Poster Presentations

P-1 – P-17 13:00 - 14:45

(Discussion Time: 13:30 – 14:30)

Educational/Main Building 2nd floor Hall

P-1
Phosphorylation of mu opioid receptor reduced morphine analgesia in inflammatory pain state
Yuta Aoki, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada
(Department of Physiology and Anatomy, Tohoku Pharmaceutical University)

P-2
Involvement of peripheral TRPV1 receptor in neuropathic pain
Yuriko Endo, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada
(Department of Physiology and Anatomy, Tohoku Pharmaceutical University)

P-3
Analysis of histamine H4 receptor-mediated scratching behavior in mice
Shota Fujiwara[1], Hirokazu Mizoguchi[1], Chizuko Watanabe[1], Akihiko Yonezawa[1], Kazuhiko Yanai[2], Hiroshi Ohtsu[3], Shinobu Sakurada[1]
([1]Department of Physiology and Anatomy, Tohoku Pharmaceutical University, [2]Department of Pharmacology, Graduate School of Medicine, Tohoku University, [3]Department of Applied Quantum Medical Engineering, School of Engineering, Tohoku University)

P-4
Total Synthesis of the Bicyclic Depsipeptides – Spiruchostatins A, B, C, and D – and Investigation of Their HDAC Inhibitory and Antiproliferative Activities
Koichi Narita[1], Yurie Fukui[1], Yui Sano[1], Takao Yamori[2], Akihiro Ito[3], Minoru Yoshida[3], Tadashi Katoh[1]
([1]Department of Chemical Pharmaceutical Science, Tohoku Pharmaceutical University, [2]Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, [3]RIKEN)

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Effect of peripherally administered of bergamot essential oil and linalool on the formalin-induced nociceptive behavior in mice
Soh Katsuyama[1], Takaaki Komatsu[2], Tsukasa Sakurada[2], Hitoshi Nakamura[1]
([1]Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University, [2]Department of Pharmacology, Daiichi College of Pharmaceutical Sciences)

P-6
Macrophage inflammatory proteins contributing to neuropathic pain were upregulated by histone modifications after peripheral nerve injury
Norikazu Kiguchi, Yuka Kobayashi, Shiroh Kishioka
(Department of Pharmacology, Wakayama Medical University)

P-7
Matrix metalloprotease 12 derived from macrophage participates in pathogenesis of neuropathic pain
Yuka Kobayashi[1], Norikazu Kiguchi[1], Yohji Fukazawa[2], Shiroh Kishioka[1]
([1]Department of Pharmacology, Wakayama Medical University, [2]Department of Amatomy, Kansai University of Health Science)

P-8
Role of antidepressant on DOI-induced head-twitch response in olfactory bulbectomized mice
Osamu Nakagawasai[1], Akira Oba[1], Hiroshi Onogi[2], Wataru Nemoto[1], Fukie Yaoita[1], Takeshi Tadano[3], Koichi Tan-No[1]
([1]Department of Pharmacology, Tohoku Pharmaceutical University, [2]Faculty of Health Science, Tohoku Fukushi University, [3]Laboratory of Environmental and Health Sciences, College of Medical Pharmaceutical and Health Sciences, Kanazawa University)

P-9
Asymmetric Synthesis of α-1-*C*-Substituted-L-*arabino*iminofuranoses and Evaluation of Biological Activities as α-Glucosidase Inhibitors
Yoshihiro Natori[1], Yuichi Yoshimura[1], Atsushi Kato[2], Isao Adachi[2], Shuichi Hirono[3], Hiroki Takahata[1]
([1]Faculty of Pharmaceutical Sciences, Tohoku Pharmaceutical University, [2]Department of Hospital Pharmacy, University of Toyama, [3]School of Pharmaceutical Sciences, Kitasato University)

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Angiotensin II induces nociceptive behavior accompanied by p38 MAPK phosphorylation mediated through spinal AT1 receptors
Wataru Nemoto[1], Osamu Nakagawasai[1], Fukie Yaoita[1], Syu-Ichi Kanno[2], Shin Yomogida[2], Masaaki Ishikawa[2], Takeshi Tadano[3], Koichi Tan-No[1]
([1]Department of Pharmacology, Tohoku Pharmaceutical University, [2]Department of Clinical Pharmacotherapeutics, Tohoku Pharmaceutical University, [3]Laboratory of Environmental and Health Sciences, College of Medical Pharmaceutical and Health Sciences, Kanazawa University)

P-11
Spinal antinociceptive effect of endomorphins in inflammatory pain and neuropathic pain
Ryo Odagiri, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada
(Department of Physiology and Anatomy, Tohoku Pharmaceutical University)

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Ken-ichi Ohno, Tamaki Matsui, Kouwa Yamashita
(Department of Bioanalytical Chemistry, Tohoku Pharmaceutical University)

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([1]Department of Pathophysiology, Tohoku Pharmaceutical University, [2]Department of Biological Psychiatry, Tohoku University Graduate School of Medicine)

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(Department of Physiology and Anatomy, Tohoku Pharmaceutical University)

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Takamitsu Sasaki[1], Yoshihiro Numata[1], Shogo Takahashi[1], Takeshi Kumagai[1], Tamihide Matsunaga[2], Kiyoshi Nagata[1]
([1]Department of Environmental and Health Science, Tohoku Pharmaceutical University, [2]Department of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Nagoya City University)

