International Symposium for Neurosciences 2013

February 26, 2013

Educational/Main Building
Tohoku Pharmaceutical University
Sendai, Japan

For Participants

Registration

Registration Desk will be in operation from 8:30 on February 26 at Educational/Main Building of Tohoku Pharmaceutical University (2nd floor).

Registration fee: 3,000 YEN

Lunch Break and Coffee Break

Lunch and soft drinks are available on Student Hall.

Instruction for Presentation

For Lecture and Symposium

The invited lectures and symposiums contain 40 min and 25 min presentation with 5 min discussion, respectively. A beam projector for "Power Point" is available for the presentation. To avoid any troubles in your presentation, the Organizing Committee encourages you to bring your own laptop PC and manage it by yourself. If you can not prepare your own laptop PC for your presentation, please contact to Organizing Committee in advance. The Organizing Committee will prepare laptop PC, Windows 7 (Power Point 2007) or Mac OS X (Power Point 2008) for your presentation. Before the presentation, you can check your presentation files with the laptop PC Organizing Committee prepared. However, in this case, there is no guarantee you can elude any font or formatting troubles in your presentation.

For Poster Presentation

All posters will be displayed in the 2nd floor hall in the Educational/Main Building of Tohoku Pharmaceutical University. The poster number will be affixed on the poster board. Poster presenters should refer to the list of "Poster Presentations" in the program and poster board number. Posters should be put up at 12:45, and taken down after 14:45. The presenters are urged to explain the poster from 13:30 to 14:30 to make valuable discussion with participants. Poster boards are 175 cm high and 87 cm wide.

Scientific Program

Welcome Address 9:25-9:30
President: Shinobu Sakurada (Tohoku Pharmaceutical University)

Invited Lecture (IL)-1 9:30-10:15
Chairman: Tsukasa Sakurada (Daiichi College of Pharmaceutical Sciences)

Antinociceptive proprieties of bergamot essential oil (BEO)
Laura Berliocchi[1], Rossella Russo[2], Maria Maiarù[1,2], Diana Amantea[2], Laura Rombolà[2], Luigi Antonio Morrone[2], Maria Tiziana Corasaniti[1], Giacinto Bagetta[2,3]
([1]Department of Health Sciences, University "Magna Græcia" of Catanzaro, [2]Department of Pharmacobiology and [3]University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria)

Coffee Break 10:15-10:30

Symposium 1 10:30-12:00
Chairman: Hirokazu Mizoguchi (Tohoku Pharmaceutical University)

S-1 10:30-11:00
Endogenous opioid system participates in the nicotine-induced physical dependence through alpha7 nicotinic acetylcholine receptor but not alpha4beta2 in mice.
Shiroh Kishioka, Norikazu Kiguchi, Yuka Kobayashi, Naoki Wakida, Chizuko Yamamoto
(Department of Pharmacology, Wakayama Medical University)

S-2 11:00-11:30
Characterization of alpha2-delta1 expression and response to chronic treatment with gabapentin in a mouse model of neuropathic pain
Damiana Scuteri[1], Laura Berliocchi[2], Maria Maiarù[1,2], Annagrazia Adornetto[1], Giacinto Bagetta[1], Maria Tiziana Corasaniti[2]
([1]Department of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, [2]Department of Health Sciences, University "Magna Græcia" of Catanzaro)

S-3 11:30-12:00
Involvement of peripheral opioid and cannabinoid receptors in antinociception induced by bergamot essential oil and β-caryophyllene
Tsukasa Sakurada, Takaaki Komatsu
(Department of Pharmacology, Daiichi College of Pharmaceutical Sciences)

Lunch Break 12:00-13:00

Poster Presentation 13:00-14:45

Coffee Break 14:45-15:00

Symposium 2 15:00-16:30
Chairman: Shiroh Kishioka (Wakayama Medical University)

S-4 15:00-15:30
Mechanisms of locus coeruleus activation by gabapentinoids
Masaru Yoshizumi, James C. Eisenach, Ken-ichiro Hayashida
(Department of Anesthesiology, Wake Forest University School of Medicine)

