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Abstract

Increasing evidence has suggested that brain orexins are
implicated in a wide variety of physiological functions. With regard to
gastrointestinal functions, orexin-A acts centrally to regulate
gastrointestinal functions such as gastric and pancreatic secretion, and
gastrointestinal motility.   Visceral sensation is also known as one of key
gastrointestinal functions which are controlled by the central nervous
system.   Little is, however, known about a role of central orexin in
visceral sensation.   The present study was therefore performed to clarify
whether brain orexin may be involved in the process of visceral sensation.
Visceral sensation was evaluated by colonic distension-induced abdominal
withdrawal reflex (AWR) in conscious rats.   Intracisternally administered
orexin-A dose-dependently increased the threshold volume of colonic
distension-induced AWR.   In contrast, neither intraperitoneal injection of
orexin-A nor intracisternal orexin-B altered the threshold volume.   While
intracisternal SB334867, an orexin 1 receptor antagonist, by itself failed to
change the threshold volume, SB334867 injected centrally completely
blocked the morphine-induced antinociceptive action against colonic
distension.   These results suggest for the first time that orexin-A
specifically acts centrally in the brain to enhance antinociceptive response
to colonic distension.   We would furthermore suggest that endogenous
orexin-A indeed mediates the antinociceptive effect of morphine on

visceral sensation through the orexin 1 receptors.　All these evidence might indicate that brain orexin play a role in the pathophysiology of functional gastrointestinal disorders such as irritable bowel syndrome because visceral hypersensitivity of the gut is considered to play a vital role in the diseases.

1.   Introduction

Orexins/hypocretins are novel neuropeptides that are localized in neurons in the lateral hypothalamus (Sakurai et al., 1998; de Lecea et al., 1998).   Despite their highly restricted origin, orexin nerve fibers are identified widely throughout the central nervous system (Payron et al., 1998; Date et al., 1998).   These evidences suggest that activation of orexin signaling probably modulates a variety of biological systems.   In fact, increasing evidence have suggested that orexins are implicated in a wide variety of physiological functions. These include feeding (Sakurai et al., 1998, Yamada et al., 2000), behavioral activity (Ida et al., 1999), sleep/awake (Chemelli et al., 1999), anxiety/depression (Suzuki et al., 2005; Brundin et al., 2007; Lutter et al., 2008; Ito et al., 2008), energy balance (Lubkin et al., 1998), neuroendocrinological response (Kuru et al., 2000) and cardiovascular functions (Shirasaka et al., 1999). In addition, orexin-A acts centrally to regulate gastrointestinal functions such as gastric and pancreatic secretion, and gastrointestinal motility (Okumura and Nozu, 2011).

Visceral sensation is also known as one of key gastrointestinal functions which are controlled by the central nervous system (Mayer and Gebhart, 1994; Longstreth et al., 2006).   Little is, however, known about a role of central orexin in visceral sensation.   The present study was therefore performed to clarify whether brain orexin may be involved in the

process of visceral sensation.   Visceral sensation was evaluated by colonic distension-induced abdominal withdrawal reflex in conscious rats.

2.   Results

First, we examined the effect of exogenously administered orexin-A on visceral sensitivity.   Figure 1 demonstrates the effect of intracisternal orexin-A on the threshold volume of colonic distension-induced abdominal withdrawal reflex (AWR) in rats. Intracisternally administered orexin-A dose-dependently increased the threshold volume of colonic distension-induced AWR.   In contrast, intraperitoneal injection of Orexin-A at a dose of 10 µg failed to alter the threshold volume (Table 1), suggesting that orexin-A acts centrally in the brain to enhance antinociceptive response to colonic distension.   In addition, intracisternal injection of orexin-B at a dose of 10 µg did not change the threshold volume (Table 1), suggesting that orexin-A specifically possesses an antinociceptive action to colonic distension.

In the next step, we tried to clarify whether endogenous orexin-A is indeed involved in the antinociceptive action against colonic distension. As shown in Figure 2, morphine, a well-known drug for pain, significantly increased the threshold volume of colonic distension-induced AWR. While intracisternal SB334867, an OX1R antagonist, by itself failed to change the threshold volume, SB334867 injected centrally completely

blocked the morphine-induced antinociceptive action against colonic distension, suggesting that endogenous orexin-A indeed mediates the antinociceptive effect of morphine.

