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Short Communication

Role of the cannabinoid signaling in the brain orexin- and ghrelin-induced visceral antinociception in conscious rats

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ABSTRACT

We hypothesized that the cannabinoid (CB) system may mediate the brain orexin- or ghrelin-induced visceral antinociception. Intraperitoneal injection of either $CB_{1/2}$ agonist, WIN 55212 or O-Arachidonoyl ethanolamine increased the threshold volume of colonic distension-induced abdominal withdrawal reflex in rats, suggesting CB could induce visceral antinociception. Pretreatment with either the CB_1 or CB_2 antagonist potently blocked the centrally injected orexin-A-induced antinociceptive action against colonic distension while CB_2 but not CB_1 antagonist blocked the brain ghrelin-induced visceral antinociception. These results suggest that the cannabinoid signaling may be involved in the central orexin- or ghrelin-induced antinociceptive action in a different mechanistic manner.

Visceral pain sensation is an important physiological function in the gastrointestinal tract. For example, visceral hypersensitivity reflected by enhanced perception of physiological signals from the gut is commonly considered to play a major role in the pathophysiology of functional gastrointestinal disorders such as irritable bowel syndrome (IBS).[1] However, the mechanisms of regulation of visceral sensation in the brain have not been fully understood. We have recently demonstrated that orexin or ghrelin acts in the brain to induce visceral hyposensitivity,[2,3] suggesting that neuropeptides may play a role in the pathophysiology of IBS.

Biological effects of cannabinoids (CB) are mediated primarily through specific cannabinoid receptors (CB_1 and CB_2).[4] Among the roles of the cannabinoid system, the CB system may be implicated in the visceral antinociception through CB_1 and CB_2 receptors.[5]

Since either orexin or ghrelin acts centrally to induce a visceral antinociception[2,3] and cannabinoid could induce visceral hyposensitivity,[5] we made a hypothesis that the CB signaling may mediate the orexin- or ghrelin-induced visceral antinociception. In the present study, we tried to clarify the above speculation.

Visceral sensation was assessed by abdominal withdrawal reflex (AWR) by colonic distention using electromyogram (EMG) in conscious rats, which was validated as quantitative measure of visceral nociception as described previously.[2,3]

Male Sprague–Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing about 200 g were housed under controlled light/dark conditions. Rats were allowed free access to standard rat chow and tap water. All of the experiments were performed in 24 h-fasted rats. Approval was obtained from the Research and Development and Animal Care committees at Asahikawa Medical University for all studies.

The specific $CB_{1/2}$ agonists, WIN 55,212 and O- Arachidonoyl Ethanolamine (Cayman Chemical, Ann Arbor, Michigan, USA), a CB_1 receptor antagonist, AM251 and a CB_2 receptor antagonist, AM630 (Wako Chemical, Osaka, Japan) were dissolved in 100% dimethyl sulfoxide (DMSO). Synthetic orexin-A and ghrelin were purchased from Peptide Institute, Osaka, Japan, and it was dissolved in normal saline.

Initially, we examined the dose-dependent effects of intraperitoneal injection of $CB_{1/2}$ agonists on the colonic distension-induced AWR threshold volume. Rats received intraperitoneal injections of several doses of WIN 55,212 or O-Arachidonoyl Ethanolamine.

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Next, to clarify whether CB is involved in the central orexin- or ghrelin-induced antinociceptive action, we examined the effect of the subcutaneous injection of either AM251 (0.5 mg/rat) or AM630 (0.5 mg/rat) on the intracisternally administered orexin-A (10 μg) or ghrelin (10 μg)-induced antinociceptive action. We selected the doses of orexin,[2] ghrelin,[3] AM251[6] or AM630[7] according to previous studies.[2,3] Next, we examined the effect of intracisternal or subcutaneous injection of CB antagonists at a much smaller dose (10 μg) on the neuropeptides-induced antinociception. Immediately after administration of CB antagonists, intracisernal injection of neuropeptides was performed. Following injections, the rats were implanted with electrodes, and the balloons were inserted, after which the rats were moved into Ballman cages to detect visceral sensitivity as below.

The data were expressed as means ± standard error (SE). The data were compared with one-way analysis of variance followed by Dunnett's multiple comparisons test. $P < 0.05$ was considered statistically significant.

First, the effects of CB receptor agonists on the visceral sensation were examined in conscious rats. Intraperitoneal injection of either $CB_{1/2}$ agonist, WIN 55212 or O-Arachidonoyl Ethanolamine increased the threshold of AWR in a dose-dependent manner (Fig. 1), suggesting that activation of CB signaling could induce an antinociceptive action against colonic distension possibly through CB receptors.