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The antinociceptive effect of morphine in neuropathic cancer pain
Ryo Sugawara, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada
(Department of Physiology and Anatomy, Tohoku Pharmacological University)

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Influence of a long-term powdered diet on the social interaction test in mice: the role of dopaminergic systems
Fukie Yaoita[1], Masahiro Tsuchiya[2], Hiroko Saito[3], Yuka Nagasawa[1], Shigeo Murai[3], Yuichiro Arai[4], Osamu Nakagawasai[1], Wataru Nemoto[1], Takeshi Tadano[1,5], Koichi Tan-No[1]
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P-1

Phosphorylation of mu opioid receptor reduced morphine analgesia in inflammatory pain state

Yuta Aoki, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada
Department of Physiology and Anatomy, Tohoku Pharmaceutical University

An inflammatory pain is a chronic pain induced by algesic or inflammatory substances. It is well established that morphine is effective against the thermal hyperalgesia during inflammatory pain state. However, we found that morphine is ineffective against the mechanical allodynia on the inflammatory pain state. In the present study, the changes in the spinal mu-opioid receptors, which is involved in the morphine analgesia, is investigated in the inflammatory pain state. To develop the inflammatory pain, complete Freund's adjuvant (CFA) was injected i.pl to the hind-paw of male ddY mice. mRNA and protein level of mu-opioid receptors were quantified by reverse transcription polymerase chain reaction (RT-PCR) and western blot, respectively. Mechanical pain threshold and analgesic effect of morphine were measured by von Frey filament test. The remarkable mechanical allodynia was observed after the CFA injection on inflamed side but not non-inflamed side. However, the analgesic effect of morphine injected s.c. was markedly decreased bilaterally after the CFA injection. mRNA level of mu opioid receptor was significantly decreased bilaterally in the dorsal root ganglion (DRG) after the CFA injection. However, the protein levels of mu opioid receptor were significantly decreased in the DRG only on inflamed side after CFA injection. The present results suggest that the reduction of morphine analgesia in inflammatory pain state on inflamed side reflect the down regulation of mu opioid receptor, whereas another mechanism is also involved in the reduction of morphine analgesia on non-inflamed side in inflammatory pain state. Interestingly, the reduced morphine analgesia after CFA injection was completely reversed on non-inflamed side, and partially reversed on inflamed side by intrathecal pretreatment with protein kinase C inhibitor, which was injected at the same time of development of inflammation by CFA injection. In conclusion, the reduction of morphine analgesia on inflamed side may mainly reflect the down regulation of mu opioid receptor, whereas the reduction of morphine analgesia on non-inflamed side may mainly reflect the inactivation of mu opioid receptor by phosphorylation.

P-2

Involvement of peripheral TRPV1 receptor in neuropathic pain

Yuriko Endo, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada

Department of Physiology and Anatomy, Tohoku Pharmaceutical University

Transient receptor potential vanilloid 1 (TRPV1) is a non-selective cation channel with high permeability for $Ca^{2+}$and expressed in central and peripheral terminals of non-myelinated primary afferent neurons. In addition to chemical ligands such as capsaicin, TRPV1 receptors are also activated by multiple stimuli such as protons, heat, and some endogenous cannabinoid. Recent reports have investigated that TRPV1 antagonists have analgesic effect on both inflammatory and neuropathic pain. In inflammatory pain, the nociceptive function of TRPV1 is well investigated. However, the roles of TRPV1 are still unclear in neuropathic pain states. Therefore, in the present study, the role of the peripheral TRPV1 in neuropathic pain was investigated.

The partial sciatic nerve ligation (PSL) model in male ddY mice was used as an animal model for neuropathic pain. The duration of licking behavior by capsaicin was measured for 15 min as TRPV1 mediated pain-related behavior. The pain threshold and analgesic effect were measured by von Frey filament test.

After the nerve ligation, the remarkable reduction of pain threshold was observed in PSL model mice, but not control model mice. The intraplanter injection of lower dose of capsaicin (0.05 nmol/20 μl) was slightly expressed nociceptive behavior in control mice, whereas nociceptive behavior was dramatically increased in PSL model mice. Those responses were blocked by the competitive TRPV1 antagonist, capsazepine. Moreover, intraplanter pretreatment with capsazepine significantly attenuated the development of mechanical allodynia is PSL model mice.

These results suggest that supersensitivity of peripheral TRPV1 is involved in both development and maintenance of mechanical allodynia in the neuropathic pain.

