

**International Symposium on Ghrelin and
Energy Metabolism Homeostasis**

April 22-23, 2017

Miyakomesse, Kyoto, Japan

Special Exhibition Hall A (B1F)

◆ Welcome Message ◆



Dear Colleagues,

It is our great honor and pleasure to invite you to “International Symposium on Ghrelin and Energy Metabolism Homeostasis”, the satellite symposium of the 90th Annual Meeting of The Japan Endocrine Society, which will be held in Kyoto, Japan from April 22 (Sat) to 23 (Sun), 2017.

We held “The 2009 International Symposium on Ghrelin” in Tokyo in 2009 to commemorate the 10th anniversary of the discovery of ghrelin. The symposium was a great chance to gather many researchers from many countries and contributed to develop ghrelin research. The number of published papers on ghrelin has been increasing to near 10 thousand in the two decades since the identification of the growth hormone secretagogue receptor in 1996. Furthermore, clinical application of ghrelin has already intended for various disorders.

In this symposium, we invite three foreign speakers, Prof. Inge Depoortere (University of Leuven) from Belgium, Prof. Suzanne Dickson (University of Gothenburg) from Sweden, and Prof. Zane Andrews (Monash University) from Australia.

We hope this symposium will provide a great opportunity for researchers from home and abroad to present their findings. This symposium will be a wonderful occasion for ghrelin researchers to discuss the latest findings and the future of ghrelin.

Sincerely,

Kenji Kangawa

International Symposium on Ghrelin and Energy Metabolism Homeostasis

April 22 (Sat), 2017

Opening Remarks

17:00

Kenji Kangawa (National Cerebral and Cardiovascular Center Research Institute)

Keynote Lecture ①

17:05-17:45

Chair : Kazuwa Nakao (Kyoto University)

Inge Depoortere (University of Leuven, Belgium)

Chemosensory signaling mechanisms of the ghrelin cell

Oral Presentation ①

17:45-18:45

Chair : Masayasu Kojima (Kurume University)

1. Takio Kitazawa (Rakuno Gakuen University)

Chicken is a unique animal model for studying function of ghrelin in gastrointestinal motility

2. Shota Takemi (Saitama University)

The important role of ghrelin on gastric contraction in *Suncus murinus*

3. Hiroyuki Kaiya (National Cerebral and Cardiovascular Center Research Institute)

Current knowledge of ghrelin in amphibians

4. Tetsuya Kouno (Shionogi & Co., Ltd.)

The role of acylated-ghrelin in the regulation of sucrose intake

April 23 (Sun), 2017

Keynote Lecture ②

9:00-9:40

Chair : Hiroshi Itoh (Keio University)

Suzanne Dickson (University of Gothenburg, Sweden)

Ghrelin and Reward-Linked Behaviour

Oral Presentation ②

9:40-10:40

Chair : Mayumi Furuya (Asubio Pharma Co. Ltd.)

1. Hiroshi Iwakura (Wakayama Medical University)

The effects of inflammatory cytokines on the expression of ghrelin

2. Ichiro Sakata (Saitama University)

The study of ghrelin secretion and acyl-modification using mice and ghrelinoma cell lines

3. Hiroshi Hashiguchi (Kagoshima University)

Direct versus indirect actions of ghrelin on hypothalamic NPY neurons

4. Daisuke Aotani (Kyoto University)

Role of ghrelin in food intake regulation

Break

10:40-10:55

Symposium ① (Sponsored by ONO PHARMACEUTICAL CO., LTD.) 10:55-12:25

Chair : Masamitsu Nakazato (University of Miyazaki)

- 1. Nobuhiro Matsumoto (University of Miyazaki)**
Clinical Application of Ghrelin for chronic respiratory failure
- 2. Koichi Takayama (Kyoto Prefectural University of Medicine)**
Clinical Application of Ghrelin in the Field of Lung Cancer: Application for Treatment of Cancer Cachexia
- 3. Yuichiro Doki (Osaka University)**
Clinical Application of Ghrelin for upper Gastrointestinal Surgery

Break 12:25-12:45

Luncheon Seminar 12:45-13:45
(Sponsored by Mitsubishi Tanabe Pharma Corporation)

Chair : Kenji Kangawa (National Cerebral and Cardiovascular Center Research Institute)

- 1. Hiroshi Itoh (Keio University)**
- 2. Masamitsu Nakazato (University of Miyazaki)**

Break 13:45-14:00

Keynote Lecture ③ 14:00-14:40

Chair : Takashi Akamizu (Wakayama Medical University)

Zane Andrews (Monash University, Australia)
Genetic dissection of ghrelin receptor neural circuits; mood motivation and metabolism

Oral Presentation ③ (Sponsored by Daiichi-Sankyo Co., Ltd.) 14:40-15:45

Chair : Nobuhiro Matsumoto (University of Miyazaki)