Next, we examined the effects of subcutaneous injection of CB_1 or CB_2 antagonist on the brain orexin- or ghrelin-induced visceral antinociception. As demonstrated in Fig. 2, subcutaneously administered AM251 or AM630 (500 μg) by it self failed to change the AWR threshold volume. Either AM251 or AM630 significantly blocked the intracisternally injected orexin-A-induced antinociceptive action against colonic distension, respectively. On the other hand, AM630 but not AM251 blocked the brain ghrelin-induced antinociception.

To clarify whether systemic administered CB antagonists act centrally, we next examined intracisternal injection of CB antagonists at a much smaller dose (10 μg). Intracisternal or subcutaneous injection of 10 μg dose of either AM251 or AM630 by itself did not change the threshold (data not shown). Intracisternally but not subcutaneously injected 10 μg dose of AM630 significantly blocked the intracisternal orexin- or ghrelin-induced antinociception while the same dose of AM251 injected intracisternally failed to block (Fig. 3).

Increasing evidence suggest that CB_1 and CB_2 receptors may be involved in the regulation of visceral sensation.[8,9] The present study demonstrated that CB agonists induced visceral hyposensitivity in rats, confirming that CB is capable of inducing an antinociceptive action against colonic distension possibly through its specific receptors such as CB_1 and CB_2 receptors in the present rat model.

Because either orexin or ghrelin acts centrally to induce visceral hyposensitivity,[2,3] we made a hypothesis that CB_1 and/or CB_2 receptors may mediate the central orexin or ghrelin-induced visceral hyposensitivity. As demonstrated in this study, the orexin-induced visceral hyposensitivity was potently blocked by either AM251, a CB_1 antagonist or AM630, a CB2 antagonist, respectively, suggesting that CB_1 and CB_2 receptors may mediate the orexin-induced visceral antinociception. On the other hand, the ghrelin-induced visceral hyposensitivity was significantly blocked by AM630 but not AM251, suggesting that CB_2 but not CB_1 receptors may mediate the ghrelin-induced visceral antinociception. These results suggest that the cannabinoid signaling may be involved in the central orexin- or ghrelin-induced antinociceptive action in a different mechanistic manner.

Intracisternal injection of 10 μg dose of AM630 significantly blocked while subcutaneous injection of 500 μg but not 10 μg dose of AM630 blocked the intracisternal orexin- or ghrelin-induced antinociception, suggesting that the site of action of CB_2 receptor antagonist may be in the brain. Li et al.[10] demonstrated recently that CB_2 receptors in the brain function to modulate pain, supporting the present speculation. With regard to the CB1 receptors,

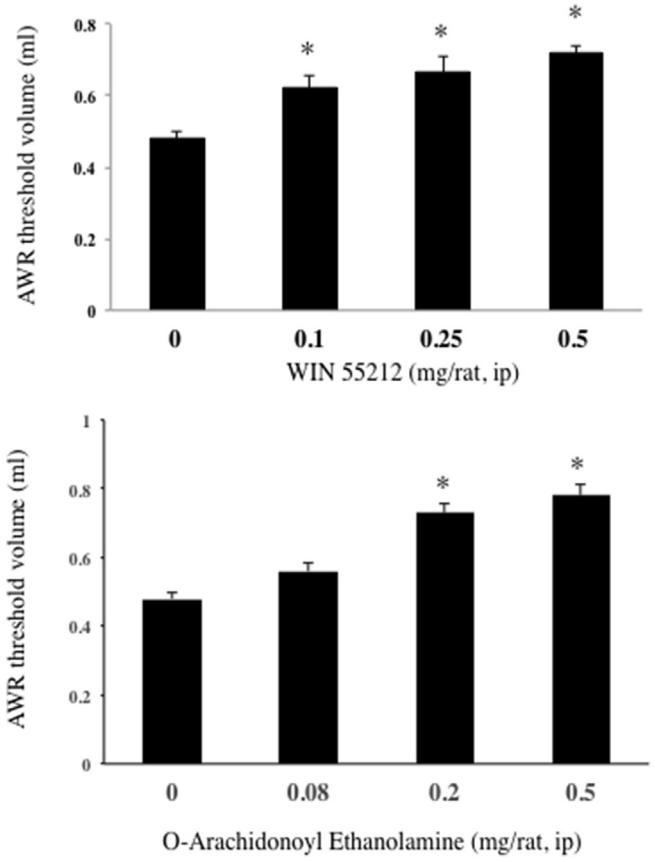

Fig. 1. Effect of intraperitoneal injection of either $CB_{1/2}$ agonist, WIN 55212 or O-Arachidonoyl Ethanolamine on the colonic distension-induced abdominal withdrawal reflex (AWR) threshold volume in conscious rat. Each column represents the mean ± S.E. Number of rats was 5–6 in each group. *P < 0.01, when compared with control.