P-3

Analysis of histamine H_4 receptor-mediated scratching behavior in mice

Shota Fujiwara[1], Hirokazu Mizoguchi[1], Chizuko Watanabe[1], Akihiko Yonezawa[1], Kazuhiko Yanai[2], Hiroshi Ohtsu[3], Shinobu Sakurada[1]

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Although histamine H_1 receptor antagonists are commonly used to treat atopic dermatitis, the treatment is not always effective. The histamine H_4 receptor, which was cloned in 2000, is expressed on several hematopoietic cells and plays important roles in the activation of mast cells, eosinophils, monocytes, dendritic cells, and T cells. Thus, histamine H_4 receptor is considered a new therapeutic target for allergic inflammation in atopic dermatitis, asthma, and rhinitis. In the present study, histamine H_4 receptor agonist 4-methylhistamine-induced scratching behavior was characterized as an itching behavior. Male ddY mice, histamine H_1 receptor-knockout mice, and histidine decarboxylase-knockout mice were used. Approximately 1 h before the injection, mice were adapted to an individual plastic cage (22.0 × 15.0 × 12.5 cm), which also served as the observation chamber. Immediately after the intradermal (i.d.) injection of 4-methylhistamine, each mouse was placed into the transparent cage and scratching behaviors induced by 4-methylhistamine were observed for 30 min. A dose-dependent increase in the total time of scratching behavior was observed following i.d. administration of 4-methylhistamine. The scratching behaviors induced by 4-methylhistamine were dose-dependently suppressed by s.c. pretreatment with histamine H_4 receptor antagonist JNJ7777120, but not histamine H_1 receptor antagonist chlorpheniramine. Moreover, in histamine H_1 receptor-knockout mice, the i.d.-administered 4-methylhistamine-induced scratching behaviors were not affected in compared with that in wild-type mice. To elucidate the involvement of endogenous histamine in the scratching behaviors induced by i.d.-administered 4-methylhistamine, histidine decarboxylase-knockout mice, which lack the endogenous histamine, were used. In the histidine decarboxylase-knockout mice, the scratching behavior induced by 4-methylhistamine was markedly suppressed. The present results suggest that the activation of histamine H_4 receptor on the skin by i.d.-injected 4-methylhistamine reveals the scratching behaviors as an itching response. The release of endogenous histamine may contribute the expression of this scratching behaviors by 4-methylhistamine.

P-4

Total Synthesis of the Bicyclic Depsipeptides – Spiruchostatins A, B, C, and D – and Investigation of Their HDAC Inhibitory and Antiproliferative Activities

Koichi Narita[1], Yurie Fukui[1], Yui Sano[1], Takao Yamori[2], Akihiro Ito[3], Minoru Yoshida[3], Tadashi Katoh[1]

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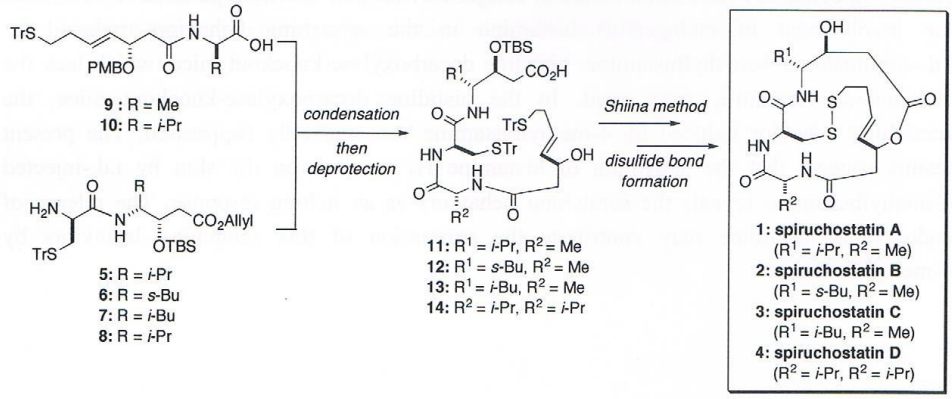
[2]Japanese Foundation for Cancer Research

[3]RIKEN

Natural products HDAC inhibitors – spiruchostatins A (**1**), B (**2**), C (**3**), and D (**4**) – are promising candidates for novel molecular-targeted anticancer agents. Structurally, these compounds are 16–membered bicyclic depsipeptides containing a characteristic disulufide bond linkage. The attractive biological activities and unique structural features prompted us to undertake the total syntheses of these natural products **1**– **4**.

The bicyclic depsipeptide HDAC inhibitors **1**– **4** were synthesized in a highly convergent and unified manner. The method involved the following three crucial steps: i) a Julia-Kocienski olefination of 1,3-propanediol-derived sulfone and a L-malic acid-derived aldehyde to access the most synthetically challenging unit, (3*S*,4*R*)-3-hydroxy-7-mercaptohept-4-enoic acid, present in carboxylic acid segment **9** or **10**, ii) amide coupling of carboxylic acid segment with amine segment (**9** and **5**, **6,** or **7**; **10** and **8**) to directly assemble the corresponding *seco*-acids, key precursors of macrolactonization, and iii) a macrocyclization of a *seco*-acid using the Shiina method to construct the requisite macrolactone. The HDAC inhibitory assay and cell-growth inhibition analysis of the synthesized depsipeptides **1**– **4** determined the order of potency of spiruchostatins A–D. Some novel aspects of structure–activity relationships (SAR) were also revealed.



