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Adenosine A1 receptors mediate the intracisternal injection of orexin-induced antinociceptive action against colonic distension in conscious rats

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ABSTRACT

We have recently demonstrated that orexin acts centrally through the brain orexin 1 receptors to induce an antinociceptive action against colonic distension in conscious rats. Adenosine signaling is capable of inducing an antinociceptive action against somatic pain; however, the association between changes in the adenosinergic system and visceral pain perception has not been investigated. In the present study, we hypothesized that the adenosinergic system may be involved in visceral nociception, and thus, adenosine signaling may mediate orexin-induced visceral antinociception. Visceral sensation was evaluated based on the colonic distension-induced abdominal withdrawal reflex (AWR) in conscious rats. Subcutaneous (0.04–0.2 mg/rat) or intracisternal (0.8–4 μg/rat) injection of *N*(6)-cyclopentyladenosine (CPA), an adenosine A1 receptor (A1R) agonist, increased the threshold volume of colonic distension-induced AWR in a dose-dependent manner, thereby suggesting that CPA acts centrally in the brain to induce an antinociceptive action against colonic distension. Pretreatment with theophylline, an adenosine antagonist, or 1,3-dipropyl-8-cyclopentylxanthine, an A1R antagonist, subcutaneously injected potently blocked the centrally injected CPA- or orexin-A-induced antinociceptive action against colonic distension. These results suggest that adenosinergic signaling via A1Rs in the brain induces visceral antinociception and that adenosinergic signaling is involved in the central orexin-induced antinociceptive action against colonic distension.

1. Introduction

Hypocretins/orexins are neuropeptides that localize in neurons in the lateral hypothalamus [1,2]. Despite their highly restricted origin, orexin nerve fibers have been widely identified throughout the central nervous system [3,4], thereby suggesting that the activation of orexin signaling modulates various biological systems. In fact, increasing evidence suggests that orexins are implicated in several physiological functions. These include feeding, sleep/awake, anxiety/depression, and neuroendocrinological responses [5]. In addition, orexin-A acts centrally to regulate gastrointestinal functions such as gastric and pancreatic secretion and gastrointestinal motility [6–11]. Therefore, we recently examined the effect of centrally administered orexin on the colonic distension-induced visceral sensation in conscious rats and demonstrated that orexin induced an antinociceptive action against colonic distension [12].

Adenosine is a ubiquitous endogenous neuromodulator or neurotransmitter, which plays an important role in pain modulation [13].

It is known that spinal or systemic administration of adenosine and its analogs leads to antinociception in various animal models [13–15]. Data obtained from pharmacological experiments using adenosine agonists and antagonists indicate that the antinociceptive effects of adenosine and its analogs are mediated by the adenosine A1 receptor (A1R) [16–18]. It has also been demonstrated that mice lacking A1Rs exhibit increased nociceptive responses [19,20]. The effects of adenosine on antinociceptive responses have been widely studied; however, little is known about whether it is also involved in the antinociceptive action related to visceral pain sensation. In the present study, we hypothesized that central adenosine signaling plays a role in visceral antinociception via orexin signaling. Therefore, we examined whether adenosine signaling mediates the orexin-induced antinociceptive action against colonic distension.

2. Methods

2.1. Ethical considerations

Approval was obtained from the Research and Development and Animal Care committees at Asahikawa Medical University (No. 13030) for all of the experiments conducted in this study.

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2.2. Animals

Male Sprague–Dawley rats (Charles River Laboratory, Atsugi, Japan) weighing about 200 g were housed under controlled light/dark conditions (lights on: 07:00–19:00), and the room temperature was regulated at 23 °C–25 °C. Rats were allowed free access to standard rat chow (solid rat chow; Oriental Yeast Co., Tokyo, Japan) and tap water. All of the experiments were performed using conscious animals, which had been deprived of food for 24 h but with free access to water until the initiation of the experiments.

2.3. Chemicals

The specific A1R agonist, *N*(6)-cyclopentyladenosine (CPA) (Abcam, Tokyo, Japan), a nonselective adenosine receptor antagonist, theophylline (Wako Chemical, Osaka, Japan), and an A1R antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Tocris Bioscience, Bristol, UK), were dissolved in 100% dimethyl sulfoxide (DMSO) immediately before the experiments. Synthetic orexin-A (human) was purchased from Peptide Institute, Osaka, Japan, and it was dissolved in normal saline immediately before the experiments.

2.4. Implantation of electrodes and placement of colorectal balloon

The electrodes used to obtain electrophysiological measurements of abdominal muscle contractions were implanted acutely on the day of the experiment, as described previously [12]. Briefly, a skin incision measuring ca. 5 mm was created while the rats were under ether anesthesia. The electrodes (Teflon-coated stainless steel, 0.05 mm diameter; MT Giken, Tokyo, Japan) were inserted approximately 2 mm into the left side of the external oblique musculature through the incision and fixed to the incised skin with cyanoacrylate instant adhesive (Aron Alpha, Toagosei, Tokyo, Japan). The electrode leads were externalized through this closed incision and threaded through a urethane tube. Immediately after implanting the electrodes, a distension balloon was inserted intraanally where the distal end was 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, Tokyo, Japan) was employed. The maximal inflation volume of 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.