3.   Discussion

The present study demonstrated for the first time that orexin-A acts in the brain to induce an antinociceptive action against colonic distension. With regard to the role of central orexin in digestive functions, it has been reported that orexin acts in the brain to stimulate gastric and pancreatic secretion, and increase gastric and colonic motility in rats (Takahashi et al., 1999; Okumura et al., 2001; Yamada et al., 2005; Nozu et al., 2011; Nozu et al., 2012; Bülbül et al., 2010a and 2010b; Kobashi et al., 2002; Krowicki et al., 2002; Miyasaka et al., 2002).   The present study therefore provided a novel evidence that central orexin plays a role in not only gastrointestinal secretion and motility but also gut sensation.   To our knowledge, CRF is an only peptide in the brain that has been reported to be implicated in the colonic distension-induced AWR in rats as following.   Gue et al. (1997) have reported that intracerebroventricular CRF enhanced the number of abdominal cramps evoked by rectal distension and alpha-helical CRF9-41 antagonized the CRF-induced enhancement of abdominal cramps in rats. In addition, centrally administered CRF induced a colonic hypersensitivity in Fisher 344 rats (Greewood-Van Meerveld et al., 2005).   Thus, CRF acts

centrally to increase visceral pain sensation.   In contrast, orexin-A

decreased the colonic distension-induced pain sensitivity as shown in this

study.   We would therefore suggest that orexin might be a unique peptide

in the brain that is capable of inducing an antinociceptive action against

colonic distension.

The present data demonstrated that an enhanced antinociceptive

action against colonic distension was seen after intracisternal injection of

orexin-A but not orexin-B, suggesting that orexin-A plays a role in the

regulation of visceral sensation in a specific manner. According to the

results by Sakurai et al. (1998), orexins binds to two specific receptors

named orexin 1 receptor (OX1R) and orexin 2 receptor (OX2R).

Orexin-A has high affinity for OX1R but orexin-B has significantly lower

affinity for OX1R. In contrast, orexin-A and orexin-B have high affinity for

OX2R equally. Based upon these results, they suggested that OX2R is a

nonselective receptor for both orexin-A and -B, while OX1R is selective

for orexin-A. Considering the characterization of orexin receptors, the

present results that centrally administered orexin-A but not orexin-B

enhanced the AWR threshold volume may indicate that OX1R mediates the

orexin-A-induced alternation of visceral sensation.

The involvement of OX1R in the enhanced AWR threshold

volume was furthermore supported by the present finding that intracisternal

SB334867, a specific OX1R antagonist, completely blocked the

antinociceptive action of morphine.   The above result additionally provided a solid evidence that endogenous orexin-A in the brain is indeed involved in the regulation of visceral sensation.   Morphine induced analgesia is mediated through spinal and supraspinal mechanisms (Barton et al., 1980).   In the current study, the subcutaneous administration of morphine produced an antinociceptive effect against colonic distension. The antinociceptive effects of morphine administration were blocked by a selective OX1R antagonist, SB-334867, suggesting that some parts of the analgesic effect of morphine on visceral sensation are mediated by orexinergic system through the activation of OX1R.

Orexin nerve fibers are identified widely throughout the central nervous system.   Among them, regions shown to play a role in pain modulation such as periaqueductal gray (PAG) and rostral ventromedial medulla (RVM) are also innervated by orexin (Peyron et al., 1998). The lateral hypothalamus that exclusively expresses orexin has a key role in the modulation of nociceptive behavior through the PAG and RVM (Behbehani et al., 1988; Holden and Pizzi, 2008).   These findings suggest that orexin may act in the PAG and/or RVM neurons to induce the antinociceptive action against colonic distension.

Irritable bowel syndrome (IBS) is a functional disorder of the gastrointestinal (GI) tract (Longstreth et al., 2006; Drossman, 2006). Brain-gut interaction plays an important role in the pathophysiology of IBS.

Several studies have shown that visceral hypersensitivity to mechanical distension of the gut in IBS is related to symptom severity and it is considered to play a role in the pathophysiology of IBS (Mayer and Gebhart, 1994; van der Veek et al., 2008; Kanazawa et al., 2008; Kuiken et al., 2005; Posserud et al., 2007).   The present findings that brain orexin plays a regulatory role in visceral sensation, suggesting that orexin in the central nervous system might be involved in the development of IBS. Based upon the above speculation, we would suggest that orexinergic signaling in the brain may be a novel therapeutic target for patients with IBS.