- 1. Miki Nonaka (National Cancer Center Research Institute)**
Therapeutic potential of ghrelin and des-acyl ghrelin for the chemotherapy-induced cardiotoxicity
- 2. Hironobu Tsubouchi (University of Miyazaki)**
Protective effect of ghrelin against pulmonary cachexia associated with lung adenocarcinoma in mice
- 3. Masanori Tamaki (Keio University)**
Ghrelin treatment improves physical decline in 5/6 nephrectomized CKD model mice through muscular enhancement and mitochondrial activation
- 4. Hiroaki Ueno (University of Miyazaki)**
Clinical application of ghrelin for diabetic peripheral neuropathy
- 5. Hiroshi Hosoda (National Cerebral and Cardiovascular Center Research Institute)**
Evaluation and optimization of blood collection and storage conditions for ghrelin measurement

Closing Remarks 15:45

Masamitsu Nakazato (University of Miyazaki)

Abstracts

Keynote Lecture

Invited Lecture

Oral Presentation

Keynote Lecture ①



Chemosensory signaling mechanisms of the ghrelin cell

**April 22, 2017
17:05-17:45**

**Inge Depoortere
University of Leuven, Belgium**

The gastrointestinal tract represents the largest key interface between the human body and the external environment. It continuously needs to monitor and discriminate between nutrients that need to be assimilated and harmful substances that need to be expelled. The different cells of the gut epithelium are therefore equipped with a subtle chemosensory system that communicates the sensory information to several effector systems involved in the regulation of appetite, immune responses, gastrointestinal motility etc. Entero-endocrine cells “taste” the luminal content through taste receptors, similar to those on the tongue to control the release of gut hormones. During my talk, I will focus on how taste receptors on the ghrelin cell may influence the release of ghrelin in response to amino acid, sweet or bitter tastants. The effect in several experimental models including a ghrelinoma cell line and studies in mice will be discussed. The results will be translated to studies with human tissue during health and disease and should help to elucidate whether targeting extra oral taste receptors with functional foods or new drugs may offer new therapies for the treatment of diseases.

Inge Depoortere is professor at the University of Leuven in Belgium. After her master studies in biochemistry she obtained her PhD in sciences at the faculty of medicine in Leuven in 1991 and continued her postdoctoral studies in Leuven. Since 2008 she is head of the “Gut Peptide Research Lab” of the Translational Research Center for Gastrointestinal Disorders (Targid) at the KU Leuven. In the past she mainly studied the role of gastrointestinal hormones in the regulation of gastrointestinal motility under normal and pathological conditions. Currently, the main focus of her research is on the nutrient sensing mechanisms in the gut and on the role of clock genes in the circadian regulation of ghrelin. She has published more than 120 papers (h-index 35) in peer reviewed international journals and has given numerous invited and abstract presentations at international meetings. Inge Depoortere has been section editor of BMC Gastroenterology (Nutrition & Metabolism) and is associate editor of Neurogastroenterology & Motility, American Journal of Physiology and Peptides and is member of the steering committee of the “International Regulatory Peptide Society” and several other societies.

Keynote Lecture ②



Ghrelin and Reward-Linked Behaviour

April 23, 2017
9:00-9:40

Suzanne Dickson
University of Gothenburg, Sweden

In recent years, we have seen the emergence of a gut-brain reward axis, through which circulating appetite-regulating hormones from the gastrointestinal tract, such as ghrelin, interact with pathways that confer reward from natural and artificial rewards. In rodents, ghrelin directly activates the mesoaccumbal dopamine pathway at the level of the ventral tegmental area (VTA) causing dopamine release in the nucleus accumbens. Indeed, ghrelin delivery to the brain ventricles or to the VTA can induce reward-linked behaviours for food and for substances of abuse (eg alcohol). Both appetitive and consummatory behaviours are affected by ventricular or VTA delivery of ghrelin, including motivated behavior for sugar (lever-pressing), anticipatory locomotor activity for a sweet treat and food reward behavior (assessed in the condition place preference test). Recently we found that ghrelin impacts on food choice, unexpectedly promoting the intake of normal chow in (i) rats with free access to high fat and sucrose and (ii) rats trained to binge on high fat diet (in a scheduled feeding paradigm). *Supported by EC grants NeuroFAST, Full4Health, Nudge-it and by the Swedish Research Council.*

1. Higher education degree
1988 BSc Pharmacology (Honours),
University of Edinburgh
2. Doctoral degree
1993 PhD (cantab)
Neuroendocrinology, University of
Cambridge, Title: Neural control of
growth hormone secretion.
Supervisor: Prof Gareth Leng.
3. Postdoctoral positions
1993 Higher Scientific Officer, The
Babraham Institute, Cambridge
4. Docent (Senior Lecturer) level
N/A. (Appointed to Senior Lecturer
at Cambridge University in 2001).
5. Current position
2004-(Tenured) Professor of
Physiology/Neuroendocrinology.
Over 90% time devoted to research.
6. Honorary Professor in
Biomedical sciences at the
University of Edinburgh, UK
(2015-)
7. Previous positions
1996-2004 (tenured) University of
Cambridge. Lecturer then Senior
Lecturer in Physiology. (Age at
appointment – 30).
1996-2004 (tenured) Fellow of
Peterhouse, Cambridge.
1999-2001 Visiting Professor, Dept
Internal Medicine, Sahlgrenska
University Hospital.
1994-1996 (tenured) King's College
London, UK. Lecturer in Anatomy.
(Age at appointment – 28).