P-5

Effect of peripherally administered of bergamot essential oil and linalool on the formalin-induced nociceptive behavior in mice

Soh Katsuyama[1], Takaaki Komatsu[2], Tsukasa Sakurada[2], Hitoshi Nakamura[1]
[1]Department of Clinical Pharmaceutics, Tohoku Pharmaceutical University
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Bergamot essential oil (BEO) consists of a volatile fraction (93 - 96 %) and a nonvolatile fraction (4 - 7 %); the former fraction contains monoterpene and sesquiterpene hydrocarbons and oxygenated derivatives such as linalool and linalyl acetate, and the latter fraction contains waxes, polymethoxylated flavones, coumarins and psoralens such as bergamottin and bergapten (Dugo P et al., *J Pharm Biomed Anal* 2000, Mondello L et al., *Flavour Fragr J* 1993). In this study, we investigated the effect of BEO containing linalool as major volatile components in the formalin test (2 % formalin). The intraplantar (i.pl.) injection of BEO or linalool into the ipsilateral hindpaw reduced both the early and late phase of formalin-induced licking response. I.pl. injection of BEO or linalool into the contralateral hindpaw did not yield antinociceptive effect, suggesting a local antinociceptive effect of BEO or linalool in formalin test. Intraperitoneal (i.p.) and i.pl. pretreatment with naloxone hydrochloride, an opioid receptor antagonist, significantly reversed BEO- and linalool-induced antinociception. Pretreatment with naloxone methiodide, a peripherally acting μ-opioid receptor-preferring antagonist, resulted in a significant antagonizing effect on antinociception induced by BEO and linalool. Our results provide evidence for the involvement of peripheral opioids in antinociception induced by BEO and linalool. These results suggest that activation of peripheral opioid receptors may play an important role in reducing formalin-induced nociception.

P-6

Macrophage inflammatory proteins contributing to neuropathic pain were upregulated by histone modifications after peripheral nerve injury

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Neuropathic pain is generated by damages of the nervous system, and characterized by allodynia and hyperalgesia. Increasing evidence indicates that neuroinflammation due to several inflammatory cytokines and chemokines largely participates in the pathogenesis of neuropathic pain. Previously, we found that chemokine macrophage inflammatory proteins (MIPs) are principal mediators in the peripheral sensitization leading to the induction of neuropathic pain. Herein, we determined the epigenetic regulation which is acquired genomic modifications underlying upregulation of MIPs in the injured peripheral nerves. Murine model of neuropathic pain was produced by partial sciatic nerve ligation (PSL). Tactile allodynia and thermal hyperalgesia were evaluated by von Frey test and Hargreaves test, respectively.

The mRNA levels of MIP-1alpha (CCL3) and MIP-2 (CXCL2) were upregulated in the injured sciatic nerve following PSL. Receptors of these MIPs (CCR1, CCR5 and CXCR2) were also increased by PSL. Circulating immune cells such as macrophages and neutrophils were recruited into the injured nerves from bone marrow, and these cells produced MIP-1alpha and MIP-2. In addition, acetylation in K9 residue and trimethylation in K4 residue of histone H3 (AcK9-H3 and MeK4-H3), which accelerate gene transcriptions associated with chromatin remodeling, on the promoter regions of MIP-1alpha and MIP-2 were enhanced in the injured nerves after PSL. Expression of AcK9-H3 and MeK4-H3 were observed in the nuclei of accumulating immune cells. Pharmacological inhibition of MIPs cascades by the perineural injection of neutralizing antibodies or antagonists prevented PSL-induced tactile allodynia and thermal hyperalgesia.

Taken together, these results suggest that augmentation of the MIPs cascades by the histone H3 modifications on their promoters in immune cells accumulated into the injured peripheral nerves contributes to neuropathic pain. Thus, these key molecules might be nominated as novel therapeutic targets for the treatment of neuropathic pain.

P-7

Matrix metalloprotease 12 derived from macrophage participates in pathogenesis of neuropathic pain

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Neuropathic pain is characterized by tactile allodynia and thermal hyperalgesia and is often accompanied by chronic neuroinflammation. Increasing evidence indicates that several types of immune cells play a crucial role in neuroinflammation and the excessive activation of immune system results in eliciting neuropathic pain. We have already reported that inflammatory cytokines and chemokines derived from macrophages and neutrophils strongly contribute to the development of neuropathic pain. Matrix metalloproteases (MMPs) are extracellular proteases and involve in tissue remodeling and cell activation. MMPs are released and activated in inflammatory cells. Recent report has shown that MMPs in the central nervous system are implicated in neuropathic pain as well as neurodegenerative disease, such as multiple sclerosis and Alzheimer disease. However, the role of MMPs in neuroinflammation of the peripheral nervous system leading to neuropathic pain remains to be clarified. In this study, we investigated the role of MMP12 in neuropathic pain, using ICR mice. To elicite neuropathic pain, mice received partial sciatic nerve ligation (PSL). After sciatic nerve (SCN) injury, macrophages, T-lymphocytes and neutrophils infiltrated in the injured SCN. By RT-PCR, the expressions of CD14 mRNA (macrophage marker), CD25 mRNA (T-lymphocyte marker) and myeloperoxidase mRNA (neutrophil marker) were increased in the injured SCN after PSL. The expressions of MMP9 mRNA and MMP12 mRNA were also increased in the injured SCN after PSL, while MMP2 mRNA was not. By immunohistochemistry, MMP12 was localized on infiltrating macrophages in the injured SCN. In cultured macrophages, the expression of MMP12 mRNA was increased by lipopolysaccharide treatment. PSL elicited tactile allodynia and thermal hyperalgesia which were evaluated by von Frey test and Hargreaves test, respectively. After the depletion of macrophages by the treatment of liposomed-clodronate, PSL-induced tactile allodynia and thermal hyperalgesia were suppressed. These results suggest that MMP12 derived from macrophages involved in pathogenesis of neuropathic pain and MMP12 may be novel therapeutic target for neuropathic pain.