S-5 15:30-16:00
Intrathecal morphine-3-glucuronide induces nociception through δ_2-opioid receptors in the spinal cord
Takaaki Komatsu[1], Shinobu Sakurada[2], Tsukasa Sakurada[1]
([1]Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, [2]Department of Physiology and Anatomy, Tohoku Pharmaceutical University)

S-6 16:00-16:30
Gangliosides produce nociceptive behavior and hyperalgesia via glutamate signals
Shun Watanabe[1], Koichi Tan-No[2], Takeshi Tadano[2], Hideyoshi Higashi[1]
([1]Division of Glyco-signal Research, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University, [2]Department of Pharmacology, Tohoku Pharmaceutical University)

Coffee Break 16:30-16:45

Invited Lecture (IL)-2 16:45-17:30
Chairman: Takeshi Tadano (Kanazawa University)

Study and development of a novel antitussive drug, TRK-851
Hiroshi Nagase
(Department of Medicinal Chemistry, School of Pharmacy, Kitasato University)

Invited Lecture (IL)-3 17:30-18:15
Chairman: Shinobu Sakurada (Tohoku Pharmaceutical University)

Is there a role for autophagy in pain processing?
Giacinto Bagetta[1], Laura Berliocchi[2], Rossella Russo[1], Maria Maiarù[1,2], Giuseppe Varano[1,3], Cristina Tassorelli[3] and Maria Tiziana Corasaniti[2]
([1]Department of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, [2]Department of Health Sciences, University "Magna Graecia" of Catanzaro, [3]IRCCS National Neurological Institute "C. Mondino" Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia)

Closing Remark 18:15-18:20
President: Shinobu Sakurada (Tohoku Pharmaceutical University)

Invited Lectures

IL-1 9:30 – 10:15

IL-2 16:45 - 17:30

IL-3 17:30 – 18:15

Educational/Main Building 2nd floor Room 20

IL-1

Antinociceptive proprieties of bergamot essential oil (BEO)

Laura Berliocchi[1], Rossella Russo[2], Maria Maiarù[1,2], Diana Amantea[2], Laura Rombolà[2], Luigi Antonio Morrone[2], Maria Tiziana Corasaniti[1], Giacinto Bagetta[2,3]

[1]Department of Health Sciences, University "Magna Græcia" of Catanzaro, [2]Department of Pharmacobiology and [3]University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria

Several essential oils and natural substances have important anti-inflammatory and analgesic proprieties. Among these, the essential oil of Bergamot (BEO, from Citrus Bergamia, Risso) has been shown from our previous works to interfere with synaptic mechanisms such as neurotransmitter release (Morrone et al., 2007) and to be neuroprotective both *in vitro* (Corasaniti et al., 2007) and *in vivo* (Amantea et al., 2009). We further investigated BEO proprieties by studying its effects on nociceptive behaviour in experimental models of pain.

To this aim, we used the spinal nerve ligation (SNL) model (Kim & Chung, 1992) and the formalin test as models of neuropathic and inflammatory pain, respectively. Male C57BL/6 mice, that had underwent SNL, were administered BEO (1 ml/kg; s.c.) in a single daily injection, 1 hour before surgery and then once daily for 14 days. The von Frey's and Haregreaves' test were then used to assess mechanical and thermal sensitivity up to 28 days after SNL.

In the formalin test, mice received a subcutaneous BEO injection 15 minute before the subcutaneous administration of formalin (s.c., 5%, 20 µl) either in the hind paw or on the scruff of the neck. Licking/biting behaviour was then monitored at intervals of 5 min for the following 60min.

In the formalin test, BEO modified either one or both phases of the liking/biting behaviour test depending on the dose and on the way of administration used. In particular, BEO administered intraplantarly significantly reduced the first phase of liking/biking behaviour with no effect on the second phase. Instead, the same dose of BEO administered subcutaneously in the scruff of the neck reduced both the first and the second phase of this inflammatory pain model. The subcutaneous administration of a lower dose in the scruff of the neck showed anti-nociceptive effect on the second but not on the first phase of the test. Following SNL, a robust mechanical allodynia developed and lasted over weeks. A daily dose of BEO (1 ml/kg; s.c.) administered daily for 7 days attenuated mechanical allodynia compared to SNL vehicle-treated animals.