Keynote Lecture ③



Genetic dissection of ghrelin receptor neural circuits; mood motivation and metabolism

April 23, 2017
14:00-14:40

Zane Andrews
Monash University, Australia

Ghrelin is a metabolic signal that is increased in response to negative energy balance. Although initially described as a hormone promoting food intake and adiposity, studies have uncovered a number of novel physiological roles including the regulation of mood, motivation and memory. Rather than consider these physiological roles as discrete and unrelated, we are examining how ghrelin might target ghrelin receptors (GHSRs) in the brain during negative energy balance to influence the adaptation to energy deficit or hunger. We are using cre-dependent tracing technology to examine GHSR neural circuits in the hypothalamus, midbrain, amygdala and hippocampus. Initial studies suggest common integrating nodes in key brain regions such as the bed nucleus of the stria terminalis, the lateral hypothalamic area and the paraventricular thalamic nucleus. We are using chemogenetic approaches to examine the behavioural and physiological output of these ghrelin receptor neural circuits in the brain and suggest that coordinated inputs into multiple brain sites is required to effectively control behaviour. Our studies point towards ghrelin signaling in the brain as a metabolic signal of energy deficit that simultaneously regulates a number of brain regions to facilitate behavioural adaptations during energy deficit or hunger.

Associate Professor Zane Andrews is a National Health and Medical Research Council Research Fellow from the Biomedicine Discovery Institute at Monash University in Melbourne, Australia. He received his PhD in New Zealand at the University of Otago in 2003 in the field of neuroendocrinology and neuroscience. From there, he went on to do postdoctoral training at Yale University, New Haven, Connecticut, USA (2004-2008). His laboratory studies how food, and the lack of food, affects the brain and behaviour. He is primarily interested in understanding the neural circuits that sense hunger and influence brain function, including energy homeostasis, mental health and neurodegeneration. It is becoming increasingly clear that a state of hunger elicits numerous effects on the brain, not just those related to food intake. This research aims to understand the complex interplay between such connected circuits may underlie co-morbidities of mental illness with metabolic dysfunction in disease such as anorexia and bulimia nervosa, obesity, Prader-Willi syndrome, binge-eating and food addictions.

Associate Professor Zane Andrews focuses on how ghrelin, as a metabolic signal of energy deficit, conveys metabolic information back to the brain to regulate energy homeostasis, anxiety and neuroprotection. He has discovered that diet-induced obesity causes ghrelin resistance and has focused on the molecular mechanisms through which ghrelin controls neuronal function and discovered important roles for mitochondrial mechanisms.

Symposium ① – 1

Clinical Application of Ghrelin for chronic respiratory failure

Nobuhiro Matsumoto¹, Hironobu Tsubouchi¹,
Yoshifumi Imazu¹, Yasuji Arimura², Shigehisa
Yanagi¹, Masamitsu Nakazato¹

¹Neurology, Respirology, Endocrinology and Metabolism,
Internal Medicine, Faculty of Medicine, University of Miyazaki

²Division of Data Management, Department of Social
Medicine, Faculty of Medicine, University of Miyazaki

April 23, 2017

10:55- 11:25

Chronic respiratory failure, which is often caused by chronic obstructive pulmonary disease, chronic lower respiratory tract infection, or interstitial pneumonia, often leads to cachexia with disease progression. Patients of chronic respiratory failure with cachexia show increased morbidity. Although cachectic status is an important clinical problem, there are no effective therapy for cachexia.

Ghrelin has various effects, which are increasing food intake, attenuating sympathetic nerve activity, anti-inflammatory effects, increasing cardiac output, and controlling fat utilization. These various effects of ghrelin are ideal targets for the treatment of severely wasting chronic pulmonary disorder.

In a few clinical studies which includes a small randomized controlled trial, ghrelin administration to cachectic patients with chronic respiratory failure showed improvements of exercise tolerance, dyspnea, and appetite. The patients in the studies gained muscle mass and weight. In another study of chronic lower respiratory tract infection with cachexia, ghrelin demonstrated suppression of airway inflammation through decreasing neutrophil accumulation in sputum, and improvements of oxygenation and exercise tolerance.

Further clinical investigations are needed to clarify the usefulness of ghrelin for cachectic patients with chronic respiratory failure.

