists in clinical management of ischemia induced mechanical allodynia.

Key Words: Sigma-1 receptor, mechanical allodynia, ischemic pain

P07-42

Follow-up of Retinal Degeneration in rd10 Mice Retina According to Postnatal Age

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Retinal prosthesis has been globally developed to restore vision for the blind with retinitis pigmentosa (RP) or age-related macular degeneration (AMD). Mammalian retinal degenerations generally progress through three phases and each phase shows respective features in morphology and physiology. In particular, widespread remodeling progresses in the remnant neural retina at phase 2 and 3. Therefore, it is necessary to investigate the retinal degenerative change according to each stage for optimal implement of retinal prosthesis. In this study, we used rd10 mice as in-vitro RP model. The spontaneous and electrically-evoked retinal ganglion cell (RGC) spikes were recorded using 8x8 multi-electrode arrays (MEA) at postnatal week (PNW) 2, 3, 4.5, 6,5, 8, 10, 15, 20, 26 and 34. Cathodic phase-first biphasic current pulses (sine and square pulse) were applied (duration: 500 us, amplitude: 5, 10, 20, 30, 40, 50, and 60 uA) in every 1 sec for 50 times. For the follow-up of the stage of retinal degeneration, mean firing rate of RGC spikes, 2nd peak latency of inter-spike interval histogram (ISIH), power spectral density (PSD) and continuous wavelet transform (CWT) images were compared according to each postnatal age groups. Regarding spontaneous RGC spikes, PNW20 retinas showed the highest mean frequency among other age groups after PNW8 ($p < 0.01$), and inter-spike intervals of RGCs showed maximum value at PNW26. These results are closely related to 4～5Hz oscillations which appear most strongly at PNW15, 20 and 26. Regarding electrically-stimulated responses, mean frequency of RGCs seems to be fixed to certain value especially in square-wave stimulation group. Main frequency of local field potentials in electrically-stimulated retina becomes higher than that of the controls at PNW 10, 15, 20, 26. However, intensities of local field potential in electrically-stimulated retinas decrease only at PNW20, and there are no changes in other age groups. These results provide useful information to determine the optimal stimulus protocols and the right timing for the prosthetic use for each phase of RP patient, eventually.

Key Words: retinal degeneration, rd10 mice, retinal prosthesis, retinal ganglion cell, and multi-electrode array

P07-43

Spinal sigma-1 receptors induce astrocyte activation via increase in gap junction protein connexin 43 expression leading to the induction of bilateral below-level mechanical allodynia following spinal cord injury in mice

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We have recently demonstrated that gap junctions contribute to the early spread of astrocyte activation in the lumbar spinal cord, which are critical to the induction of bilateral below-level neuropathic pain in spinal cord injury (SCI) rats. The present study determined whether spinal gap junction protein connexin 43 (Cx43) expression and astrocyte activation are modulated by sigma-1 receptor (Sig-1R) activation, which is known as a critical role in the induction of neuropathic pain, leading to the SCI-induced induction of bilateral below-level chronic neuropathic pain. SCI was performed by transverse hemisection of the right thoracic spinal cord between T11-12 vertebral segments in mice. Below-level mechanical allodynia and thermal hyperalgesia were evaluated in bilateral hindpaws of SCI mice. Immunohistochemistry and western blotting were performed to determine potential changes in Cx43, glial fibrillary acidic protein (GFAP), and Sig-1R expression in spinal cord. Gap junction protein Cx43, GFAP, and Sig-1R expression were increased not only in at level (injury site) but also in below level (lumbar enlargement site) spinal cord dorsal horn following SCI. Administration with the gap junction decoupler, carbenoxolone, or Sig-1R antagonist, BD1047, suppressed SCI-induced increase in GFAP expression and dose-dependently ameliorated bilateral below-level mechanical allodynia, but not thermal hyperalgesia. Furthermore, repeated administration with BD1047 significantly reduced SCI-induced increase in Cx43 expression. These findings demonstrate that spinal Sig-1R activation modulates astrocyte activation via increase in gap junction protein Cx43 expression not only in the injury site but also in the remote region of spinal cord dorsal horn, ultimately contributing to the induction of bilateral below-level chronic mechanical allodynia in SCI mice.

Key Words: Astrocyte activation, Bilateral below-level chronic mechanical allodynia, Connexin 43, Sigma-1 receptor, Spinal cord injury

P07-44

3 weeks of heat acclimation reduces sudomotor activity in human subjects

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Sweating response is modulated in two different ways depending on heat adaptation period; enhanced or suppressed sweating. We examined body temperature, sudomotor activity and serum levels of endogenous pyrogens before and after heat acclimation (HA) to test the hypothesis that body temperature and endogenous pyrogens would be associated with sudomotor activity. Nine tennis players were recruited. The subjects were exposed to half-body immersion in hot water (42±0.5°C) at the same time of day on alternate days for 3 weeks. The HA protocol included 10 bouts of 30 min immersion. All experiments were performed in an automated climate chamber (temperature, 26.0±0.5°C; relative humidity, 60±3.0%; air velocity, < 1 m/sec). Tympanic and skin temperatures were measured, and mean body temperatures were calculated. Serum levels of PGE2, COX-2, and orexin were analyzed before and after HA. Sudomotor activity, including onset time, sweat rate (SR) and volume (SV), active sweat gland density (ASGD), and sweat gland output (SGO), was tested in four areas of skin. Body temperature decreased significantly after HA. There was an increase in local sweat onset time, but there was a decrease in local SR, SV, and SGO after HA. Serum levels of PGE2, COX-2, and orexin were reduced after HA and positively correlated with body temperatures. Our data suggest that decreased body temperature after HA is associated with decrease in endogenous pyrogens, which contributed to reduction of sudomotor activity under the heat load.

Key Words: body temperature, sudomotor activity, endogenous pyrogens, heat acclimation

P07-45(PO-17)

Age and sex related differences in sudomotor function evaluated by the quantitative sudomotor axon reflex test in healthy humans

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The aim of the present study was to quantitatively investigate the age and sex-related differences in sudomotor function in healthy humans. The quantitative sudomotor axon reflex test (QSART) with iontophoresis (2 mA for 5 min) and 10% acetylcholine (ACh) was performed to determine axon reflex-mediated (AXR), with and without iotophoresis (AXR(1) and AXR(2), respectively), and directly activated (DIR) sweating. All experiments were conducted under thermoneutral conditions (temperature 24.0±0.5°C; relative humidity 40±3%). In general, men had enhanced values of onset time of AXR, sweat rates, activated sweat gland density (ASD) and activated sweat gland output (SGO) than women, but not in all cases. The onset time of AXR (r2=0.567; $P<0.001$) was positively correlated with advancing age, whereas sweat rates of AXR(1) and AXR(2) (r2=0.571 and r2=0.486, respectively; $P<0.001$), DIR (r2=0.594; $P<0.001$), ASD (r2=0.496; $P<0.001$) and SGO (r2=0.551; $P<0.001$) were negatively correlated in both men and women with advancing age. The results demonstrate that an attenuation of sudomotor function occurs with aging in both genders. Moreover, the findings showed a progressive increase in onset time and a decrease in sweat rates, ASD and SGO with increasing age in both genders. A variation in sweat function was found between genders, but not in all age groups.