P-8

Role of antidepressant on DOI-induced head-twitch response in olfactory bulbectomized mice

Osamu Nakagawasai[1], Akira Oba[1], Hiroshi Onogi[2], Wataru Nemoto[1], Fukie Yaoita[1], Takeshi Tadano[3], Koichi Tan-No[1]

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Olfactory bulbectomy (OBX) in rodents represents a valuable experimental model of depression. This study was designed to shed further light on the impact of putative serotonergic neuronal degeneration in OBX mice and to assess the effect of a widely used antidepressant on serotonergic related behavioral changes induced by OBX. Adult male ddY mice were subject to bilateral OBX or sham surgery. The serotonin (5-HT) $_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) enhanced a head-twitch response (HTR) in OBX mice. Effects of 5-HT_{2A}, 5-HT_{2C} antagonists and fluvoxamine were observed in OBX mice following DOI administration. The HTR elicited by the administration of DOI (0.5mg/kg and 1mg/kg, i.p.) was increased about twofold in OBX mice when compared with controls on the 14th day after the surgery. The injection of ketanserin (0.025mg/kg, i.p.), a 5-HT_{2A} receptor antagonist, inhibited the enhancement of the DOI-induced HTR after OBX. Likewise, the administration of SB 242084 (1mg/kg, s.c.), a 5-HT_{2C} receptor antagonist, also inhibited the DOI-induced HTR in OBX mice. Chronic but not acute treatment with the antidepressant fluvoxamine, a selective serotonin reuptake inhibitor (SSRI), suppressed the enhancement of DOI-induced HTR after OBX. These findings indicate that OBX, and the subsequent degeneration of neurons projecting from the olfactory bulb, caused a supersensitivity of 5-$HT_{2A/2C}$ receptors which may be involved in symptoms of depression.

P-9

Asymmetric Synthesis of α-1-*C*-Substituted-L-*arabino*iminofuranoses and Evaluation of Biological Activities as α-Glucosidase Inhibitors

Yoshihiro Natori[1], Yuichi Yoshimura[1], Atsushi Kato[2], Isao Adachi[2], Shuichi Hirono[3], Hiroki Takahata[1]

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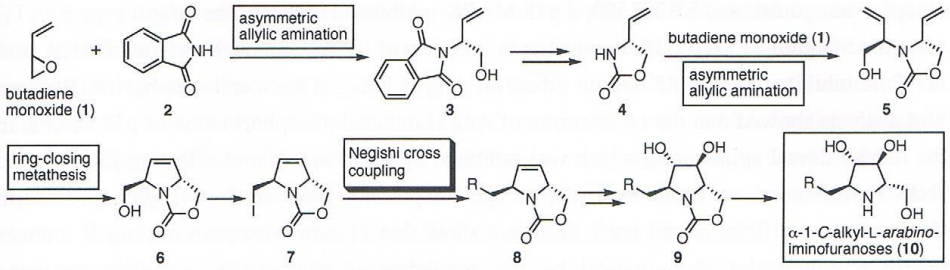
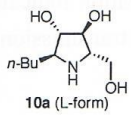
Iminosugars have emerged as potent inhibitors of glucosidases and glucosyltransferases, due to their transition state analogues in the enzymatic reactions. Therefore, many enantioselective syntheses of iminosugars have been reported in recent years. A much attention has been focused on the synthesis of their D-forms. There were few reports of systematic studies of the biological properties of the L-forms of iminosugars. Our attention was focused on the synthesis of both enantiomers of D- and L-iminofuranoses, and we have been interested in their biological evaluations.

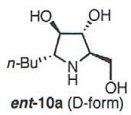
The desired iminofuranoses were synthesized via asymmetric allylic aminations, ring-closing metathesis and Negishi cross coupling. Negishi cross coupling as key reaction enabled us to provide a lot of α-1-*C*-alkyl-L-*arabino*iminofuranose derivatives **10**. The synthetic method of L-*arabino*iminosugars **10** was shown below, and D-*arabino*iminosugars ***ent*-10** were provided via similar route.



We measured the α-glucosidase inhibitory activities of prepared D- and L-*arabino*iminofuranose derivatives. α-1-*C*-butyl-L-*arabino*iminofuranose **10a** showed a greater inhibition against intestinal sucrase activity relative to commercially drugs. On the other hand, the activity of α-1-*C*-butyl-D-*arabino*iminofuranose ***ent*-10a** was interestingly over 1000-fold weaker than L-form **10a**.



10a (L-form)



***ent*-10a** (D-form)

IC_{50} (μM) values for the rat intestinal α-glucosidases

compound	maltase	sucrase
10a (L-form)	0.2	**0.032**
***ent*-10a** (D-form)	NI	392
acarbose	0.16	0.24
voglibose	0.18	0.37
miglitol	0.59	1.0

NI : No Inhibition
(less than 50% inhibition at 1000 μM)

We describe the details of asymmetric synthesis and oral biological evaluation of L-*arabino*iminofuranose derivatives **10**. Additionally, we also report the molecular docking properties of α-1-*C*-butyl-L-*arabino*iminofuranose **10a** as the novel class of α-glucosidase inhibitor for improvement of postprandial hyperglycemia with maltase.