Symposium ①–2

Clinical Application of Ghrelin in the Field of Lung Cancer: Application for Treatment of Cancer Cachexia

**April 23, 2017
11:25-11:55**

Koichi Takayama

Department of Respiriology, Kyoto Prefectural University of Medicine

Cachexia is a frequent disorder in cancer patients and may also be accompanied by poor prognosis and poor quality of life (QOL). Despite worsening prognoses with the symptoms, clinical factors involved and the effect of body weight (BW) loss to the overall status remain unexplained. We have conducted a prospective cohort study, Japan Nutrition and QOL survey in patients with advanced non-small cell lung cancer (NSCLC) study (JNUQ-LC study) to investigate changes in BW loss in relation to hand grip strength (HGS), QOL, and clinical parameters to understand their effects on prognosis. This study demonstrated that BW loss in advanced NSCLC patients correlated with worsening of QOL and Karnofsky Performance Scale and a decrease in HGS. Furthermore, patients with BW loss reported more early deaths than those without.

There were some published studies testing the effect of ghrelin in cancer patients and ghrelin administration increased appetite with some benefit on QOL and meal appreciation, without affecting tumor growth. Currently, anamorelin, an orally administered low-molecular-weight ghrelin receptor agonist is developed for the treatment of NSCLC-related cachexia. Two completed phase 3 studies of anamorelin conducted in North America, Europe, and Australia. Ghrelin and ghrelin mimetics might be an option for the pharmacotherapies for multimodal treatment of cancer.

Symposium ①–3

Clinical Application of Ghrelin for upper Gastrointestinal Surgery

April 23, 2017
11:55-12:25

Yuichiro Doki
Dept. Gastroenterological Surgery, Osaka University

There are several evidences that ghrelin is not always indispensable for the maintenance of normal body weight and appetite. For example, chronic atrophic gastritis by H. Pylori infection results in extreme reduction of ghrelin producing cells, however normal daily life is not strongly impaired in the long term. Upper gastrointestinal surgery strongly affect the ghrelin secretion. After total gastrectomy more 90% of ghrelin disappear from the peripheral blood and ghrelin decline to almost half after distal gastrectomy and esophagectomy. After long term rehabilitation, patients can adjust themselves to the status of low or without ghrelin. However in the short term after surgery, patients suffer from various symptoms cause by the lack of ghrelin. Therefore we considered ghrelin supplementation in the early post-operative phase may useful to recover patients after major surgery.

We administrated synthetic ghrelin after total gastrectomy and esophagectomy with gastric tube reconstruction, when patients re-start oral food intake after surgery. Only 10 days ghrelin administration significantly prevented from body weight loss after surgery; -3 to -4% BW in control group versus -1% in the ghrelin group. In the body composition loss of muscle is effectively suppressed in the ghrelin group.

Our final purpose is reimbursement of ghrelin as a drug which stimulates recover after upper gastro-intestinal surgery. It is a great problem that body weight and appetite may not regarded to be worth for reimbursement by health insurance in Japan. We have started clinical trials to expect anti-inflammatory effect of ghrelin after esophagectomy. Anti-inflammatory reaction of ghrelin is different from appetite stimulation. Various approach could be considered for ghrelin clinical application in the field of surgery.

Oral Presentation ① – 1

Chicken is a unique animal model for studying function of ghrelin in gastrointestinal motility

Takio Kitazawa¹, Hiroki Teraoka¹, Hiroyuki Kaiya²

**April 22, 2017
17:45-18:00**

¹Sch. of Vet Med. Rakuno Gakuen Univ.

²Dept. of Biochem. Natl Cerebr Cardiovascul Ctr Res Inst.

Ghrelin has been identified in various vertebrates from fish to mammals. Structural similarity of ghrelin and its receptor with motilin and the motilin receptor prompt us to clarify functional roles of ghrelin in gastrointestinal (GI) motility. We used some non-mammalian vertebrates, and examined the effect of ghrelin on contractility of GI tract as well as the expression of ghrelin receptor mRNA to determine whether motor-stimulating action of ghrelin is common through vertebrates. Expression level of ghrelin receptor mRNA differed depending on the animal species and on GI regions. GI region-dependent expression of ghrelin receptor was remarkable in chickens, and the expression levels changed depending on age. In comparative study, ghrelin did not cause GI contraction in goldfish, rainbow trout, bullfrog and Japanese fire belly newts even using the homologous ghrelin, whereas ghrelin only contracted the chicken GI tract. The contraction of ghrelin changed with the age. Our results show that motor-stimulating action of ghrelin is not conserved across vertebrates, and that chicken is a unique animal species for evaluation of GI-stimulating action of ghrelin with different age.