Key Words: acetylcholine, activated sweat gland density, activated sweat gland output, aging, quantitative sudomotor axon reflex test (QSART), sweating

P07-46

Trans-anethole Allevates Trimethyltin Chloride-Induced Impairments in Long-term Potentiation

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Trans-anethole is a kind of aromatic compound, studied on anti-inflammation, anticonvulsant, antinociceptive and anticancer. Recently, it was reported that trans-anethole had neuroprotective effect on brain. Trimethyltin chloride (TMT) is a kind of organometallic compounds, known to be neurotoxicity especially in hippocampus. However, little is known about the effects of trans-anethole and TMT in long-term potentiation (LTP) induction. In the present study, we investigated the effects of trans-anethole and TMT on NMDA receptor-dependent and NMDA receptor-independent LTP in mice hippocampal slices. We also examined the role of trans-anethole in TMT-induced LTP impairment. As results, trans-anethole enhanced both NMDA receptor-dependent and NMDA receptor-independent LTP. Further, it alleviated TMT-induced LTP impairments. The present results suggest that trans-anethole modulates hippocampal LTP induction and it may be a therapeutic target of Alzheimer's disease.

Key Words: Trans-anethole, Trimethyltin chloride, long-term potentiation (LTP), synaptic plasticity, hippocampus

P07-47

Effect of Sodium Nitroprusside on Neuronal Excitability in Rat Substantia Gelatinosa Neurons

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Nitric oxide (NO) is an important signaling molecule involved in nociceptive transmission. It can induce analgesic and hyperalgesic effects in the central nervous system. In this study, patch-clamp recording was used to investigate the effect of NO on neuronal excitability in substantia gelatinosa (SG) neurons of the spinal cord. Different concentrations of sodium nitroprusside (SNP; NO donor) induced a dual effect on the excitability of neuronal membrane: 1mM of SNP evoked membrane hyperpolarization and an outward current, whereas 10 μM induced depolarization of the membrane and an inward current. These effects were prevented by hemoglobin and 2-(4-carboxyphenyl)-4, 4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt (c-PTIO) (NO scavengers), phenyl N-tert-butylnitrone (PBN; nonspecific reactive oxygen species scavenger), and through inhibition of soluble guanylyl cyclase (sGC). Pretreatment with n-ethylmaleimide (NEM; thiol-alkylating agent) also decreased effects of both 1mM and 10 μM SNP, suggesting that these responses were mediated by direct S-nitrosylation. Charybdotoxin (CTX) and tetraethylammonium (TEA) (large-conductance Ca^{2+}-activated K^+ channel blockers) and glybenclamide (ATP-sensitive K^+ channel blocker) decreased SNP-induced hyperpolarization. La3+ (nonspecific cation channel blocker), but not Cs+ (hyperpolarization-activated K^+ channel blocker), blocked SNP-induced membrane depolarization. In conclusion, NO dually affects neuronal excitability in a concentration dependent manner via modification of various K^+ channels.

Key Words: Nitric oxide, SNP, neuronal excitability, dual effect

P07-48

Roles of Reactive Oxygen Species Generated by Xanthine Oxidase in Rat Trigeminal Caudal Neurons

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The caudal subnucleus of the spinal trigeminal nucleus (medullary dorsal horn; MDH) is implicated in the processing of nociceptive information in the orofacial region. Recent studies indicate that reactive oxygen species (ROS) have been involved in persistent pain primarily through spinal mechanisms. In this study, to investigate the role of xantine/xantine oxidase (X/XO) system, a well known generator of superoxide anion (O2・-), on membrane excitability in rat MDH neurons, patch clamp recording and confocal imaging were used. An application of X/XO (300 μM /30 mU) induced a membrane depolarization and inward currents. When slices were pretreated with ROS scavengers such as phenyl N-tert-butylnitrone (PBN), superoxide dismutase (SOD) and catalase, X/XO-induced responses were decreased. An anion channel blocker, 4,4-diisothiocyanostilbene-2,2-disulfonic acid (DIDS) significantly decreased X/XO-induced depolarization. X/XO elicited an inward current associated with a linear current-voltage relationship that reversed near -40 mV, suggesting a voltage-independent nonselective cation channel. X/XO-induced depolarization was reduced in the presence of La3+, a nonselective cation channel blocker, and by lowering the external sodium concentration, indicating that membrane depolarization and inward current are induced by influx of Na+ ions. X/XO-induced responses were suppressed by both 1,2-bis(o-aminophenoxy) ethane-N,N,N', N'-tetraacetic acid (BAPTA), a chelater of intracellular Ca^{2+}, and thapsigargin, an inhibitor of the endoplasmic reticulum Ca^{2+}-ATPase. Moreover, Ru360, a specific inhibitor of the mitochondrial Ca^{2+} uniporter, decreased X/XO-induced responses. In calcium imaging technique using the confocal laser microscope in single cell of MDH, X/XO-induced increase of fluorescence intensity was reduced in external Ca^{2+} free solution and by pretreatment with BAPTA-AM. In conclusion, X/XO-induced ROS modulate the membrane excitability of MDH neurons, which was related to the increase of cytosolic Ca^{2+} and activation of nonselective cation channel. These results suggest that O2・-, in addition to its role as a neurotoxin, also can be considered a neuronal signaling molecule for pain transmission.

Key Words: spinal trigeminal nucleus neurons, xantine/xantine oxidase, reactive oxygen species, membrane excitability

P07-49

Isolation of Early Responses of Retinal Ganglion Cells Using Moving Average Filter-Based Spike Sorting Algorithm

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Epiretinal approach to retinal prosthesis aims at direct stimulation of the retinal ganglion cells (RGCs) using electrodes on the inner limiting membrane of the retina. Epiretinal stimulation can evoke both, the short-latency, early (direct) responses and long-latency, late (network-mediated) responses. Previously, in our study we have been using 400 ms range RGC responses criterion which contains short- and long-latency responses together due to the limitation to isolate short-latency spikes. For harvesting short-latency RGC spikes free from stimulus artifacts, in this study, we applied moving average filter (MAF)

algorithm for artifact subtraction. A moving average is used with time series data to reduce short-term fluctuations, therefore, it can be viewed as an example of a low-pass filter used in signal processing. MAF algorithm analyzes data points by creating a series of averages of different subsets of the full data set. The average value is taken from an equal number of data on either side of a central value. As a well-known method in engineering, but hardly used in electrophysiology, we want to establish MAF-based algorithm for spike detection like other well-known artifact subtraction methods (template subtraction, SALPA; subtraction of artifacts by local polynomial approximation algorithm). In rd10 mice, we effectively identified the latency of early RGC spikes as 23.5±3.2 ms (n=3 retinas, 22 channels) after stimulus. However, our MAF-based algorithm has a weakness that it hardly discriminate stimulus evoked-RGC spike and spike looking-residual artifact. Therefore, we are making TTX subtraction-based algorithm now, and we will compare the latency of early spike harvested from MAF-based algorithm and TTX subtraction-based algorithm.