4.   Experimental procedures

4.1.   Animals

Male Sprague-Dawley rats weighing about 200 g were housed under controlled light/dark conditions (lights on: 07:00−19:00) with the room temperature regulated to 23−25°C. Rats were allowed free access to standard rat chow (Solid rat chow, Oriental Yeast Co., Tokyo, Japan) and tap water.   All experiments were performed in conscious animals deprived of food for 24 h but with free access to water up to the initiation of the experiments.

4.2.   Chemicals

Synthetic orexin-A (human/bovine/rat/mouse) and orexin-B (rat/mouse) was purchased from Peptide Institute Inc., Osaka, Japan and were dissolved in normal saline just before the experiments. Selective orexin-A antagonist, SB-334867 (Tocris Bioscience, Ellisville, MO) (Smart et al., 2001) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO). Morphine hydrochloride (Hokuyaku Co., Hokkaido, Japan) was dissolved in saline.

4.3.   Implantation of electrodes and placement of colorectal balloon

Electrodes for measuring abdominal muscle contractions electrophysiologically were acutely implanted on the day of the experiment as described previously (Nozu et al., 2014).   Briefly, under ether anesthesia, skin incision about 5 mm in length was made in rats. The electrodes (Teflon coated stainless steel, 0.05 mm diameter, MT Giken, Tokyo, Japan) were inserted approximately 2 mm into left side external oblique musculature through the incision and fixed by cyanoacrylate instant adhesive (Aron Alpha, TOAGOSEI, Tokyo, Japan) together with the incised skin. The electrode leads were externalized through this closed incision and threaded through a urethane tube.   Immediately after implantation of electrorodes, a distension balloon was inserted intra-anally with the distal end positioned 2 cm proximal to the anus.   A 6-Fr (2 mm external diameter) disposable silicon balloon-urethral catheter for pediatric

use (JU-SB0601, Terumo Co., Tokyo, Japan) was used. The maximal inflation volume for the balloon was 1.5 mL and the length of the maximally inflated balloon was 1.2 cm. The balloon was secured in place by taping the catheter to the tail.

4.4. Detection of visceral sensitivity

Visceral pain in response to colonic distension was assessed by abdominal muscle contractions in conscious rats, which was validated as quantitative measure of visceral nociception (Ness and Gebhart, 1988). After completing the surgery for electrodes implantation and balloon placement, the rats were placed in Bollman cages while still sedated, and were allowed to recover and adjust for 20 min before testing. Then electrode leads were connected to a custom made electromyogram (EMG) amplifier. EMG signals were amplified, filtered (3000 Hz) and digitized by a PowerLab system, and stored by computer software (LabChart 7). Colonic distension was performed according to a previous publication (Tang et al., 2013). After the animals were fully awake and adjusted to the environment, ascending-limit phasic distension was applied in increments of 0.1 ml. (0.1-1.2 mL) was applied for 5 s every 10 s to induce colonic distension until a visible contraction of the abdominal wall was observed by an investigator blinded to the treatment. The balloon was distended with water and the abdominal withdrawal reflex (AWR) was

detected to examine the pain threshold volume by EMG as previously

described (Qin et al., 2012). The pain threshold volume was defined as

the intensity of colonic distension that elicited an observable AWR, i.e., a

sudden and persistent abdominal muscle contraction.

4.5   Experimental procedures

We initially examined the dose-related effects of intracisternal

injection of orexin-A on the colonic distension-induced AWR threshold

volume. Rats received intracisternal injections of several doses of

orexin-A. The doses used in this study were basically selected according

to previous reports (Takahashi et al., 1999). Control animals were

injected with normal saline (10 μL) intracisternally. Intracisternal

injection was performed under brief ether anesthesia with a

10-μL-Hamilton microsyringe after rats were mounted in a stereotaxic

apparatus (David Kopf Instruments, Ti- junga, CA) as reported previously

(Okumura et al., 1994). Intraperitoneal injection of 10 μg of orexin-A

dissolved in 0.5 mL of saline was performed to examine the possibility that

centrally administered orexin-A was acting periphery by leaking into the

circulation. As a control, normal saline (0.5 mL) alone was injected

intraperitoneally. Next, we examined the effects of intracisternal injection

of orexin-B on visceral sensation. To clarify whether endogenous orexin

may be involved in the regulation of visceral sensation, we examined the

effect of intracisternal injection of SB334867, a specific OX1R antagonist,

on the subcutaneously administered morphine-induced antinociceptive

action against colonic distension. Following the intracisternal or

intraperitoneal injection, and/or subcutaneous injection, rats received

implantation of electrorodes and placement of balloon, and were moved to ballman cages to evaluate the AWR threshold volume as described above.