Oral Presentation ①–2

The important role of ghrelin on gastric contraction in *Suncus murinus*

Shota Takemi¹, Ichiro Sakata¹, Kayuri Kuroda¹, Takafumi Sakai^{1,2}

¹Area of Regulatory Biology, Division of Life Science, Graduate School of Science and Engineering, Saitama University, ²Area of Life-NanoBio, Division of Strategy Research, Graduate School of Science and Engineering, Saitama University

April 22, 2017
18:00-18:15

Ghrelin, a peptide hormone produced in the stomach, has been known to be involved in the regulation of gastric contraction in humans and rodents. To elucidate the detailed mechanisms of ghrelin on gastric contractions, we used *Suncus murinus*, a recently established small animal model for gastrointestinal motility. *S. murinus* produces motilin, a family peptide of ghrelin, and its stomach anatomy and physiological patterns of gastric contractions, in fed and fasted states, are closely similar to humans. Ghrelin administration in phase II, and latter half of phase I, of the migrating motor complex (MMC) enhanced gastric motility in *S. murinus*. In addition, we showed that ghrelin and motilin coordinately stimulated strong gastric contractions *in vitro* and *in vivo*. We also demonstrated that a pretreatment with a ghrelin antagonist, D-lys3-GHRP6, inhibited the effects of motilin-induced gastric contractions, and a γ -aminobutyric acid (GABA) antagonist reversed this inhibition. Our results suggest that ghrelin is essential for motilin-induced gastric contractions and that ghrelin-mediated GABAergic neurons are involved in this neural pathway.

Oral Presentation ①–3

Current knowledge of ghrelin in amphibians

April 22, 2017
18:15-18:30

Hiroyuki Kaiya, Kenji Kangawa, Mikiya Miyazato
National Cerebral and Cardiovascular Center Research
Institute

We have identified cDNA encoding prepro-ghrelin and growth hormone secretagogue-receptor 1a (GHS-R1a, ghrelin receptor) in various amphibians. Amphibian ghrelin has a unique structure feature that the acylated amino acid is threonine (Thr-3) instead of serine (Ser-3) only in the Family, Ranidae. We have identified the ghrelin receptor from three species of amphibians, bullfrog (*Rana catesbeiana*) and Japanese tree frog (*Hyla japonica*) and Japanese fire belly newt (*Cynops pyrrhogaster*). GHS-R1a mRNA was expressed in the brain, gastrointestinal tract and gonad in frogs and newt. Interestingly, GHS-R1a mRNA expression in the pituitary was not detected in the two frogs but in the newt. In frogs, gastric GHS-R1a mRNA expression increased by fasting, but not in the brain. A dehydration treatment increased GHS-R1a mRNA expression in the brain, stomach and ventral skin in tree frog. Intracerebroventricular injection of ghrelin in dehydrated tree frog did not affect water absorption from the ventral skin. In functional analyses of the receptor, ghrelin and GHS-R1a agonists increased intracellular Ca^{2+} concentration in HEK293 cell that transfected each receptor cDNA, but ligand selectivity of ghrelin with Ser-3 and Thr-3 was not observed in the receptor of frogs, but in the newt. These results suggest that ghrelin is involved in energy homeostasis and possibly in osmoregulation in amphibians.

Oral Presentation ①-4

The role of acylated-ghrelin in the regulation of sucrose intake

Tetsuya Kouno, Nobuteru Akiyama, Takahito Ito, Kumiko Fujieda, Isamu Nanchi, Tomohiko Okuda, Mitsuru Notoya, Takanori Iwasaki, Hideo Yukioka

Shionogi Pharmaceutical Research Center, Shionogi & Co., Ltd.

**April 22, 2017
18:30-18:45**

The octanoyl modification of ghrelin by ghrelin O-acyltransferase (GOAT) is essential for exerting its physiologic actions. Since exogenous acylated-ghrelin has shown to stimulate food intake in humans and rodents, GOAT has been regarded as a promising target for modulating appetite, thereby treating obesity and diabetes. However, GOAT-knockout (KO) mice have been reported to show no meaningful body weight reduction, when fed a high-fat diet. Here, in this study, we sought to determine whether GOAT has a role in the regulation of body weight and glucose metabolism when fed a dietary sucrose. We first found that peripherally administered acylated-ghrelin stimulated sucrose consumption, and GOAT KO mice consumed less sucrose solution compared to WT littermates. Then, we examined the effect of dietary composition of sucrose on food intake and body weight in GOAT KO and WT mice. As a result, when fed a high-fat diet, food intake and body weight were comparable between GOAT KO and WT mice. However, when fed a high-fat/high-sucrose diet, GOAT KO mice showed significantly reduced food intake and marked resistance to obesity, resulting in amelioration of glucose metabolism. These results suggest that blockade of acylated-ghrelin production offers therapeutic potential for obesity and metabolic disorders caused by overeating of palatable food.