Key Words: Retinal ganglion cell, Moving average filter-based spike sorting algorithm, stimulus artifact, short-latency spike, and TTX subtraction-based algorithm

P07-50

Serotonin receptors in vagal and splanchnic sensory nerves innervating the liver

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Unlike primary somatic sensory transmitted to the spinal cord, the primary visceral sensory information from intra-abdominal organs is transmitted to the spinal cord and medulla oblongata via splanchnic nerve and vagus nerve, respectively. These two nerves have different sets of terminal sensory receptor profiles. This study is to see the difference in the responsiveness to serotonin of both nerves innervating the liver and to elucidate the activation mechanism of the serotonin receptors. Fluorescent dye (DiI or DiD) was injected into the liver in an anesthetized guinea pig. One week later, the animal was sacrificed and vagal ganglia and dorsal root ganglia were harvested. The neurons labeled with fluorescent dye (hepatic sensory nerve) were certified under a fluorescent microscope. Intracellular calcium measurement, patch clamp recording and single cell rt-PCR were done in the labeled neurons. About 10% of vagal nerve and 20% of splanchnic nerves (Th 9, 10, 11) innervated into the liver. Serotonin (10 μM) increased the intracellular calcium in 54% of labeled vagal sensory neuron but 2% of splanchnic neurons. Receptor antagonists for 5-HT2 and 5-HT3 blocked the serotonin response of vagal hepatic neurons by 90% and 40%, respectively. However, the receptor antagonists for 5-HT1 or 5-HT4 did not affect at all. In single rt-PCR we also found mRNA of 5-HT2 and 5-HT3 in over 50% of captured labeled neuron. We found that the sensory information for serotonin in liver could be transmitted exclusively via vagus nerve into medulla oblongata and that 5-HT2 and 5-HT3 receptors involved in serotonin response of hepatic vagal nerves. Vagal sensory information is one of major factors to elicit parasympathetic reflex (vago-vagal reflex) and spinal nerves information is to elicit sympathetic reflex. To discriminate the visceral sensory input to elicit parasympathetic or sympathetic reflex is important to understand the homeostasis in the body

Key Words: liver, vagus nerve, splanchnic nerve, serotonin

P07-51

Establishment of Visually Evoked Potentials (VEP) Recording in wild-type Mice Using the BioPAC System

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For the development of feasible retinal prosthesis, one important element is judging tool about patient's perception to electrical stimulus. If there is VEP like response to electrical stimulus in animal, it means that the stimulus effectively evokes response in visual pathway. Previously, in our lab, we have been focusing in-vitro experiment to optimize electrical stimulus parameters. In-vivo VEP recording on V1 cortex provides better information about animal's perception than in-vitro retinal recording. Therefore, in this study, we established VEP recording on V1 cortex using BioPAC system first, and now this system is used for electrically evoked potential (EEP) recording to harvest optimal electrical stimulus for animal. After anesthesia, wild-type (C57BL/6J strain) mice (n=5) were secured to stereotaxic apparatus (Harvard Apparatus, USA). For the recording of VEP, the stainless steel needle electrode (impedance: 2-5 kΩ) was positioned on the surface of the cortex through the burr hole at 2.5 mm lateral and 4.6 mm caudal to bregma (1). In the EEP recording, reference electrode was positioned on the MPtA (Medial Parietal Association Cortex) through the burr hole at 1 mm lateral and 2 mm caudal to bregma. To localize the electrical stimulus, a ring-shaped ground electrode was located above the right eye and coated steel needle electrode (impedance: 2-5 k Ω) was inserted in the right eye. DA 100C and EEG 100C BioPAC modules were used for the trigger signal recording and VEP recording, respectively. And STM 100C module and STMISOC were used for approving electrical stimulus. Cathodic phase 1st biphasic square pulse was applied with the range of 0.6 V~4 V. When left eye was blocked by black cover and right eye was stimulated by flash light (2) using HMsERG (RetVet Corp, USA), VEP response at left V1 cortex was detected, but no response at right V1 cortex. Amplitude and latency of N1 peak of VEP recording varied according to the depth of the electrode on V1 cortex. From the surface upto 600 um depth, N1 peak amplitude increased (Gain: 20,000, 142.9 μV, n=4), while deeper than 600 um, N1 peak amplitude decreased. The deeper the insertion depth of the electrode is, the latency of N1 peaks tends to be delayed. However, there was no

statistically significant difference among the latencies of N1 peaks. In EEP recording data, EEP N1 response (Gain: 10,000, 152.83 μV, n=1) was found from 1 V stimulus and varied according to the stimulus amplitude. We are analyzing EEP responses, now.
Key Words: VEP, EEP, retinal prosthesis, V1 cortex

P07-52

IKKβ Suppresses Adult Hippocampal Neurogenesis through NF-κB and Wnt/β-catenin Signaling

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Hippocampal neurons are continuously generated from radial glial stem cells which are lined in the subgranular zone (SGZ) of the dentate gyrus (DG) and represent GFAP-positive cells. Toll-like receptors (TLRs) are known to be involved in regulating adult hippocampal neurogenesis. However, the molecular mechanism of IKKβ, a TLRs down-stream molecule, in regulating adult hippocampal neurogenesis have been not elucidated. To investigate the role of IKKβ in the process, we used tamoxifen induced IKKβ conditional knock-down mice (GFAP-CreERT2/IKKβF/F mice) in which the IKKβ gene was specifically ablated in GFAP-positive neural stem cells. The knock-down of IKKβ had the enhanced proliferation of adult neural stem cells (NSCs) with increasing the number of BrdU positive cells in the hippocampal DG through activating cell cycle signaling. The knock-down of IKKβ was also augmenting the neural differentiation of NSCs with increasing DCX/BrdU-double positive cells and NeuN/BrdU-double positive cells through upregulation of β-catenin and NeuroD1. Moreover, the knock-down of IKKβ increased the survival of new born NSCs mediated by NF-κB, while did not affect the glial differentiation of adult NSCs. These results demonstrate that IKKβ inhibits adult neurogenesis through suppressing the proliferation of NSCs by regulating cell cycles and cell survival by NF-κB signaling and the neural differentiation of the NSCs by Wnt/β-catenin signaling.
Key Words: Neural Stem Cell, Hippocampus, IKKβ, Wnt/β-catenin, Neurogenesis