2.5. Detection of visceral sensitivity

The abdominal withdrawal reflex (AWR) test was performed as described previously to detect the pain threshold, which was defined as the intensity of colorectal distension that elicited AWR [21]. Previously, Tang et al. [22] evaluated the antinociceptive effect of a drug on visceral hypersensitivity in rats and demonstrated that changes in the AWR score reflected the balloon volume used for colonic distension and that the intracolonic pressure had a linear association with the intraballoon volume used in experiments. The balloon used in their study was quite similar to that used in the present study. Al-Chaer et al. [21] also demonstrated that the contraction of the abdominal muscles in rats increased in response to the graded enhancement of colonic distension. Lifting of the abdomen was consistently observed as a characteristic of AWR, and it was assumed to be accompanied by a strong contraction of the abdominal muscles [21]. Visual observations of the AWR in response to graded colonic distension were slightly difficult in the Ballman cages employed by Al-Chear et al. compared with platforms, as described previously [21]. Based on these findings, we considered the threshold volume (AWR threshold volume) to induce sudden and apparent abdominal muscle contractions, which were detected by an electromyogram (EMG), as a parameter for evaluating AWR in the present study. Briefly, colonic distension was performed as described previously [12], that is, an ascending method for limited phasic distension was applied by inflating the balloon manually with water using a syringe until the AWR was detected by the EMG. After completing the surgery for electrode implantation and balloon placement, the sedated rats were placed in Ballman cages where they were allowed to recover and adjust for 20 min before testing. Next, the electrode leads were connected to a custom-made EMG amplifier. The EMG signals were amplified, filtered (3000 Hz) and digitized using a PowerLab system and recorded using computer software (LabChart 7).

The pain threshold was assessed two times (2-min interval), and the mean of the threshold was calculated as the results for the animals. In the majority of animals, the pain threshold in the first test was consistent with that in the second test.

2.6. Experimental procedures

Initially, we examined the dose-dependent effects of subcutaneous or intracisternal injection using an A1R agonist, CPA, on the colonic distension-induced AWR threshold volume. Rats received subcutaneous or intracisternal injections of several doses of CPA. Control animals were injected with DMSO subcutaneously (0.5 ml) or intracisternally (10 μl). Intracisternal injection was performed under brief ether anesthesia using a 10-μl Hamilton microsyringe after the rats were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA), as reported previously [23]. Next, to clarify whether adenosine is involved in the central CPA- or orexin-induced antinociceptive action against colonic distension, we examined the effect of the subcutaneous injection of theophylline or DPCPX on the intracisternally administered CPA- (4 μg/10 μl) or orexin-A (10 μg/10 μl)-induced antinociceptive action against colonic distension. We selected the doses of theophylline (1 mg/kg) [24] and DPCPX (1 mg/kg) based on previous studies [25]. Recently, we demonstrated that intracisternal injection of orexin-A increased the threshold volume of colonic distension-induced AWR in a dose-dependent manner [12]; thus, the dose of orexin-A (10 μg/10 μl) was selected according to our previous study [12]. Following intracisternal and/or subcutaneous injection, the rats were implanted with electrodes, and the balloons were inserted, after which the rats were moved into Ballman cages to evaluate the AWR threshold volume as described above. In rats that received subcutaneous and intracisternal injections, immediately after injecting the chemicals subcutaneously, we injected orexin-A intracisternally, and we then completed the electrode implantation surgery and balloon placement. The procedures on each rat were performed within 5 min.

2.7. Statistical analysis

The data were expressed as means ± standard error (SE). The data were compared with the Student's *t*-test and one-way analysis of variance followed by Dunnett's multiple comparisons test. $P < 0.05$ was considered statistically significant.

3. Results

Fig. 1 shows the dose–response effects of CPA, an A1R agonist, on visceral sensation. Subcutaneous administration of CPA (0.04–0.2 mg/rat) increased the threshold of AWR in a dose-dependent manner (Fig. 1A). Intracisternal injection of CPA also increased the threshold of AWR in a dose-dependent manner (0.8–4 μg/rat; Fig. 1B). There was a significant dose-dependent difference in the antinociceptive response, thereby suggesting that the activation of central A1R by CPA induced the antinociceptive action against colonic distension. The antinociceptive action against colonic distension caused by intracisternal CPA at a dose of 4 μg/rat was blocked significantly by the subcutaneous injection of either theophylline or DPCPC (Fig. 2).

Next, to clarify whether central orexin-induced visceral antinociception is mediated by adenosine signaling, we examined the effects of an adenosine receptor antagonist, theophylline, or an A1R