4.6. Statistical analysis

For statistical analysis of the data, data were expressed as means ± S.E. Student's t-test and one-way ANOVA followed by Dunnett's multiple comparison test were used. Values of $P < 0.05$ were considered statistically significant.

4.7. Ethical considerations

The approval of the Research and Development and Animal Care committees at the Asahikawa Medical University was obtained for all studies.

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Figure legends

Figure 1.

Effect of intracisternal injection of orexin-A on the colonic distension-induced abdominal withdrawal reflex (AWR) threshold volume in conscious rat. Each column represents the mean ± S.E. Number of rats was shown in the parenthesis. *P < 0.01, when compared with control.

Figure 2.

Effect of intracisternal injection of SB334867, an OX1R antagonist, on the subcutaneously administered morphine-induced antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± S.E. Number of rats was shown in the parenthesis. *P < 0.01, when compared with saline. **P < 0.01, when compared with DMSO + morphine. SB; SB334867.
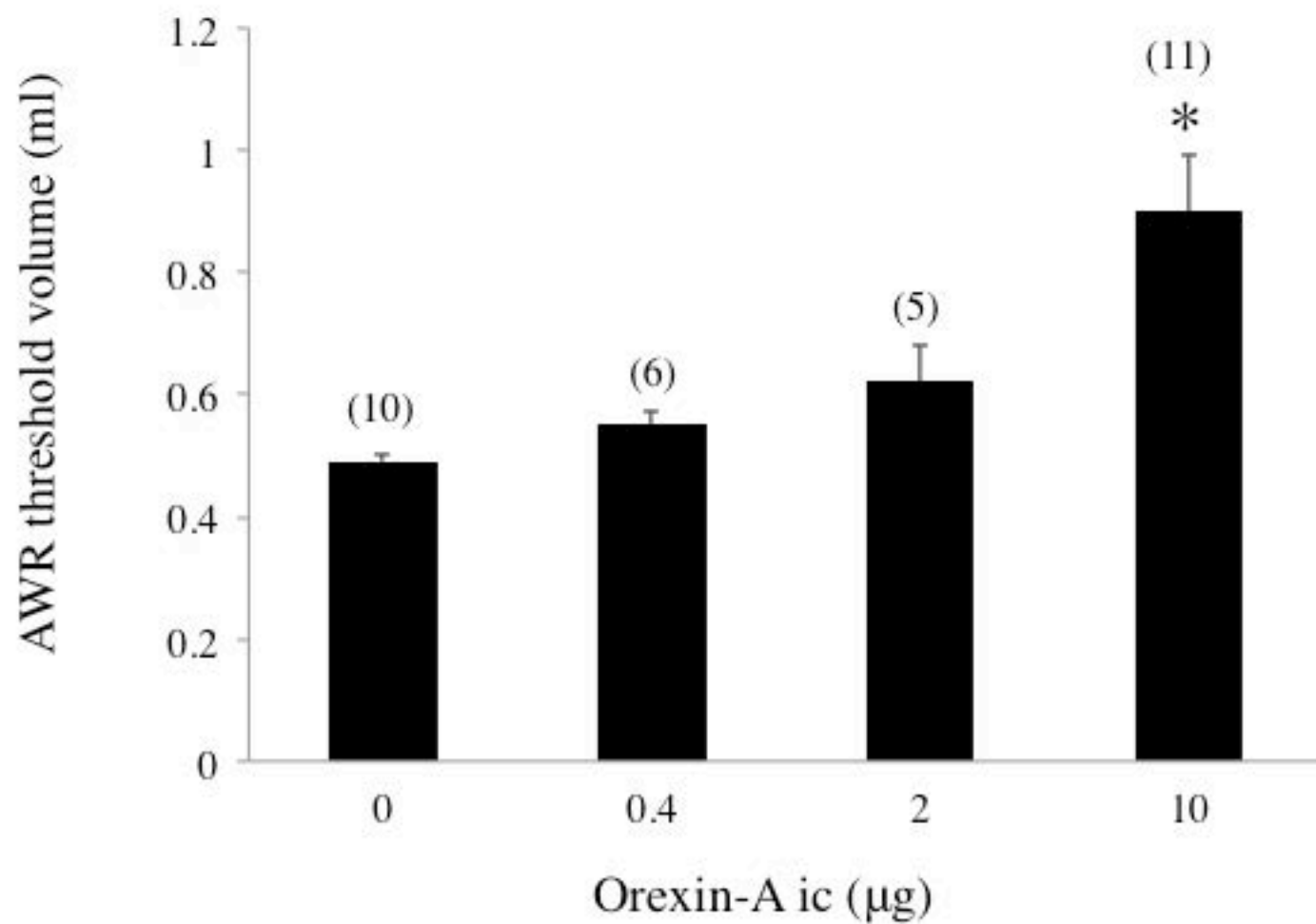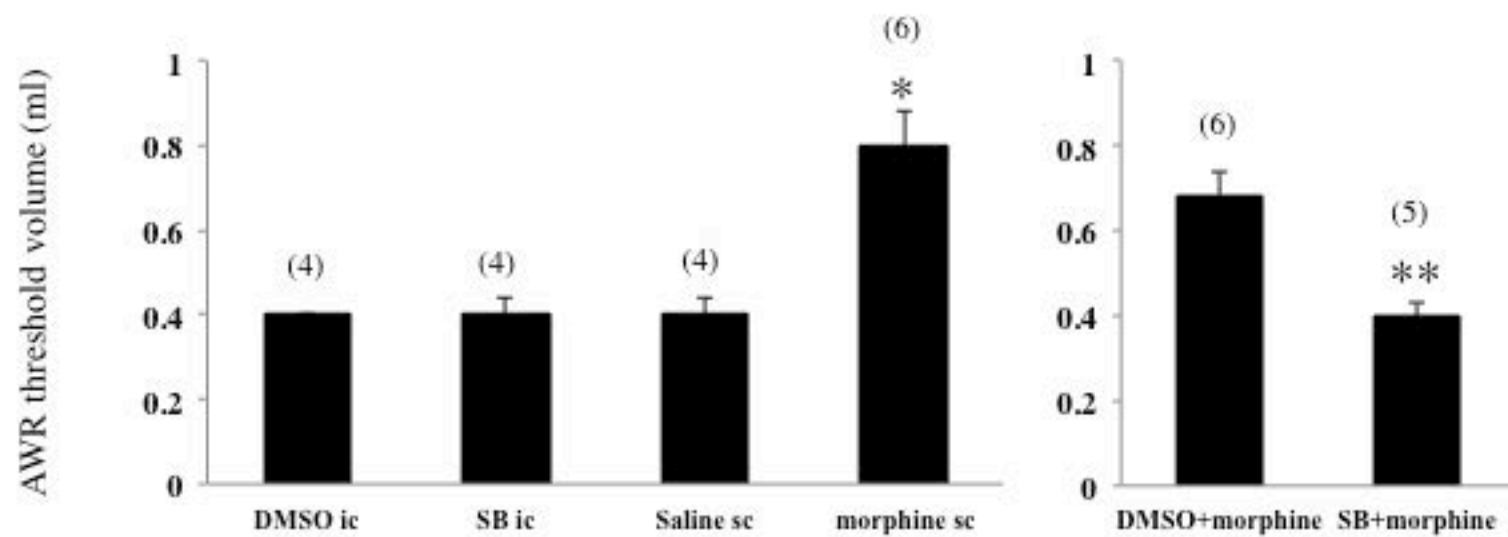
Figure 1

Figure 2

Table 1. Effect of intraperitoneal orexin-A and intracisternal orexin-B on colonic distension-induced visceral pain in rats

	N	AWR threshold volume (ml)
Saline (ip)	5	0.48 ± 0.02
Orexin-A 10 µg (ip)	6	0.47 ± 0.03
Saline (ic)	4	0.43 ± 0.03
Orexin-B 10 µg (ic)	5	0.46 ± 0.04

Each data represents the mean ± S.E. AWR; abdominal withdrawal reflex. ip; intraperitoneal, ic; intracisternal