P07-53(PO-18)

Ginger and Its Pungent Constituents Non-Competitively Inhibit Serotonin Currents on Visceral Afferent Neurons

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Nausea and emesis are a major side effect and obstacle for chemotherapy in cancer patients. Employ of antiemetic drugs help to suppress chemotherapy-induced emesis in some patients but not all patients. Ginger, an herbal medicine, has been traditionally used to treat various kinds of diseases including gastrointestinal symptoms. Ginger is effective in alleviating nausea and emesis, particularly, for cytotoxic chemotherapy drug-induced emesis. Ginger-mediated antiemetic effect has been attributed to its pungent constituents-mediated inhibition of serotonin (5-HT) receptor activity but its cellular mechanism of action is still unclear. Emetogenic chemotherapy drugs increase 5-HT concentration and activate visceral vagal afferent nerve activity. Thus, 5-HT mediated vagal afferent activation is essential to provoke emesis during chemotherapy. In this experiment, water extract of ginger and its three major pungent constituent's effect on 5-HT-evoked responses were tested on acutely dispersed visceral afferent neurons with patch-clamp methods. The ginger extract has similar effects to antiemetic drug ondansetron by blocking 5-HT-evoked responses. Pungent constituents of the ginger, [6]-shogaol, [6]-gingerol, and zingerone inhibited 5-HT responses in a dose dependent manner. The order of inhibitory potency for these compounds were [6]-shogaol > [6]-gingerol > zingerone. Unlike well-known competitive 5-HT_3 receptor antagonist ondansetron, all tested ginger constituents acted as non-competitive antagonist. Our results imply that ginger and its pungent constituents exert antiemetic effects by blocking 5-HT-induced emetic signal transmission in vagal afferent neurons.
Key Words: 5-HT receptor, Antiemetic, Chemotherapy, Shogaol, Vagal afferent nerves

P08-01

The optimization of spinal cord contusion injury parameters on sensory and motor abnormalities

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Several SCI devices provide reliable bioinformatic data for SCI research in rodent animal models, however, the roles of SCI parameters on sensory and motor abnormalities is not clear. In this study, we compared the roles of SCI parameters on locomotion and below-level neuropathic pain in rat SCI models. SCI was produced by T10 contusion (1 sec dwell time, Infinite Horizon Impactor) injury in three ages with 200 (young), 300 (adult) and 500 (mature) g male SD rats. To compare the roles of SCI parameters, locomotion, and mechanical- and heat-induced withdrawal responses at hindlimbs were compared by Injury Force (146-150, 151-155 and 156-160 Kdyne), Displacement (701-900, 901-1,100 and 1,101-1,300 μm) and Velocity (116-120, 121-125 mm/s), respectively. In comparison by ages, 200 g group showed faster recovery of locomotion. All SCI age groups showed mechanical allodynia compared to before SCI, but 200 and 300 g group was greater than the 500 g group. In heat hyperalgesia, the 200 and

300 g group showed significant differences compared to before SCI ($p<0.05$). In comparison by Force, the 200 g group showed faster recovery of locomotion. All ranges of Force showed mechanical allodynia compared to before SCI, but 200 and 300 g groups showed greater changes than the 500 g group ($p<0.05$). In addition, 156-160 Force group in all aging groups showed significant differences to heat stimuli compared to before SCI. In comparison by Displacement, locomotion recovery did not show significant differences. All ranges of Displacement showed mechanical allodynia compared to before SCI, but the 200 and 300 g groups showed greater changes. In addition, 700-1100 Displacement group showed significant differences to heat stimuli compared to before SCI ($p<0.05$). In comparison by velocity, the 200 g group showed faster recovery of BBB. All ranges of Velocity showed mechanical allodynia compared to before SCI, but the 200 and 300 g group showed greater changes. In addition, the 200 and 300 g groups showed significant differences to heat stimuli compared to before SCI ($p<0.05$). The present study suggests that aging is the most important factor in post SCI sensory and motor abnormalities and mechanical allodynia is more a common outcome than heat hyperalgesia in below-level region following T10 SCI. This works was supported by the NIH 11255 and NS39161 (United States), and the National Research Foundation of Korea (NRF) grant (MSIP, No 2011-0030124) and NRF-2014R1A1A4A0 1004179 funded by the Korea goverment

Key Words: Spinal cord injury, neuropathic pain, locomotion, aging

P08-02

A combined computational model of beta-adrenergic signaling and action potential in rat ventricular cardiomyocyte

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Action potential(AP) and β-adrenergic signaling are important in the contraction mechanism of cardiomyocytes. In this study, we developed a computational model to simulate the AP, Calcium-induced Calcium release(CICR) and β-adrenergic signaling in rat ventricular cardiomyocyte. We used MATLAB (Mathworks, Natick, MA) coding for this model. Contraction mechanism of cardiomyocytes is initiated by β-adrenergic signaling. Sympathetic nerve stimulation causes norepinephrine to bind to β-adrenergic receptor (β-AR). When the β-AR interacts with the Gs alpha subunit of Gs-protein, it leads to the activation Adenylyl cyclase that converts ATP into cAMP. cAMP then activates protein kinase A(PKA) which then phosphorylates L-tpye Ca^{2+} channel(LCC), ryanodine receptor (RYR), and phospholamban(PLB). This results to the Ca^{2+} induction into the intracellular from the LCC, which finally leads to the release into the cytosol from SR. Action potential occurs when neurotransmitter binds to the voltage-gated Na+ channel receptor. It leads to intracellular induction of Na+. Voltage-gated Na+ channel is closed by ion concentration gradient, which then opens the voltage-gated K^+ channel. Lastly, Ca^{2+} is released into the intracellular by voltage-gated Ca^{2+} channel before finally returning to its resting membrane potential. The resulting combined model, referred to as the AP and β-adrenergic signaling model, successfully reconstructs range of experimental data and models, including Na+ current (INa), the Ca^{2+}-independent transient outward K^+ current (It), the sustained K^+ current (Iss), the Na-Ca^{2+} exchanger (INaCa), the L-type Ca^{2+} current (ICaL) in response to voltage-clamp stimuli from the sarcoplasmic reticulum(SR), voltage-dependence of excitation-contraction coupling gain, graded release, and role of PKA in β-adrenergic signaling. As a result, the model would simulate the process of β-adrenergic signaling pathway where it shows that β-adrenergic signaling activate PKA which in turn activates LCC by phosphorylating it. This phosphorylation leads to the intracellular induction of Ca^{2+}. When combined, the mathematical model of physiological parameters in rat ventricular cardiomyocyte can predict the physiological change in ventricular cardiomyocyte.