P-10

Angiotensin II induces nociceptive behavior accompanied by p38 MAPK phosphorylation mediated through spinal AT1 receptors

Wataru Nemoto[1], Osamu Nakagawasai[1], Fukie Yaoita[1], Syu-Ichi Kanno[2], Shin Yomogida[2], Masaaki Ishikawa[2], Takeshi Tadano[3], Koichi Tan-No[1]

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It has been demonstrated that angiotensin II (Ang II) participates in either the inhibition or the facilitation of nociceptive transmission depending on the brain area. However, the role of spinal Ang II in nociceptive transmission remains unclear. Therefore, in order to elucidate the role of Ang II in nociceptive transmission in the spinal cord, we examined the effect of intrathecal (i.t.) administration of Ang II into mice. I.t. administration of Ang II produced a behavioral response in mice mainly consisting of biting and/or licking of the hindpaw and the tail along with slight hindlimb scratching directed toward the flank. The behavior induced by Ang II was dose-dependently inhibited by intraperitoneal injection of morphine, suggesting that the behavioral response is related to nociception. The nociceptive behavior was also inhibited dose-dependently by i.t. co-administration of losartan, an Ang II type 1 (AT_1) receptor antagonist, and SB203580, a p38 MAPK inhibitor. However, the Ang II type 2 (AT_2) receptor antagonist PD123319, the upstream inhibitor of ERK1/2 phosphorylation U0126, and the JNK inhibitor SP600125 had no effect on Ang II-induced nociceptive behavior. Western blot analysis showed that the i.t. injection of Ang II induced phosphorylation of p38 MAPK in the lumbar dorsal spinal cord, which was inhibited by losartan, without affecting ERK1/2 and JNK. Furthermore, we found that AT_1 receptor expression was relatively high expressed in the lumbar superficial dorsal horn. Our data show that i.t.-administration of Ang II induces nociceptive behavior accompanied by the activation of p38 MAPK signaling mediated through AT_1 receptors. This observation indicates that Ang II may act as a neurotransmitter and/or neuromodulator in the spinal transmission of nociceptive information.

P-11

Spinal antinociceptive effect of endomorphins in inflammatory pain and neuropathic pain

Ryo Odagiri, Hirokazu Mizoguchi, Chizuko Watanabe, Akihiko Yonezawa, Shinobu Sakurada

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Endomorphin-1 (EM-1: Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (EM-2: Tyr-Pro-Phe-Phe-NH_2) are carboxy-amidated tetrapeptides that are identified from bovine brain extracts. With high affinity and selectivity to mu opioid receptors, endomorphins have been considered to be endogenous ligands for mu opioid receptors. As mu-opioid receptor agonists, endomorphins show potent antinociceptive effect and their antinociceptive profile is quite distinct from that of traditional mu-opioid receptor agonist morphine or DAMGO. However, little is known about the effect of endomorphins on chronic pain, like an inflammatory pain or neuropathic pain. Therefore, in the present study, the antinociceptive effect of endomorphins on mechanical allodynia in inflammatory pain and neuropathic pain were investigated.

For inflammatory pain model, male ddY mice were treated intraplantarly with complete Freund's adjuvant (CFA) on the right hind-paw. Neuropathic pain model was produced by tightly ligating a half of the left sciatic nerve in male ddY mice. Antinociceptive effect of endomorphins and morphine was measured by von Frey filament test.

The remarkable mechanical allodynia was observed after CFA injection or nerve ligation on ipsilateral side but not contralateral side. Antinociceptive effect of intrathecally (i.t.) -administered morphine was bilaterally decreased in inflammatory pain model, but significantly decreased on only ipsilateral side in neuropathic pain model. On the other hand, antinociceptive effect of endomorphins injected i.t. was significantly decreased on only ipsilateral side in inflammatory pain model. However, endomorphins bilaterally produced potent and significant antinociceptive effect in neuropathic pain model. In conclusion, the endomorphins have potent antinociceptive effect against morphine-resistant mechanical allodynia in neuropathic pain.

P-12

Development of estradiol ELISA for efficient screening of aromatase inhibitors

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Aromatase is a cytochrome P-450 enzyme which catalyzes the conversion of the androgens such as androstenedione and testosterone into estrogens, estrone (E1) and estradiol (E2), respectively. Therefore, aromatase should be one of the primary targets for the treatment of the estrogen-dependent breast cancer, and its inhibitors have been reported by various researchers, including us. In the current inhibition assay, however, radioactive substrate such as tritium-labeled androgens are mainly used, and the radioactivity resulting from the enzymatic products is detected. In order to establish a non-radioactive and efficient screening of aromatase inhibitors, therefore, we developed a competitive E2 enzyme-linked immunosorbent assay (ELISA) in this study.

The horseradish peroxidase (HRP)-conjugated E2 as a labeled antigen was prepared by use of N-hydroxysuccinimide ester for the competitive immunoassay. After immunological reaction on the secondary antibody-immobilized 96 well microplate, the enzymatic activity of HRP was detected by the colorimetric assay using tetramethylbenzidine (TMB) and hydrogen peroxide. Under the optimized conditions, the calibration curve for E2 was obtained with the measurable concentration range of 9.77-2500 pg/mL with the relative standard deviations (RSD) ranged from 2.96 to 4.35% (n = 8). The cross-reactivity of antibody to the related molecules such as E1, estriol, androstenedione and testosterone was tested. On the basis of 50% inhibition concentration (IC_{50}), it was found to show a cross-reactivity to estriol of 0.5%. However, E1, androstenedione and testosterone showed less than 0.05% cross-reactivity.