Altogether, our data suggest that BEO is able to interfere with pain sensitivity possibly acting via two different mechanisms (peripheral and central) and may be a useful adjuvant drug for pain treatment (Bagetta et al., 2010). However, BEO toxic profile on cell survival and proliferation suggest a cautionary approach to the use of inappropriate dilutions of the oil (Berliocchi et al., 2011).

IL-2

Study and development of a novel antitussive drug, TRK-851

Hiroshi Nagase
Department of Medicinal Chemistry, School of Pharmacy, Kitasato University

We discovered potent antitussive activity of NTI, a selective δ opioid antagonist by s.c. injection. Although codeine is used as antitussive drug, the drug is well known to afford addiction. As NTI is the δ antagonist, it is expected to be no addictive antitussive drug. So we started to develop the δ antagonist as antitussive drug. However, NTI was the highly polar molecule and hard to penetrate through blood brain barrier.

In the course of our study for obtaining the δ antagonists, easy to penetrate the barrier, we designed and synthesized a novel δ antagonist, TAN-615. Unfortunately, the antagonist was extremely fast to metabolite by oral administration. We examined precisely the structure of the metabolites and tried to block the metabolic positions. Finally, we obtained TRK-851 which was stable for the metabolic enzymes and showed potent antitussive activity by p.o. administration. Furthermore, TRK-851 showed highly selective δ antagonist activity. The antagonist also attenuated severe cough in mouse models which was not inhibited by codeine and another antitussive drugs.

Finally, TRK-851 progressed to preclinical trial I.

IL-3

Is there a role for autophagy in pain processing?

Giacinto Bagetta[1], Laura Berliocchi[2], Rossella Russo[1], Maria Maiarù[1,2], Giuseppe Varano[1,3], Cristina Tassorelli[3] and Maria Tiziana Corasaniti[2]

[1]Department of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, [2]Department of Health Sciences, University "Magna Graecia" of Catanzaro, [3]IRCCS National Neurological Institute "C. Mondino" Foundation and University Centre for the Study of Adaptive Disorders and Head Pain, University of Pavia

Chronic pain is a debilitating condition affecting life quality and has dramatic economic impact on national health systems. However, our ability to treat this condition is currently limited and there is an urgent need for more effective treatments. This goal can be achieved through a better understanding of the neurobiology of pain and its mechanisms.

We focussed our research on autophagy, a physiological mechanism contributing to protein and organelles degradation, cellular remodelling and survival. Because of its critical homeostatic role, any imbalance in the autophagic flux can impact basal functions leading to cellular dysfunction. Both insufficient and excessive autophagy have therefore differently implicated in several human pathologies such as neurodegenerative diseases and cancer.

Changes occurring during spinal processing of sensory information consequent to a peripheral lesion are important for chronic pain conditions. We have recently shown that autophagy is impaired in the spinal cord following peripheral nerve injury and suggested a potential role for this degradative pathway in spinal pain processing (Berliocchi et al., 2011; Berliocchi et al., 2012). The formation of the autophagic marker LC3-II, together with the accumulation of autophagy substrates like p62 detected by western blot in the spinal dorsal horn following spinal cord ligation (SNL; Kim and Chung, 1992), indicates that autophagy is impaired in this experimental model of neuropathic pain. The increased expression of these autophagic markers is restricted to the spinal cord side ipsilateral to the ligation in SNL mice, correlates with the up-regulation of the calcium channel subunit α2δ-1, and is not present in mice that underwent sham surgery. Immunofluorescence and confocal microscopy analysis suggest that these changes in the expression of the autophagic markers occur within specific cell populations in the spinal dorsal horn. Seven days after surgery, increased p62 immunoreactivity is detectable in the most superficial laminae of the spinal dorsal horn on the injury side of SNL mice and is less evident in mice that underwent sham surgery, thus confirming the western blot data. Double immunostainings with some main cellular markers, indicate the presence of p62 mainly in NeuN-positive cell bodies, occasionally in GFAP-positive processes, but not in Iba1-positive cells, thus suggesting a predominant expression in the neuronal compartment. Analogous changes were observed also in the formalin test, a model of acute inflammatory pain characterised by a peripheral and a central component.