Key Words: Action potential(AP), β-adrenergic signaling, computational model

P08-03

Genomic Physiology of Breast Cancer Compared with Normal Breast Tissue

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A malignant tumor is a group of cells that exhibit several common traits, such as abnormal cell growth, evasion of apoptosis, induction of angiogenesis, and tissue invasion, named as cancer hallmarks. These traits have been mostly observed at the cellular level, but how these traits emerge has not been studied well at the genome level. Cancer genome research has been focused on the identification of genes that undergo in the change of genome, such as sequence variants, mutations, etc. However, we should be careful whether DNA mutation is the cause or the result of cancer. In a viewpoint of systems biology, cancer is a well-organized system expressing phenotypes that have emerged by networking genes in a different way of the host system. In this study, we investigated the genetic networks of breast cancer using gene expression datasets of breast invasive carcinoma and normal breast tissue in The Cancer Genome Atlas (TCGA) Data Portal and mouse breast cancer model in the NCBI GEO database (GSE3165). The genetic network of normal breast has more spreading branches for implementing phenotypes of breast tissue than the network of breast cancer. We identified that most systems involving in cell cycle regulation and DNA check & repair program in normal breast are disassembled in breast cancer, and even the rest are segregated from the main system of breast tissue. In addition, the systems of angiogenesis and cell matrix formation, integrated together as a

system in normal breast, are dissociated in breast cancer, which suggests that the tortuous shape of blood vessels in cancer is from the failure of communication between angiogenesis and cell matrix remodeling. We also found the buildup of immune systems in breast cancer, which are rarely developed in normal breast tissue. The breakdown of the cell cycle regulation and DNA check & repair systems was also identified in the mouse breast cancer models induced by c-Myc overexpression in lactating mammary gland luminal cells. However the immune systems we found from the human breast cancer are not developed, which suggests that the cancer induction cannot totally imitate the spontaneous development of cancer. These results show that the breakdown of DNA check & repair system and the reckless activation of cell cycle cannot prevent cells having damaged genome from continuing to divide, and increase the mutation rate as a result.

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Key Words: Genomic Physiology, Breast, Cancer, System, Cell Cycle

P09-01

Tumor Suppression by Klotho through Downregulation of IGF-1 Receptor in Clear Cell Renal Cell Carcinoma

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Klotho is a membrane protein predominantly produced in the kidney that exerts anti-aging and organ protection effects. Klotho functions as a tumor suppressor via regulating growth factor receptors including IGF-1 receptor. Aberrant activation of IGF-1 signaling has been implicated in the tumor progression of highly invasive metastatic clear cell renal cell carcinoma (ccRCC) which is the most common kidney malignancy. However, the link between Klotho and IGF-1 receptor in progression of ccRCC remains elusive. In the present study, Klotho expression is correlated with favorable prognostic factors of ccRCC, whereas IGF-1 receptor is highly expressed and is correlated with poor clinicopathological features of ccRCC patients. The overall survival rate of Klotho positive patients is much higher than that of Klotho negative patients. Functionally, Klotho suppresses IGF-1-induced cancer hallmarks including ccRCC cell migration. Phosphoinositide-3-kinase/Akt and MAPK pathways, downstream signaling cascades of IGF-1 receptor, are blunted by Klotho treatment. Moreover, Klotho reduces IGF-1-stimulated Ca^{2+} signaling which is a key mediator of tumor progression. Altogether, Klotho ameliorates tumor progression of ccRCC via inhibiting IGF-1 signaling. Thus, endogenous Klotho not only serves as a prognostic marker for ccRCC but also functions as a tumor suppressor with therapeutic potential.

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Key Words: Klotho, IGF-1 receptor, clear cell renal cell carcinoma, prognostic marker, Ca^{2+} signaling

P09-02

Angiotensin-(1-9)-AT2R Axis Protects Monocrotaline-induced Pulmonary Hypertension in rat

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Angiotensin-(1-9) [Ang-(1-9)], generated from angiotensin I by angiotensin converting enzyme II, has been reported to have protective effects on cardiac and vascular remodeling. However, there is no investigation about its effect on pulmonary vasculature under pulmonary hypertension [PH]. Thus, the aim of present study is to investigate whether Ang-(1-9) protects right ventricular hypertrophy and vascular remodeling in monocrotaline [MCT] -induced PH rat models. Sprague-Dawley rats received Ang-(1-9) (576ug/kg/day) or saline via osmotic minipumps. Three days after implantation of osmotic minipumps, 50mg/kg MCT or vehicle were subcutaneously injected. Three weeks after MCT injection, right ventricular [RV] hypertrophy and right ventricular systolic pressure [RVSP] were increased in MCT-treated rats. However, Ang-(1-9) attenuated RV hypertrophy and RVSP. Bronchoalveolar fluid of PH rat exhibited excessive pro-inflammatory cytokines, such as TNF-a, IL-6 and IL-1, which was attenuated by administration of Ang-(1-9). In addition, immunohistochemical analysis and western blot data revealed that Ang-(1-9) reduced medial hypertrophy of pulmonary arterioles and endothelial damage of MCT-injured vessels. All these effects were inhibited by angiotensin type 2 receptor blocker [AT2R] administration, not by Mas receptor blocker administration. In survival rates study Ang-(1-9) improved the survival rates from 33% to 83%. These results suggest that Ang-(1-9) prevents MCT-induced lung injury and right heart hypertrophy, which is mediated by AT2R.

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Key Words: Angiotensin-(1-9), Pulmonary hypertension, vascular remodeling, monocrotaline

P09-03

DJ-1 protein participates in regulation of the (pro)renin receptor expression via ROS-mediated epigenetic modification

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A multifunctional protein DJ-1 is involved in transcriptional regulation and scavenging oxidative stress. Thus, DJ-1 may be associated with the development of renal disorders. We investigated whether DJ-1 protein regulates expression of (pro)renin receptor (PRR), a member of renin-angiotensin system (RAS). The levels of mRNA and protein were analyzed by real-time PCR and western blot, respectively. H2O2 production was measured by using fluorescence probe. Histone modification was determined by chromatin immunoprecipitation. Expression of PRR was higher in kidney from DJ-1 knockout mice (DJKO) compared with wild-type mice (DJWT). Histone deacetylase 1 (HDAC-1) recruitment at the PRR promoter was lower, and histone H3 acetylation and RNA polymerase II recruitment were higher in DJ-1KO than in DJWT. Knockdown or inhibition of HDAC-1 enhanced PRR expression in mesangial cells from DJWT. H2O2 production was greater in DJKO cells compared with DJWT cells. These changes in PRR expression and epigenetic modification in DJKO cells were induced by treatment with H2O2 and reversed completely by addition of an antioxidant reagent. Prorenin- stimulated ERK1/2 phosphorylation was greater in DJKO cells than in DJWT controls and this in DJKO was inhibited by a PRR-inhibitory peptide, but not by AT1 and AT2 receptor inhibitors. Expression of renal fibrotic genes was higher in DJKO than in DJWT. These results suggest that DJ-1 protein may regulate expression of renal PRR through H2O2-mediated epigenetic modification. Therefore, renal DJ-1 protein may be an important molecule in acceleration of renal pathogenesis through PRR regulation.