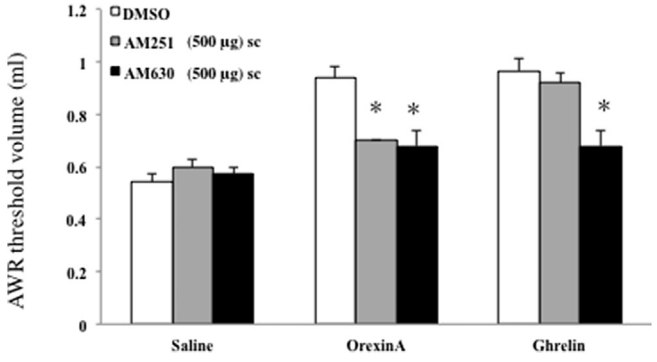

Fig. 2. Effects of the subcutaneous (sc) injection of CB_1 receptor antagonist, AM251 or CB_2 receptor antagonist, AM630 at a dose of 500 μg on the intracisternally (ic) administered orexin-A (10 μg/10 μl)- or ghrelin (10 μg/10 μl) -induced antinociceptive action against colonic distension in conscious rats. Immediately after administration of CB antagonists, intracisernal injection of neuropeptides was performed. Each column represents the mean ± SE. Number of rats was 5–8 in each group. *P < 0.01 compared with DMSO.

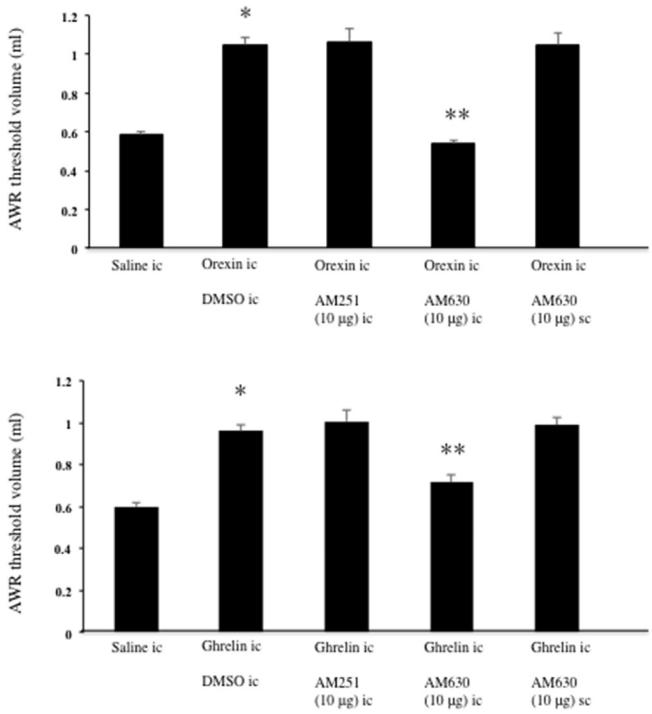

Fig. 3. Effects of the intracisternal (ic) or subcutaneous (sc) injection of CB_1 receptor antagonist, AM251 or CB_2 receptor antagonist, AM630 at a dose of 10 μg on the intracisternally (ic) administered orexin-A (10 μg/10 μl)- or ghrelin (10 μg/10 μl) -induced antinociceptive action against colonic distension in conscious rats. Immediately after administration of CB antagonists, intracisernal injection of neuropeptides was performed. Each column represents the mean ± SE. Number of rats was 5–7 in each group. *P < 0.01 compared with saline. **P < 0.01 compared with orexin ic + DMSO ic or ghrelin ic + DMSO ic, respectively.

the present findings that intracisternal injection of AM251 could not block the antinociception by orexin, central CB_1 receptors might not mediate the orexin-induced visceral antinociception.

We recently reported that centrally injected ghrelin induces visceral antinociception is blocked by orexin-1 receptor antagonist, suggesting that endogenous orexin may mediate the ghrelin-induced antinociception (Okumura et al., Brain Res 2018). On the other hand, the present results demonstrated that the antinociceptive action by ghrelin was significantly blocked by CB_2 but not CB_1 antagonist while the orexin-induced antinociception was blocked by not only CB_2 but CB_1 antagonist. The discrepancy may be explained by following speculation. Endogenously released orexin in the brain may preferentially activate the CB2 signaling while exogenously administered orexin may activate equally both CB1 and CB2 signaling. The above speculation may explain the discrepancy. Further studies should be needed to clarify the speculation.

In conclusion, the CB system may mediate the central orexin- or ghrelin induced antinociceptive action against colonic distension through CB_2 and/or CB_1 receptors. These findings may help in understanding the pathophysiology of altered visceral sensation in IBS.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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