Finally, we investigated the consequences of autophagy impairment on pain behaviour. Cloroquine is known to increase lysosomal pH disrupting the normal autophagic flux and resulting in autophagosome accumulation. When injected intrathecally in naïve mice, cloroquine (CQ, 100μM) was able to induce a spinal accumulation of LC3-II and p62 indicative of a block in autophagosome clearance. This was not associated to cell death, but induced an increased mechanical sensitivity suggesting a participation of the autophagic flux into spinal mechanisms of central sensitization.

Altogether, our data indicate that spinal autophagy is impaired in pain states and suggest that this event may be playing a relevant role in pain processing, thus providing a potential novel target for pain control.

Symposium

S-1 – S-3 10:30 – 12:00

S-4 – S-6 15:00 – 16:30

Educational/Main Building 2nd floor Room 20

S-1

Endogenous opioid system participates in the nicotine-induced physical dependence through alpha7 nicotinic acetylcholine receptor but not alpha4beta2 in mice

Shiroh Kishioka, Norikazu Kiguchi, Yuka Kobayashi, Naoki Wakida, Chizuko Yamamoto
Department of Pharmacology, Wakayama Medical University, Wakayama 641-0012 Japan

A single injection of nicotine or morphine elicits analgesia and serum corticosterone (SCS) increase in mice. Nicotine-induced analgesia was prevented by both nicotinic acetylcholine receptor (nAChR) antagonist (mecamylamine 1 mg/kg; MEC) and opioid receptor antagonist (naloxone 1 mg/kg; NLX), and also by repeated administration of morphine or nicotine. On the other hand, the nicotine-induced SCS increase was suppressed by MEC, but not NLX. Furthermore, nicotine-induced analgesia was reduced by both alpha4beta2 nAChR antagonist (dihydro-beta-erythroidine; DH-beta-E) and alpha7 nAChR antagonist (methyllycaconitine; MLA), while nicotine-induced SCS increase was suppressed by DH-beta-E, but not MLA. Thus, we predicted that endogenous opioid system might be located on the downstream of alpha7 nAChR, but not alpha4beta2. On the other hand, it is well known that nicotine has physical dependence liability, but its mechanism is still unclear. In this study, we examined whether physical dependence on nicotine was developed by the stimulation of endogenous opioid system through alpha7 nAChR in ICR mice. We evaluated SCS increase as an indicator of nicotine withdrawal, because SCS is a stress hormone. NLX (0.01-5 mg/kg) elicited SCS elevation in mice receiving repeated nicotine (3 mg/kg, twice a day for 7 days) in a dose-dependent manner, and the NLX-induced SCS increase was correlated with the doses (1-5 mg/kg, twice a day for 9 days) and the days (3 mg/kg, twice a day for 3-9 days) of repeated nicotine. When opioid antagonist, naltrexone (1–10 mg/kg), was administered together with repeated nicotine (5 mg/kg, s.c., twice a day for 5 days), NLX-induced SCS increase was significantly suppressed, indicating that endogenous opioid system might participate in the development of physical dependence on nicotine. Concomitant administration of MLA with repeated nicotine, but not DH-beta-E, suppressed the SCS increase by NLX. These results suggest that endogenous opioid system was located on the downstream of alpha7 nAChR, and that physical dependence on nicotine was, at least in part, developed by the stimulation of endogenous opioid system.

S-2

Characterization of alpha2-delta1 expression and response to chronic treatment with gabapentin in a mouse model of neuropathic pain

Damiana Scuteri[1], Laura Berliocchi[2], Maria Maiarù[1,2], Annagrazia Adornetto[1], Giacinto Bagetta[1], Maria Tiziana Corasaniti[2]

[1]Department of Pharmacobiology and University Center for Adaptive Disorders and Head Pain, Section of Neuropharmacology of Normal and Pathological Neuronal Plasticity, University of Calabria, [2]Department of Health Sciences, University "Magna Græcia" of Catanzaro

Neuropathic pain is a form of chronic pain arising from a lesion or disease affecting the somatosensory pathways within the peripheral and central nervous system. Almost 20% of the European population is affected by chronic or intermittent pain with consequent strong reduction of the patient's life quality and high societal costs.