Oral Presentation ② – 1

The effects of inflammatory cytokines on the expression of ghrelin

April 23, 2017
9:40-9:55

Hiroshi Iwakura, Mika Bando, Yoko Ueda,
Takashi Akamizu
The First Department of Medicine, Wakayama Medical
University

Previously, we established a ghrelin-producing cell line MGN3-1, originated from a gastric ghrelinoma developed in ghrelin-promoter SV40 Tag transgenic mice, in order to explore the mechanism of ghrelin production and secretion in molecular basis. We then revealed that catechol amines, oxytocin, prostaglandin E2, and tryptophan stimulated ghrelin secretion, while lactate, palmitate, somatostatin suppressed it from the cells.

In the current study, we examined the effects of LPS and inflammatory cytokines including IL-1 β , TNF- α , and IL-6 on the expression of ghrelin in MGN3-1 cells. We found that IL-1 β , and TNF- α with lesser extent, significantly suppressed ghrelin mRNA expression in the cells. MGN3-1 cells expressed IL-1 β receptor and IL-1 β significantly stimulated NF- κ B, p38, JNK, and ERK pathways. Knockdown of IKK2 by siRNA significantly attenuated the suppression of ghrelin mRNA by IL-1 β .

These results indicate that IL-1 β directly suppressed ghrelin mRNA via NF- κ B pathway at least partially, which may have a role in the regulation of appetite during inflammation.

Oral Presentation ②-2

The study of ghrelin secretion and acyl-modification using mice and ghrelinoma cell lines

Ichiro Sakata¹, Zhi Gong¹, Chika Ikenoya¹, Shota Takemi¹, Takafumi Sakai^{1,2}

April 23, 2017

9:55-10:10

¹Area of Regulatory Biology, Division of Life Science, Graduate School of Science and Engineering, Saitama University, ²Area of Life-NanoBio, Division of Strategy Research, Graduate School of Science and Engineering, Saitama University

Ghrelin is a peptide hormone with a unique structure comprising a medium chain fatty acid modification. Ghrelin cells are known to be abundantly localized in the gastric mucosa and are released into the blood stream to exert their multifunctional physiological effects. To elucidate the regulatory mechanisms of ghrelin secretion and acyl modification, we developed novel ghrelin-producing cell lines. Using ghrelinoma cell lines, we focused on the mechanisms of ghrelin secretion and found that several GPCRs were highly expressed in ghrelin cells. Then, we showed that noradrenaline treatment stimulated ghrelin secretion via β_1 -adrenergic receptor, and fasting-induced ghrelin elevation was completely inhibited by the β_1 -adrenergic receptor antagonist in mice. In addition, we demonstrated that long chain fatty acids, glucose, and L-glutamate significantly inhibited ghrelin secretion. Furthermore, we recently revealed that the genes involved in fatty acid synthesis and long chain fatty acid metabolism were expressed in ghrelin cells, and that CPT-1 inhibitor treatment dramatically decreased the levels of acyl-modified ghrelin. Here, we introduce the current knowledge of the mechanisms involving ghrelin secretion and its acyl modification.

Oral Presentation ②–3

Direct versus indirect actions of ghrelin on hypothalamic NPY neurons

Hiroshi Hashiguchi¹, Zhenyu Sheng², Vanessa Routh², Volodymyr Gerzanich³, J. Marc Simard³, Yoshihiko Nishio¹, Joseph Bryan⁴

¹Department of Diabetes and Endocrinology, Kagoshima University Graduate School of Medicine and Dental Science Kagoshima, ²Department of Pharmacology, Physiology and Neuroscience Rutgers, The State University of New Jersey, ³Departments of Neurosurgery, Pathology, and Physiology, University of Maryland School of Medicine, ⁴Pacific Northwest Diabetes Research Institute

April 23, 2017
10:10-10:25

Objective: Assess direct versus indirect action of ghrelin on hypothalamic NPY neurons.

Methods: Electrophysiology was used to measure ion channel activity in NPY-GFP neurons in slice preparations. Ca²⁺ imaging was used to monitor ghrelin activation of isolated NPY GFP neurons. Immunohistochemistry was used to localize Trpm4, SUR1 and Kir6.2 in NPY-GFP neurons in slice.

Results: Depolarization by acetylated ghrelin under Tetrodotoxin application of neurons in slice was accompanied by a decreased input resistance (IR) in ~70% of neurons(15/22) or increased IR in the remainder (7/22), consistent with direct opening or closing of ion channels, respectively. Ca²⁺ imaging studies demonstrated reducing [Na⁺]_o suppressed activation suggesting a role for non-selective cation channels. SUR1 and two channel partners, Kir6.2 and Trpm4, were identified immunologically in neurons *in situ* and the actions of SUR1 and Trpm4 modulators proved informative. SUR1 agonist elevated [Ca²⁺]_c and SUR1 antagonist suppressed ghrelin action. Selective Trpm4 antagonists blocked ghrelin action on isolated neurons.