Finally, the model samples for aromatase reaction containing excess amount of androstenedione and sodium borohydride were prepared and subjected to the proposed immunoassay. As a result, the excellent calibration curve for E2 was obtained, and it was consisitent with that of E2 alone, indicating that the excess amount of androstenedione and sodium borohydride in the model samples have little effect on the proposed immunoassay. The results strongly suggest that the proposed immunoassay is available for the efficient screening of aromatase inhibitors.

P-13

The involvement of glucocorticoids in asthma exacerbations induced by psychological stress

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Bronchial asthma is a chronic airway disease characteristic of eosinophilic inflammation tightly regulated by Th2 immunity. Psychological stress such as low socioeconomic status, academic examination and loss of jobs has been demonstrated to be associated with asthma symptom aggravation. However, how psychological stress perceived by brain leads to the exacerbation of the disease in lung remains unclear. HPA axis activation by stress exposure results in the increase of glucocorticoid (GC) levels in serum. While in the short time, the effect of GC may be beneficial for the treatment of asthma, their long-term effects are thought to sustain the increased vulnerability to the allergic condition through preventing the development of Th1 cells and regulatory T cells counterbalancing Th2 immune responses. Therefore, we hypothesized that the increase of GC levels evoked by chronic stress exposure could primed immune regulation preferring to more Th2 predominance in adaptive immunity, thereby eventually aggravating asthmatic responses to antigen challenge subsequent to stress exposure. To assess this hypothesis, we investigated the effects of a GC receptor antagonist, RU-486, and a GC synthesis inhibitor, metyrapone, on asthmatic airway responses using our murine model of stress asthma. Mice exposed to restraint stress on 3 consecutive days followed by forced swim stress on 4 consecutive days showed the increase of inflammatory cell counts accompanied with the increased contents of Th2 cytokines in airways, and also the worsening of airway responsiveness to inhaled methacholine and epithelial mucus secretion compared to non-stressed mice. Administration of RU-486, as well metyrapone, before each stress exposure abolished these exacerbations in stressed mice. These findings indicated that GC released upon stress exposure was involved in the stress-induced exacerbations of allergic asthma. The further exploration of underlying mechanisms linking GC release to the worsening of Th2-driven immune responses in airways would promote the clarification of the pathogenic role of neuroendocrine-immune axis in stress-induced asthma exacerbations.

P-14

Different effectiveness of narcotic analgesics on multiple sclerosis-related chronic pain

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Multiple sclerosis (MS) is an autoimmune disease that inflammatory demyelinating lesion arises in the central nervous system. Although the primary symptoms of MS are losses of sensory and motor functions, it is now recognized that chronic pain is a major symptoms for MS patients. However, the mechanisms of chronic pain associated with MS haven't been investigated. In the present study, the effects of narcotic analgesics, morphine and oxycodone on the MS-related chronic pain were investigated using the experimental autoimmune encephalomyelitis (EAE) model for MS. Female C57BL/6J mice were received the s.c. immunization with myelin oligodendrocyte glycoprotein 35-55 (MOG) emulsified in complete Freund's adjuvant. As an immunoenhancer, pertussis toxin was administered i.p. at the time of induction and again 48 h later. Control mice were received same treatment except MOG. The pain threshold and analgesic effects were measured by von Frey filament test at 6 days after immunization. After the immunization, the remarkable reduction of pain threshold was observed in EAE model mice but not control mice. Morphine expressed potent analgesic effect in control mice but not EAE model mice, whereas oxycodone expressed potent analgesic effect in both control mice and EAE model mice. Interestingly, analgesic effect of oxycodone in control mice was not inhibited by pretreatment with κ opioid receptor antagonist, nor-binaltorphimine (nor-BNI), whereas that in EAE model mice was significantly inhibited by nor-BNI. The result suggested that κ opioid receptor is involved in analgesic effect of oxycodone in EAE model mice. It is known that activation of N-methyl-D-aspartate (NMDA) receptor is important mechanism underlying on neuropathic pain and its morphine resistance. Therefore we investigated the involvement of NMDA receptor on the MS-related chronic pain using noncompetitive NMDA receptor antagonist, MK-801. As a result, reduction of pain threshold after immunization was completely eliminated by i.p. injection of MK-801. Moreover, reduced morphine analgesia in EAE model mice was recovered to the level of that in control mice by pretreatment with MK-801. The present results suggest that activating the κ opioid receptors oxycodone is effective for treatment to morphine-resistant MS-related chronic pain. Moreover, the activation of NMDA receptor is associated with the development of and morphine resistance in MS-related chronic pain, therefore NMDA receptor antagonist may be effective combination drug of morphine treatment against MS-related chronic pain.