Key Words: DJ-1, (pro)renin receptor, epigenetic modification, ERK1/2, Oxidative stress

P09-04

Unaltered Airway Smooth Muscle Sensitivity to Methacholine in Precision-Cut Lung Slices (PCLS) from Ovalbumin-induced Asthmatic Mice

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Asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness (AHR) and reversible airway obstruction. Methacholine (MCh) is widely used in broncho-provocation test to evaluate airway resistance. For experimental investigation, ovalbumin-induced sensitization is frequently used in rodents (Ova-asthma). However, albeit the inflammatory histology and AHR in vivo, it remains unclear whether the MCh sensitivity of airway smooth muscle isolated from Ova-asthma is persistently changed. The contractions of airways in precision-cut lung slices (PCLS) from control, Ova-asthma, and IL-13 overexpressed transgenic mice (IL-13TG) were compared by analyzing the airway lumen space (AW). The airway resistance in vivo was measured using plethysmograph. AHR and increased inflammatory cells in BAL fluid were confirmed in Ova-asthma and IL-13TG mice. In the PCLS from all three groups, MCh concentration-dependent narrowing of airway lumen (ΔAW) was observed. In contrast to the AHR in vivo, the EC50 of MCh for ΔAW from Ova-asthma and IL-13TG appeared higher than control, indicating decreased sensitivity while statistically insignificant. Although the AW recovery upon MCh-washout showed sluggish tendency in Ova-asthma, the change was statistically insignificant. Membrane depolarization-induced ΔAW by 60 mM K^+ (60K-contraction) was larger in IL-13TG than control, whereas 60K-contraction of Ova-asthma was unaffected. Furthermore, serotonin-induced ΔAW of Ova-asthma was smaller than control and IL-13TG. The AHR in Ova-asthma and IL-13TG are not reflected in the contractility of isolated airways measured in PLCS. The AHR of the disease model animals seems to require intrinsic agonists or inflammatory microenvironment that is washable during tissue preparation.

Key Words: Asthma, Airway smooth muscle, Methacholine

P10-01

Involvement of Heme Oxygenase-1 Induction in the inhibition of vascular inflammation by Xanthoceras sorbifolia in Human Umbilical Vein Endothelial Cells

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Xanthoceras sorbifolia Bunge, which belongs to the Sapindaceae family, is a shrub in Inner Mongolia and China. It has been used as a folk medicine for various diseases including hypertension and enuresis. However, the vascular protective effects of this compound are not fully understood. In the present study, ethanol extract of seed from the Xanthoceras sorbifolia (EXS) Bunge treatment was found to show potent inhibitory effect on vascular inflammation process by TNF-α in human umbilical vein en-

dothelial cells (HUVEC). EXS significantly decreased TNF-α-induced expression of cell adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial cell selectin (E-selectin) in a dose-dependent manner. Pre-treatment of EXS significantly inhibited the translocation and transcriptional activity of NF-κB increased by TNF-α. In addition, EXS also significantly inhibited the formation of intracellular reactive oxygen species (ROS). Furthermore, we found that the vascular protective effects of EXS were linked to the up-regulation of heme oxygenase-1(HO-1) and nuclear factor E2-related factor-2 (Nrf2) expressions in HUVEC. The inhibitory effects of EXS on the TNF-α-induced vascular inflammation were partially reversed by an inhibitor of HO-1, tin protoporphyrin IX (SnPP), while increased by an inducer of HO-1, cobalt protoporphyrin IX (CoPP). In conclusion, these results suggested that EXS ameliorated TNF-α-induced vascular inflammation, possibly through the disturbing ROS/NF-κB pathway and activation of Nrf-2/HO-1. Hence, EXS plays a key role in the vascular inflammation and further prevents the development of atherosclerosis.

Key Words: Xanthoceras sorbifolia Bunge (EXS); Vascular inflammation; Cell adhesion molecules; HO-1.

P10-02

Galgeundanggwitang improves diabetic vascular complication in apolipoprotein E knockout mice fed a Western diet

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Galgeundanggwitang (GGDGT), an herbal medicine, is used to treat hypertension, stroke, and other inflammatory disorders in the clinical setting. Recently, GGDGT was recognized by the Korea Institute of Oriental Medicine. This study aimed to evaluate the effects of GGDGT in a diabetic atherosclerosis model using apolipoprotein E knockout (ApoE-/-) mice fed a Western diet. The mice were divided into four groups: control group, C57BL6J mice receiving a regular diet (RD); ApoE-/- group, ApoE-/- mice receiving a Western diet (WD); rosiglitazone group, ApoE-/- mice receiving rosiglitazone (WD+10 mg/kg/day); GGDGT group, ApoE-/- mice receiving GGDGT (WD+200 mg/kg/day). Treatment with GGDGT significantly improved glucose tolerance and plasma lipid levels. In addition, GGDGT ameliorated acetylcholine-induced vascular relaxation of the aortic rings. Immunohistochemical staining showed that GGDGT suppressed intercellular adhesion molecule (ICAM)-1 expression; however, expression of endothelial nitric oxide synthase (eNOS) and insulin receptor substrate (IRS)-1 were restored in the thoracic aorta and skeletal muscle, respectively. These findings suggest that GGDGT attenuates endothelial dysfunction via improvement of the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signalling pathway and improves insulin sensitivity in diabetic atherosclerosis.

Key Words: Galgeundanggwitang, Diabetes, Atherosclerosis, Insulin resistance, eNOS

P10-03

Combination treatment with 2-methoxyestradiol overcomes bortezomib resistance of multiple myeloma cells

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Bortezomib is a proteasome inhibitor used for the treatment of relapsed/refractory multiple myeloma (MM). However, intrinsic and acquired resistance to bortezomib has already been observed in MM patients. In a previous report, we demonstrated that changes in the expression of mitochondrial genes lead to changes in mitochondrial activity and bortezomib susceptibility or resistance, and their combined effects contribute to the differential sensitivity or resistance of MM cells to bortezomib. Here we report that the combination treatment of bortezomib and 2-methoxyestradiol (2ME), a natural estrogen metabolite, induces mitochondria-mediated apoptotic cell death of bortezomib-resistant MM KMS20 cells via mitochondrial reactive oxygen species (ROS) overproduction. Bortezomib plus 2ME treatment induces a higher level of cell death compared with treatment with bortezomib alone and increases mitochondrial ROS and $Ca^{(2+)}$ levels in KMS20 cells. Pretreatment with the antioxidant N-acetyl-L-cysteine scavenges mitochondrial ROS and decreases cell death after treatment with bortezomib plus 2ME in KMS20 cells. Moreover, we observed that treatment with bortezomib plus 2ME maintains the activation of c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase kinase kinase 4/7 (MKK4/7). Collectively, combination treatment with bortezomib and 2ME induces cell death via JNK-MKK4/7 activation by overproduction of mitochondrial ROS. Therefore, combination therapy with specific mitochondrial-targeting drugs may prove useful to the development of novel strategies for the treatment of bortezomib-resistant MM patients.