Gabapentin is a widely used drug in the treatment of neuropathic pain likely acting via the voltage-dependent calcium channel subunit alpha2-delta1. In particular, gabapentin and its parent compound pregabalin are first choice drugs in management of neuropathic pain in the elderly. It has been shown that the anti-allodynic effect of gabapentin depends on the expression of this target molecule and that parameters like circadian oscillation can affect a2δ-1 expression and consequently gabapentin's efficacy. Aim of this study was to verify the anti-allodynic effect of a chronic treatment with gabapentin and to correlate it with a2δ-1 expression in an experimental model of neuropathic pain. Furthermore, a2δ-1 expression was characterized in mice of different age.

The study was conducted in male mice (C57Bl/6J, 22-25g) that underwent Spinal Nerve Ligation (SNL), according to the model described by Kim & Chung (1992). Behavioural tests were used to assess mechanical (von Frey's test) and thermal (Hargreaves' test) sensitivity in both sham (n=2) and SNL animals (n=8), whereas Western blot experiments were conducted to assess the expression of alpha2-delta1 subunit of voltage gated Ca^{2+} channels in mice of different age (2-6-11 months).

In SNL, but not sham animals, severe mechanical allodynia is fully developed at day 1-3 after ligature and peaks at day seven after surgery, lasting for up to thirty days. Western blotting results demonstrate that behavioural effects are paralleled by a2δ-1 overexpression in the spinal cord sections ipsilateral to the nerve injury and this is detectable on day 3 and 7 after surgery; the latter observation is conserved in SNL animals of 6 and 11 months of age. No sensitization to thermal sensitivity develops in SNL animals. Under these experimental conditions, administration of gabapentin (100 mg/kg given i.p. once daily 1 h before behavioural tests), starting from day seven after surgery, minimizes allodynia assessed for up to two weeks. By contrast, this treatment schedule does not affect allodynia measured in later phases of neuropathic pain.

In conclusion, our data provide further evidence to the rational use of gabapentin in the early treatment of neuropathic pain in view of its action on a2δ-1 subunit, which is overexpressed in the initial stages of neuropathic state development. Also, these results open new horizons to further investigations on the role of a2δ-1 subunit in the mechanisms underlying neuropathic pain development in aged animals.

S-3

Involvement of peripheral opioid and cannabinoid receptors in antinociception induced by bergamot essential oil and β-caryophyllene

Tsukasa Sakurada, Takaaki Komatsu
Department of Pharmacology, Daiichi College of Pharmaceutical Sciences

Bergamot essential oil (BEO) is one of the most common essential oil containing linalool and linalyl acetate as major volatile components. This study investigated the effect of intraplantar (i.pl.) BEO or linalool on neuropathic hypersensitivity induced by partial sciatic nerve ligation (PSNL) in mice. The i.pl. injection of BEO or linalool into the ipsilateral hindpaw to PSNL reduced PSNL-induced mechanical allodynia. Peripheral (i.pl.) injection of BEO or linalool into the contralateral hindpaw did not yield anti-allodynic effects, suggesting a local anti-mechanical allodynic effect of BEO or linalool in PSNL mice. We also examined the possible involvement of spinal extracellular signal-regulated protein kinase (ERK) in BEO or linalool-induced anti-allodynia. In western blotting analysis, i.pl. injection of BEO or linalool resulted in a significant blockade of spinal ERK activation induced by PSNL. These results suggest that i.pl. injection of BEO or linalool may reduce PSNL-induced mechanical allodynia followed by decreasing spinal ERK activation. β-Caryophyllene (BCP) is also a common constitute of the essential oils of numerous spice, food plants and major component in *Cannabis*. We investigated the contribution of peripheral cannabinoid (CB) and opioid systems in the antinociception produced by i.pl. injection of BCP in mice. The i.pl. injection of BCP produced a dose-dependent antinociceptive effect in the capsaicin test. BCP-induced antinociception was prevented by subcutaneous (s.c.) and i.pl. pretreatment with AM630, a selective CB_2 receptor antagonist, but not by AM251, a selective CB_1 receptor antagonist. Pretreatment with naloxone hydrochloride, an opioid receptor antagonist, and β-funaltrexamine, a selective μ-opioid receptor antagonist, reversed the antinociceptive effect of BCP in a dose-dependent manner. Pretreatment with naloxone methiodide (s.c.), a peripherally acting antagonist for opioid receptors and i.pl. pretreatment with β-endorphin antisera, an endogenous opioid peptide, resulted in a significant antagonizing effect on BCP-induced antinociception. Our results provide evidence for the involvement of peripheral CB_2 and opioid receptors in the peripheral, local nature of the antinociception induced by i.pl. BCP. In conclusion, the present results have shown that BEO, linalool and BCP are effective after local treatment, and that their peripheral use may be of therapeutic interest in acute and chronic pain.