Conclusions: Ghrelin can directly depolarize neurons either by activating a depolarizing conductance or inhibiting a hyperpolarizing conductance. Results with isolated NPY-GFP neurons suggest ghrelin-activated Trpm4/SUR1 non-selective cation channels.

Oral Presentation ②-4

Role of ghrelin in food intake regulation

April 23, 2017
10:25-10:40

Daisuke Aotani¹, Hiroyuki Ariyasu², Kazuwa Nakao¹

¹Kyoto University, Medical Innovation Center, ²The first department of Medicine, Wakayama Medical University

To elucidate the role of ghrelin in the regulation of food intake, we have been trying to generate variable models of transgenic (Tg) mice overexpressing ghrelin using different promoters. Almost all of these animals produced only des-acyl ghrelin rather than ghrelin. We then tried to generate Tg mice expressing a ghrelin analogue, which possessed ghrelin activity (Trp3-ghrelin Tg mice). Although Trp3-ghrelin Tg mice exhibited approximately 6-fold greater ghrelin activity than wild type mice, they did not show any phenotype in growth and food intake. After the identification of ghrelin O-acyltransferase (GOAT) in 2008, we generated doubly Tg mice overexpressing both human GOAT and des-acyl ghrelin in the liver by cross-mating the two kinds of Tg mice. The plasma ghrelin concentration of the ghrelin Tg mice was approximately 1.9-fold higher than that of wild type mice under normal dietary conditions; however, the ghrelin Tg mice showed approximately 11.3-fold increase in the plasma ghrelin concentration compared with that of wild type mice when they were fed on 40% of medium-chain triglycerides diet. The ghrelin Tg mice showed no apparent phenotypic changes in body weight, length and food intake. Further studies are intensively ongoing in our laboratory.

Oral Presentation ③ – 1

Therapeutic potential of ghrelin and des-acyl ghrelin for the chemotherapy-induced cardiotoxicity

Miki Nonaka¹, Nagomi Kurebayashi², Takashi Murayama², Masami Sugihara³, Seiji Shiraishi¹, Kanako Miyano¹, Hiroshi Hosoda⁴, Shosei Kishida⁵, Kenji Kangawa⁶, Takashi Sakurai², Yasuhito Uezono^{1,7}

¹Division of Cancer Pathophysiology, National Cancer Center Research Institute, ²Department of Cellular and Molecular Pharmacology, Juntendo University Graduate School of Medicine, ³Department of Clinical Laboratory Medicine, Juntendo University School of Medicine, ⁴Department of Regenerative Medicine and Tissue Engineering, National Cerebral and Cardiovascular Center Research Institute, ⁵Department of Biochemistry and Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, ⁶National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan, ⁷Division of Supportive Care Research, National Cancer Center, Exploratory Oncology Research & Clinical Trial Center

April 23, 2017
14:40-14:55

In Japan, a half of Japanese becomes cancer during their whole life and one-third of Japanese people die because of cancer. The cancer has been recognized as an incurable disease, however, it has been becoming as curable diseases owing to development of anticancer drugs and state-of-the art operational technology. On the other hand, cardiovascular diseases in patients with cancer or caused by cancer chemotherapy have recently become a great concern. To date, some anticancer drugs and molecular targeted therapies cause cardiotoxicity, which limits cancer treatment and significantly decrease the quality of life in cancer patients. Among them, an anthracycline doxorubicin (DOX), which has been used for cancer chemotherapy, cause cardiotoxicity. Cellular mechanisms of DOX-induced cardiotoxicity include free-radical damage to cardiac myocytes, leading to mitochondrial injury and subsequent myocyte cell death. Recently some reports indicate that ghrelin and des-acyl ghrelin inhibit DOX-induced cardiotoxicity. However, little is known about molecular mechanisms how ghrelin and/or des-acyl ghrelin prevent DOX-induced cardiotoxicity. In the present study, we show the possible mechanisms of the effects of ghrelin and des-acyl ghrelin on the DOX-induced cardiotoxicity, and further explore to identify the receptors for des-acyl ghrelin through *in vitro* and *in vivo* basic researches.

Oral Presentation ③-2

Protective effect of ghrelin against pulmonary cachexia associated with lung adenocarcinoma in mice

Hironobu Tsubouchi, Shigehisa Yanagi, Ayako Miura, Nobuhiro Matsumoto, Masamitsu Nakazato
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April 23, 2017
14:55-15:10

Cancer cachexia is a refractory syndrome characterized by loss of skeletal muscle and fat mass. The reductions in body weight, skeletal muscle and fat mass are important prognostic indicators for cancer patients with cachexia. Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, has multifaceted effects, such as stimulation of food intake and adiposity, mitigation of inflammation and prevention of muscle catabolism. However, the therapeutic effect and molecular mechanism of ghrelin treatment on cancer cachexia, such as muscle wasting, remain unknown.