Key Words: bortezomib, combination therapy, multiple myeloma, 2-methoxyestradiol, resistance, superoxide dismutase 2

P10-04

Finite Element Modeling of Pulsatile Blood Flow in a Simplified Human Aorta Model: A Study on the Prediction of the Changes in Biomechanical Properties of the Aorta

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To understand the biomechanical properties of the artery can provide important insight into arterial biology under physiological and pathological conditions. Vascular diseases such as atherosclerosis or aneurysm are closely associated with vascular geometry and flow condition in the blood. In this study, the effects of aortic torsion, curvature, and taper on blood flow and wall stress were simulated numerically in which the physiological flow through the aorta was solved using commercial software COMSOL Multiphysics 4.4. Applying incompressible Navier-Stokes equation, the blood was assumed to follow a fully developed Newtonian laminar flow, and pressure boundary condition was regulated in the inlet and outlet portions of the aorta. Aortic wall was assumed to be a rigid solid, non-permeable, and non-slip; with a blood density value (ρ) of 1060 kg/m3 and dynamic viscosity value (μ) of 0.005 Pa.s. The results showed that the flow velocity was higher in the ascending and abdominal portions of the aorta. At systolic peak, the maximum velocity is 0.62 m/s on the outer part of the abdominal aorta (greater curvature of the aorta). Pressure distribution through aortic wall was higher in the curvature of the aorta. Average pressure values in the aorta range from 9 kPa to 12 kPa. Using the simplified aorta model, the degree of distortion of the aorta due to blood flow was assessed. Upon blood entry into the aorta, the distortion of the aorta was observed, with the highest wall shear stress occurring at the curvature. Moreover, the average values of von Mises stress in the aorta range from 20 kPa to 26 kPa during one cardiac cycle. The tapering of the aorta was another important feature of the aorta that influences the type of blood flow. Our study demonstrates that the computational model of the aorta can be used to predict the blood pressure dependent aortic wall stress alteration. The model will be helpful to understand biomechanical properties of the aorta.

Key Words: Computational model, Vascular diseases, Von Mises stress, Biomechanical properties

P10-05

Valproic acid-induced autophagy protects apoptosis in lung cancer cells

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Valproic acid (VPA) is a short-chain fatty acid and has been identified as a potent histone deacetylase inhibitor. In the present study, we evaluated the effect of VPA on lung cancer cells (A549, SK-LU-1, NCI-H460 and NCI-H1299) and normal lung (human pulmonary fibroblast) cells in relation to apoptosis and autophagy. VPA inhibited the growth of lung cancer cells, and induced apoptotic cell death in these cancer cells. However, SK-LU-1 cells were strongly resistant to VPA. This agent also did not significantly inhibit the growth of normal lung cells. VPA-induced apoptosis was accompanied by the loss of mitochondrial membrane potential (MMP), PARP-1 cleavage and caspase-3 activation in lung cancer cells. In contrast, SK-LU-1 and normal lung cells did not show the activation of caspase-3. While the level of LC3 B was increased in lung cancer cells by VPA, this level did not alter by this agent in SK-LU-1 and normal lung cells. Interestingly, SK-LU-1 and normal lung cells already showed the activation of autophagy in VPA-untreated control cells. In addition, hydroxychloroquine (an inhibitor of autophagy) enhanced cell death in VPA-treated A549 cells. In conclusion, VPA induced apoptosis and autophagy in lung cancer cells. The activation of autophagy might be a mechanism for defense cell death.

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Key Words: Valproic acid, Apoptosis, Autophagy, Lung cancer

P10-06

Oxidative stress induced by auranofin triggers cell death in human mesothelioma cells

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Mesothelioma is an aggressive tumor that has been associated with asbestos exposure. Auranofin as an inhibitor of thioredoxin reductase (TrxR) affect many biological processes such as inflammation and proliferation. In this study, we investigated the effects of auranofin on patient-derived mesothelioma cells (ADA, CON, Hmeso, Mill, Phi, REN and ROB) in relation to cell growth, cell death and oxidative stress. The level of TrxR1 was increased in mesothelial cells (HM69 and HM 72) and some mesothelioma cells (Mill, Phi, REN and ROB). ADA, CON and Hmeso cells showed low expression level of TrxR1. Auranofin inhibited the growth of mesothelioma cells. Among mesothelioma cells, ADA and CON cells were sensitive to auranofin. This agent also induced apoptosis as well as necrosis in mesothelioma cells. In relation to reactive oxygen species (ROS) level, auranofin increased ROS level including O2・-. It also decreased the expression levels

and activities of cellular antioxidants such as TrxR and superoxide dismutase. In conclusion, auranofin inhibited the growth of human mesothelioma cells via apoptosis and necrosis, and the inhibition was closely related to oxidative stress.

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Key Words: Auranofin, Mesothelioma, Thioredoxin Reductase, Oxidative stress

P10-07

Transduction of PEP-1-heme oxygenase-1 protects insulin-producing INS-1 cells from cytokine-induced cytotoxicity

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Pro-inflammatory cytokine, such as IL-1β, IFN-γ and TNF-α plays an important role in the destruction of pancreatic β cells, thereby triggering the development of type 1 diabetes mellitus. In the present study, we examined whether this cytokine-induced pancreatic β cell damage is effectively ameliorated by the PEP-1, a protein transduction domain that is able to deliver exogenous molecules to living cells, mediated transduction of heme oxygenase-1 (HO-1), which is one of the cytoprotective enzymes in response to a variety of harmful stimuli including cellular oxidative stress. Recombinant PEP-1-HO-1 fusion protein was successfully delivered into insulin-producing INS-1 cells in time- and dose-dependent manners and was maintained in the cells for at least 48 h. The cytokine-induced cell death, reactive oxygen species production, NO production and lipid peroxidation of INS-1 cells were significantly reduced in the cells pretreated with PEP-1-HO-1 for 1 h. This protective effect of PEP-1-HO-1 against cytokine toxicity partly correlated with the inhibition of an inflammatory signaling pathway (decreased activation of the transcriptional factor p65 and reduced expression of inducible NO synthase and COX-2). Moreover, the cytokine-induced activation of caspase-3 and PRAP were significantly suppressed by pretreatment of PEP-1-HO-1. These results suggest that the transduction of PEP-1-HO-1 may provide a new strategy to protect the cytokine-induced pancreatic β cell destruction in Type 1 diabetes by relieving inflammation and oxidative stress.