S-4

Mechanisms of locus coeruleus activation by gabapentinoids

Masaru Yoshizumi, James C. Eisenach, Ken-ichiro Hayashida
Department of Anesthesiology, Wake Forest University School of Medicine

We have demonstrated that gabapentin (GBP) activates locus coeruleus (LC) neurons via glutamatergic signaling and that the analgesic effects of GBP in rats with peripheral nerve injury relies on this activation and subsequent spinal noradrenaline release. To investigate the mechanisms of GBP in the LC, we tested the following hypotheses: 1) GBP increases glutamate concentrations in the LC by disinhibition through reduced pre-synaptic GABA release; 2) GBP increases glutamate in the LC directly and indirectly by actions on astrocytes.

Basal GABA concentration and glutamate decarboxylase expression increased in the LC but decreased in the spinal dorsal horn after spinal nerve ligation (SNL). In microdialysates from the LC, intravenously administered GBP decreased GABA concentrations in normal and SNL rats. In synaptosomes from the LC, GBP and other α2δ ligands inhibited KCl-evoked GABA release in normal and SNL rats. In microdialysates from the spinal dorsal horn, intravenous GBP did not alter GABA concentration in normal but slightly increased in SNL rats. In synaptosomes from the spinal dorsal horn, neither GBP nor other α2δ ligand affected KCl-evoked GABA release in normal and SNL rats. Riluzole (RIL) and GBP facilitated glutamate-induced glutamate release from astrocytes and increased glutamate uptake, the latter being completely blocked by the glutamate transporter blocker, DL-TBOA. RIL and GBP enhanced glutamate-induced intracellular Ca^{2+} response. The enhancement of glutamate-induced Ca^{2+} response by RIL and GBP was blocked by the DL-TBOA and an inhibitor of Na^{+}/Ca^{2+} exchanger, KB-R7943.

These results suggest that peripheral nerve injury induces plasticity of GABAergic neurons differently in the LC and spinal dorsal horn, and that GBP reduces pre-synaptic GABA release in the LC but not in the spinal dorsal horn. These results also demonstrate that RIL and GBP enhance Na^{+}-glutamate co-transport through glutamate transporters, and subsequent Ca^{2+} influx via the reverse mode of Na^{+}/Ca^{2+} exchange enhances glutamate-induced glutamate release in astrocytes. The present study supports the idea that GBP activates descending noradrenergic inhibition via disinhibition of LC neurons, and that GBP directly acts on glutamate regulation in astrocytes.

S-5

Intrathecal morphine-3-glucuronide induces nociception through δ_2-opioid receptors in the spinal cord

Takaaki Komatsu[1], Shinobu Sakurada[2], Tsukasa Sakurada[1]

[1]Department of Pharmacology, Daiichi College of Pharmaceutical Sciences

[2]Department of Physiology and Anatomy, Tohoku Pharmaceutical University

Morphine-3-glucuronide (M3G), a main metabolite of morphine, has been proposed as a responsible factor when patients are presented with the neuroexciatory side effects (nociceptive behavioral responses such as hyperalgesia and allodynia) observed following systemic administration of large doses of morphine. Indeed, both M3G (3 nmol/5 μl) and high-dose morphine (60 nmol/5 μl) elicit a serve hindlimb scratching followed by biting and licking when administered intrathecally (i.t.) into mice.

Our previous research has demonstrated that both M3G and high-dose morphine may stimulate a simultaneous release of substance P and glutamate from primary afferent terminals, which induces secondary activation of the spinal extracellular signal-regulated kinase (ERK) signaling through the nitric oxide (NO)-cGMP-PKG pathway.