P-15

Hepatic differentiation of human induced pluripotent stem cells by using factors involved in liver function and development

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Human induced pluripotent stem cells (iPSCs) are a valuable source of hepatocytes for use in drug metabolism studies. However, the current protocols for generating iPSC-derived hepatocyte-like cells (iPSHCs) are inefficient, and thus, they have insufficient hepatocyte-specific features, which include expression of a series of hepatocyte-specific genes, such as those encoding cytochrome P450 (*CYP*). In this study, we investigated whether the introduction of human hepatocyte nuclear factor 6 (HNF6) and microRNAs (miRNAs), which are implicated in the regulation of liver development and metabolic pathways, could modulate the expression of *CYP* genes in iPSHCs.

The iPSCs were induced to differentiate into hepatocyte-like cells by a three-stage method (sequential addition of activin A, dimethylsulfoxide, and cytokines; total 25 days of culturing). The iPSHCs were infected with human HNF6-expressing adenovirus (AdhHNF6) or transfected with miRNAs, which are expressed in higher levels in an adult liver than in an embryonic liver. To assess the effect of over-expression of the factors on the gene expression in iPSHCs, the expression levels of *CYP* genes were measured using real-time PCR.

CYP3A4 mRNA was detected in iPSHCs. The expression level was lower than that in HepG2 cells or hepatocytes. However, AdhHNF6 infection dramatically increased the expression level of *CYP3A4* mRNA, and the increased level was 1450-fold higher than that in non-infected iPSHCs. Further, the testosterone 6 β -hydroxylase activity in AdhHNF6-infected iPSHCs was assessed. Minor increases in the expression levels of *CYP1A2* and *CYP3A7* mRNA were observed but not in those of *CYP1A1*, *CYP2C9*, and *CYP2D6*. Moreover, the expression level of *CYP3A4* mRNA was significantly upregulated in let-7c-transfected iPSHCs, with a 30-fold increase when compared with the *CYP3A4* mRNA levels in the non-transfected iPSHCs.

The above results suggest the possibility that HNF6 and miRNAs might be useful in inducing the differentiation of iPSCs into hepatocytes.

P-16

The antinociceptive effect of morphine in neuropathic cancer pain

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Approximately 80% of cancer patients complain of a pain. Cancer pain can be divided into acute pain and chronic pain. Chronic pain in cancer patients is a persist pain so called neuropathic cancer pain. The neuropathic cancer pain is a one of the worst symptom for cancer patients to reduce their QOL. In the present study, the antinociceptive effect of morphine in the neuropathic cancer pain was determined. The neuropathic cancer pain was developed following the methods by Shimoyama et al (2002). Male C57BL/6J mice were inoculated tumor S180 cells to the immediate proximity of the left sciatic nerve. The mechanical pain threshold and morphine analgesia after inoculation were determined by von Frey filament test. After the inoculation of tumor cells, the mechanical pain threshold was gradually decreased on ipsilateral paw, but not contralateral paw. The decrease of mechanical pain threshold was significant at 5 days after the inoculation, and peaked at 7 and 10 days after the inoculation. However, after that, the mechanical pain threshold on ipsilateral paw significantly increased, indicating mechanical hyposensitivity. At 3 days after inoculation of tumor cells, the antinociceptive effect of morphine (5 mg/kg, s.c.) was maintained in both contralateral paw and ipsilateral paw. However, at 5 days after inoculation, the antinociceptive effect of morphine was significantly reduced in ipsilateral paw. Interestingly, at 7 days after inoculation, the antinociceptive effect of morphine was significantly reduced in both contralateral paw and ipsilateral paw. In conclusion, after inoculation of tumor cells, the antinociceptive effect of morphine ipsilaterally reduced at 5 days after inoculation, but bilaterally reduced at 7 days after inoculation. The neuropathic cancer pain may lead the bilateral neural plasticity to reduce morphine analgesia.

P-17

Influence of a long-term powdered diet on the social interaction test in mice: the role of dopaminergic systems

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It is well known that the characteristics of mastication are important for the maintenance of our physical well-being. In this study, to assess the importance of the effects of food hardness during mastication, we investigated whether a long-term powdered diet might cause changes in emotional behavior tests, including spontaneous locomotor activity and social interaction (SI) tests, and the dopaminergic system of frontal cortex in mice. Mice fed a powdered diet for 17 weeks from weaning were compared with mice fed a standard diet (control). The dopamine turnover and expression of dopamine receptors in the frontal cortex were also evaluated. Spontaneous locomotor activity, SI time and dopamine turnover of the frontal cortex were increased in powdered diet-fed mice. On the other hand, the expression of dopamine-4 (D4) receptors in the frontal cortex was decreased in powdered diet-fed mice. Moreover, we examined the effect of PD168077, a selective D4 agonist, on the increased SI time in powdered diet-fed mice. Treatment with PD168077 decreased the SI time. These results suggest that changes in the dopaminergic system, especially the D4 receptor subtype in the frontal cortex may be involved in the increased SI time induced by a long-term powdered diet.

株式会社ブレストシーブ

「国際交流学術シンポジウム 2013」の運営にあたり、上記会社の賛助をいただきました。ここに深甚なる感謝の意を表します。

"International Symposium for Neurosciences 2013" is supported by a MEXT (Ministry of Education, Culture, Sports, Science and Technology)-Supported Program for the Strategic Research Foundation at Private Universities (2010-2014)

「国際交流学術シンポジウム 2013」は、文部科学省「私立大学戦略的研究基盤形成支援事業」(東北薬科大学大学院薬学研究科:2010 年−2014 年)の後援により開催いたします。

国際交流学術シンポジウム 2013
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