To investigate the efficacy of ghrelin treatment against the syndrome of cancer cachexia, we used mice with a bronchioalveolar epithelium-specific null mutation of Pten (Pten-KO). All of Pten-KO mice develop lung adenocarcinoma after intraperitoneally urethane injection.

Ghrelin treatment mitigated the reductions in food intake, body weight, skeletal muscle weight and intraabdominal fat weight in Pten-KO mice. In addition, ghrelin administration reduced the inflammatory cytokine levels in plasma, retained the skeletal muscle mass and muscle contraction force and suppressed the p38-MAPK and NF- κ B signaling in skeletal muscles. Our results indicate that ghrelin may be a therapeutic agent on cancer cachexia by ameliorating skeletal muscle wasting and regulating systemic inflammation.

Oral Presentation ③-3

Ghrelin treatment improves physical decline in 5/6 nephrectomized CKD model mice through muscular enhancement and mitochondrial activation

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**April 23, 2017
15:10-15:25**

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Objective: Chronic kidney disease (CKD) impairs physical performance and the decrease consists a risk for cardiovascular diseases. In our previous study, we demonstrated that a reduction in muscle mitochondria rather than muscle mass was a major cause of physical decline in 5/6 nephrectomized (5/6Nx) CKD model mice. Because ghrelin treatment has been expected to cause both muscular hypertrophy and mitochondrial oxidation effect, we examined the usefulness of ghrelin for a recovery of physical decline in 5/6Nx mice, focused on the epigenetic modification of PGC-1 α , a master regulator of mitochondrial biogenesis.

Methods: 5/6Nx C57Bl/6 mice were intraperitoneally administered acylated ghrelin (0.1 nmol/gBW; 3 times per week) for a month. Muscle strength and exercise endurance were measured by using a dynamometer and treadmill, respectively. Mitochondrial DNA copy number was determined by quantitative PCR. The methylation level of the cytosine residue at 260 base pairs upstream (C-260) of initiation point of PGC-1 α gene, which has been demonstrated to decrease the expression, was evaluated by methylation specific PCR and bisulfite sequencing methods after the ghrelin treatment.

Results: Ghrelin treatment improved both muscle strength and exercise endurance in 5/6Nx mice associated with an increase in muscle mass and muscle mitochondrial amount. Ghrelin treatment decreased methylation ratio of C-260 of PGC-1 α gene in the skeletal muscle and increased the expression.

Conclusions: Ghrelin treatment effectively improved physical decline in 5/6Nx mice, associated with muscle enhancement and epigenetic modification of muscle PGC-1 α gene, and mitochondrial activation.

Oral Presentation ③–4

Clinical application of ghrelin for diabetic peripheral neuropathy

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April 23, 2017

15:25-15:35

Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes, and its progression is associated with deterioration in quality of life. Although several drugs are available for DPN, all of them provide only symptomatic relief. We investigated the therapeutic effects of ghrelin for DPN based on its various physiological functions.

Seven type 2 diabetic patients with typical clinical signs and symptoms of DPN were hospitalized. Synthetic human ghrelin (1.0 $\mu\text{g}/\text{kg}$) was administered intravenously for 14 days. Motor conduction velocity (MCV) of the posterior tibial nerves improved significantly after the treatment compared to baseline (35.1 ± 4.7 to 38.6 ± 4.8 m/s, $P = 0.016$), while MCV in six control diabetic patients did not change throughout hospitalization. The subjective symptoms assessed by total symptom score also significantly improved (15.6 ± 8.2 to 11.1 ± 5.8 , $P = 0.047$). Although sensory conduction velocity (SCV) of the sural nerves could not be detected in three of the seven patients at baseline, it was detected in two of the three patients following the treatment. SCV in the remaining four patients did not change. Plasma glucose, but not serum C peptide, levels during liquid meal tolerance test significantly improved after the treatment.

Although this is an open-label and small-sized study, our findings suggest that ghrelin may be a novel therapeutic target for DPN.

Oral Presentation ③–5

Evaluation and optimization of blood collection and storage conditions for ghrelin measurement

**April 23, 2017
15:35-15:45**

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Ghrelin, a 28-amino acid peptide with an n-octanoyl modification indispensable for its biological activity, is synthesized principally in the stomach and released in response to acute and chronic energy imbalances. As ester bonding is both chemically and enzymatically unstable, ghrelin deacylation rapidly occurs in circulation as well as in whole blood and plasma samples. Previously we recommended a standard procedure for the collection of blood samples: blood samples is collected with EDTA-aprotinin and separated immediately, and acidification is the best method for the preservation of plasma ghrelin. Recently a new method of blood collection for ghrelin measurement is reported that alkyl fluorophosphonate can protect against ghrelin deacylation in biological samples. The present study examined the effect of alkyl fluorophosphonate on the stability of ghrelin octanoylation in whole-blood collection, storage and processing.

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