Key Words: Type 1 Diabetes, β cell destruction, cytokine, Transduction, PEP-1-HO-1

P10-08(PO-13)

Passive heating improved lipolysis and regulation of fibroblast growth factor-21

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There is relativley little information on serum biomarkers of heat stress. Therefore, the goal of this study was to verify the effect of passive heating on the expression of fibroblast growth factor-21 (FGF21) and free fatty acids (FFAs). Four passive heating protocols based on intensity (39°C vs. 43°C, leg immersion, 30 min) and type (leg vs. half immersion, 42°C, 30 min) were used. Each protocol was applied on a 2 day cycle to 12 healthy adult males (age, 22.4±2.9 years; height, 174.1±4.6 cm; weight, 71.3±5.6 kg; body mass index, 23.1±3.0). The subjects were categorized into two groups according to the study design (randomized, with a parallel trial). Body temperature, FGF21 and FFAs were determined prior to passive heating, and immediately and 60 min after passive heating. Body temperature was significant higher (43°C) than the 39°C measured under identical passive heating type (leg immersion). Passive heating was effective for the expression of FGF21 and for lipolysis. The quantitative levels of FGF21 and FFA increased with increasing temperature (39°C < 42°C < 43°C). A significant difference in the quantitative levels of FGF21 and FFAs was also evident based on the type of passive heating (leg < half immersion) even when passive heating was applied at the same temperature (42°C). In conclusion, passive heating was effective for expressing FGF21 and for lipolysis. Therefore, passive heating may be expected to help in the degradation of body fat. Additionally, when the identical type (leg immersion) of passive heating is applied, a loading temperature of 43°C is more effective for expressing FGF21 and for lipolysis than 39°C and 42°C, and half immersion is more effective than leg immersion at 42°C.

Key Words: FGF21, Lipolysis, Passive heat loading, Leg immersion, Half immersion

P10-09

Gross Morphological Features of the Organ Surface Primo-Vascular System Revealed by Hemacolor Staining

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The primo-vascular system (PVS) which, consist of primo-vessels (PVs) and primo-nodes (PNs), is a novel anatomical network and new circulatory system. In the 1960s, Bong-Han Kim claimed that the PVS represented the meridians of acupuncture. Various observational methods have been used to clarify its anatomical properties. In this study, we tested Hemacolor reagents, a rapid staining system used in hematology and clinical cytology to determine whether the staining can be suitable for PVS studies. Hemacolor staining, a system of three dyes solutions, was performed for the rapid cellular identification of PVS cell's WBCs and RBCs. The overall staining procedure of the PVS is as follows: either a PVS slice (200 μn) or the whole PVS tissue was transferred into a drop of Hank's balanced salt solution (HBSS; Sigma, St. Louis, MO, USA) on slide glass and air-dried completely without water for 1-3 min. In this study, using Hemacolor staining, we confirmed the channel structures composed of a few sinuses (20-50 μm) within PNs and PVs, and several lines of ductules (3-5 μm) filled with single cells or granules ($\sim$1 μm) in PVs. In a PN slice, there was a honeycomb-like structure containing granules with pentagonal lumens ($\sim$10 μm). At the cellular level, the PVS was densely filled with WBCs (90.3%), RBCs (5.9%), and putative MCs (3.8%). Granules were also found within the putative MCs at various degranulation stages, and some granules showed spontaneous vibrating movements. The results of the present study indicate that Hemacolor is a promising staining system for the rapid identification and characterization of PVS cells and structures. This study shows that Hemacolor staining is useful in identifying as well as in characterizing cellular and structural properties of the PVS by confirming typical morphological features of PVs and PNs with a simple light microscope in a short time period. Our results provide two pieces of morphological evidence supporting the circulatory nature of the PVS and its roles in relation to immune functioning. (1) There are two major channel structures in the PVS: sinuses and ductules. (2) The PVS is unique in its large population of immune cells, including a cellular composition of 90.3% WBCs, 5.9% RBCs, and 3.8% putative MCs. Of note, the MC population is high, and RBCs are present in PNs. These findings and the experimental approaches used in this study may help to elucidate the structure and function of the PVS in normal and disease states in future studies.

Key Words: Primo-vascular system, Primo-node, Primo-vessel, Hemacolor staining

P10-10

Comparative proteomic profile after human bone marrow-derived mesenchymal stem cell transplantation in ischemic rat and OGD- induced SH-SY5Y cell

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Many investigations have reported that bone marrow-derived mesenchymal stem cells (BMMSCs) can facilitate brain repair after cerebral ischemia. The present study involved a proteomic analysis of brain tissue samples from rats that received transplanted human BMMSCs (hBM-MSCs) after transient middle cerebral artery occlusion (MCAo). A proteomic analysis of the brain was performed using quantitative 2-dimensional (2D) gel electrophoresis followed by mass spectrometry in the following three animal groups: sham-operated rats, MCAo rats, and hBM-MSC-treated MCAo (MCAo+hBM-MSCs) rats. Significantly altered 2D gel spots ($p < 0.05$) were identified from normalized intensity patterns on the imaged gels, where approximately 300 proteins showed changes in expression. Among them, 30 proteins were excised and further analyzed by peptide mass fingerprinting. The levels of some proteins in brain tissues from the MCAo and MCAo+hBM-MSCs groups were significantly different from those in the control group. In comparison to the MCAo group, the MCAo+hBM-MSCs group had 10 up-regulated proteins, including vacuolar ATP synthase subunit B2, annexin A3 (ANXA3), and glucose-regulated protein precursor 78. In the same group, there were 20 down-regulated proteins, including calretinin, pyridoxal phosphate phosphatase, and synaptosomal-associated protein 25 (SNAP-25). Among the up- and downregulated proteins, ANXA3 and SNAP-25 had their expression confirmed by Western blotting in brain tissues and real-time PCR using an oxygen-glucose deprivation system. In conclusion, we observed that multiple proteins were over- or under-expressed in hBM-MSCtreated rats after cerebral ischemia. Further investigation of the identified proteins will prove useful for the development of stem cell therapies for the treatment of stroke

Key Words: Annexin A3, Bone marrow-derived mesenchymal stem cells, Ischemia, Synaptosomal-associated protein 25, Two-dimensional electrophoresis

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- *Monograph*

2. **Eyre HJ, Lange DP, Morris LB.** Informed decisions: the complete book of cancer diagnosis, treatment, and recovery. 2nd ed. Atlanta: American Cancer Society; c2002 May. 768 p.

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3. **Armstrong EJ, Armstrong AW, Rocco TP.** Integrative cardiovascular pharmacology: Hypertension, ischemic heart disease, and heart failure. In: Golan DE, Tashjian AH, Armstrong EJ, Armstrong AW, editors. Principles of pharmacology: The pathophysiological basis of drug therapy. 3rd ed. Baltimore: Lippincott Williams & Wilkins; 2012. p.437-463.

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