Elevation of spinal dynorphin content has been observed during the continuous spinal infusion of morphine. Although dynorphin has a relatively high affinity for the κ-opioid receptor and may act as an endogenous antinociceptive peptide, i.t. dynorphin has also been reported to produce nociceptive behaviors. Here, we investigated the possible involvement of spinal dynorphin in M3G-induced behavioral responses consisting of scratching, biting and licking (SBL). Pretreatment with antisera against dynorphin inhibited i.t. M3G-induced behavioral responses and ERK activation. Dynorphin is rapidly degraded into [Leu^5]enkephalin by dynorphin-converting enzymes (cystein protease). The behavioral responses and ERK activation evoked by i.t. M3G were significantly suppressed by i.t. injection of *p*-hydroxymecuribenzoate, an inhibitor of cystein proteases. Furthermore, M3G-induced behavior and ERK activation was inhibited dose-dependently by i.t. co-administration of the non-selective δ–opioid receptor antagonist naltridole or the selective δ_2-opioid receptor antagonist naltriben, while the selective δ_1-opioid receptor antagonist BNTX had no effect. We further showed that [Leu^5]enkephalin co-administered with [Leu^5]enkephalin-converting enzyme inhibitors, phosphoramidon, (an endopeptidase 24.11 inhibitor) and bestatin, (a general aminopeptidase inhibitor), produced both a series of SBL behaviors and ERK activation. [Leu^5]enkephalin in combination with peptidases inhibitors-induced behavior and ERK activation were blocked by NMDA receptor antagonists, nitric oxide synthase inhibitors, the non-selective δ–opioid receptor antagonist and the selective δ_2-opioid receptor antagonist. These results suggest that M3G-induced SBL behaviors may be triggered through the δ_2-opioid receptor activated by [Leu^5]enkephalin which is formed from dynorphin in the spinal cord.

S-6

Gangliosides produce nociceptive behavior and hyperalgesia via glutamate signals

Shun Watanabe[1], Koichi Tan-No[2], Takeshi Tadano[2], Hideyoshi Higashi[1]

[1]Division of Glyco-signal Research, Institute of Molecular Biomembrane and Glycobiology, Tohoku Pharmaceutical University

[2]Department of Pharmacology, Tohoku Pharmaceutical University

Gangliosides are abundant glycolipids in neural tissue and play essential roles in axonal elongation, synaptic transmission, and neuron-glia interactions. Gangliosides are sialic acid containing glycosphingolipids synthesized from ceramide and divided into several groups (asialo-, a-, b-, and c-series gangliosides) based on their biosynthetic pathway. *St8sia1* (b- and c-series gangliosides precursor biosynthesis enzyme) knockout mice, which lack b- and c-series gangliosides but not asialo and a-series gangliosides, exhibit altered nociceptive responses(1). The mechanism underlying this defect, however, remains unclear. To address this issue, we first investigated whether gangliosides in peripheral tissues are involved in nociception. Intraplantar injection of a b-series ganglioside GT1b, but not a-series gangliosides such as GM1, produced nociceptive responses and enhanced low-dose formalin-induced nociception. Next, we examined the effects of several receptor antagonists on GT1b-induced hyperalgesia. Among the tested antagonists, only glutamate receptor antagonists reduced GT1b-induced hyperalgesia. Furthermore, subcutaneous microdialysis analysis revealed that GT1b injection increased intraplantar glutamate levels. These findings suggested that GT1b induced extracellular glutamate to accumulate in subdermal tissues, thereafter activating glutamate receptors, which in turn resulted in hyperalgesia and nociception.

On the other hand, transient deletion of endogenous sialyl conjugates, such as gangliosides, by sialidase treatment reduced nociceptive behavior during the late phase of 2% formalin-induced nociception. Thus, these results indicated that endogenous complex b-series gangliosides are involved in nociceptive responses involving glutamate signaling in peripheral tissues. These results suggest that gangliosides play important roles in nociceptive responses originating in peripheral tissues(2).

(1) Handa Y et al., (2005) *Pain,* **117**(3): 271-279.

(2) Watanabe S et al., (2011) *Pain*, **152**(2): 327-334.