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ABSTRACT

Levodopa possesses antinociceptive actions against several somatic pain conditions. However, we do not know at this moment whether levodopa is also effective to visceral pain. The present study was therefore performed to clarify whether levodopa is effective to visceral pain and its mechanisms. Visceral sensation was evaluated by colonic distension-induced abdominal withdrawal reflex (AWR) in conscious rats.

Subcutaneously (80 mg/rat) or intracisternally (2.5 μg/rat) administered levodopa significantly increased the threshold of colonic distension-induced AWR in conscious rats. The dose difference to induce the antinociceptive action suggests levodopa acts centrally to exert its antinociceptive action against colonic distension. While neither sulpiride, a D2 dopamine receptor antagonist, nor SCH23390, a D1 dopamine receptor antagonist by itself changed the threshold of colonic distension-induced AWR, the intracisternally injected levodopa-induced antinociceptive action was significantly blocked by pre-treatment with subcutaneously administered sulpiride but not SCH23390. Treatment with intracisternal SB334867, an orexin 1 receptor antagonist, significantly blocked the subcutaneously administered levodopa-induced antinociceptive action. These results suggest that levodopa acts centrally to induce an antinociceptive action against colonic distension through activation of D2 dopamine receptors and the orexinergic system in the brain.

1. Introduction

Levodopa is the drug to treat for Parkinson's disease (PD) (1) and also used clinically for the relief of pain from bone metastasis (2), pain induced by diabetic neuropathy and helps zoster (3,4). In experimental animals, levodopa induced an analgesic action. Shimizu et al. have demonstrated that intrathecal injection of levodopa attenuated the substance-P-induced nociceptive behavior in mice (5). Cobacho et al. (6) have shown an antiallodynic action of intrathecal levodopa in a rat model of painful mononeuropathy. Thus clinical and experimental studies have suggested that levodopa possesses antinociceptive actions against several somatic pain conditions.

Visceral pain sensation is also known as one of vital sensory functions. 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) (7–10). It has been reported that the same chemical is not necessarily capable of reducing not only somatic pain but visceral pain because Ide et al. (11) have showed that (−)-Pentazocine induces visceral chemical antinociception, but not thermal, mechanical, or somatic chemical antinociception, in μ-opioid receptor knockout mice. Since the association between levodopa and visceral pain perception has not been investigated while levodopa does possess antinociceptive actions against somatic pain conditions as described above, the present study was therefore performed to clarify whether levodopa is also effective to visceral pain and its mechanisms.

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2. Materials and methods

2.1. 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) 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.

2.2. Chemicals

Levodopa (Tokyo Chemical Industry, Tokyo, Japan) was dissolved in 100% dimethyl sulfoxide (DMSO) (Sigma–Aldrich, St. Louis, MO, USA). SCH23390, a D1 dopamine receptor antagonist and sulpiride, a D2 dopamine receptor antagonist (Wako Chemical, Osaka, Japan), were dissolved in 100% DMSO. Synthetic orexin-A (human/bovine/rat/mouse) was purchased from Peptide Institute Inc., Osaka, Japan and dissolved in normal saline. Selective orexin 1 receptor (OX1) antagonist, SB334867 (Tocris Bioscience, Ellisville, MO, USA) (12) was dissolved in 100% DMSO.

2.3. 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 (13). Briefly, with the rats under ether anesthesia, a ~5-mm incision skin was created. 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 the implantation of electrodes, a distension balloon was inserted intraanally 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, 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.

2.4. Detection of visceral sensitivity

Abdominal withdrawal reflex (AWR) test was performed as previously described to detect the pain threshold, which was defined as the intensity of colorectal distension that elicited AWR (14). Tang et al. (15) have evaluated an antinociceptive effect of a drug on visceral hypersensitivity in rats and demonstrated that the changes in AWR score paralleled the balloon volume for colonic distension and that intracolonic pressure was linearly associated with intraballoon volume in the experiments. The balloon used in the study is quite similar to the balloon used in the present study. Al-Chaer et al. (14) have demonstrated that a stronger contraction of the abdominal muscles in rats in response to graded colonic distension in rats. Lifting of abdomen was consistently observed as a characteristic of AWR and is supposed to be accompanied with a strong contraction of the abdominal muscles (14). Visual observations of the AWR in response to graded colonic distension were a little bit difficult in Ballman cages in this study when compared on platforms Al-Chear et al. (14) have shown. Based on these findings, we considered the threshold volume (AWR threshold volume) to induce sudden and apparent abdominal muscle contractions detected by EMG as a parameter for evaluating AWR as described previously (13,16). In briefly, colonic distension was performed, i.e., ascending method of limits phasic distension was applied by inflating the balloon by water using a syringe manually until the abdominal withdrawal reflex (AWR) was detected by EMG. After completing the surgery for electrode implantation and balloon placement as described above, the sedated rats were placed in Ballman cages 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 recorded using a computer software (LabChart 7).

The pain threshold was assessed two times (2 min interval) and the mean of the threshold was calculated as the data of the animals. In a large majority of the animals, the pain threshold at the first time was consistently equal to the second one.

2.5. Experimental procedures

We initially examined the dose-related effects of subcutaneous or intracisternal injection of levodopa on the colonic distension-induced AWR threshold volume. Rats received subcutaneous (0.5 ml) or intracisternal (10 μl) injections of several doses of levodopa. 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, Tujunga, CA) as reported previously (17). Next, to clarify whether dopamine receptors may be involved in the levodopa-induced antinociceptive action against colonic distension, we examined the effect of subcutaneous injection of D1 (SCH23390, 0.2 mg/rat) or D2 (sulpiride, 40 mg/rat) dopamine receptor antagonist on the intracisternally administered levodopa (10 μg/10 μl) – induced antinociceptive action against colonic distension. The doses of dopamine receptor antagonists were selected according to the previous report (16). To clarify whether endogenous orexin may be involved in the levodopa-induced antinociceptive action against colonic distension, we examined the effect of intracisternal injection of SB334867, a specific OX1R antagonist, (80 μg/10 μl) on the subcutaneously administered levodopa-induced antinociceptive action against colonic distension. Following the intracisternal and/or subcutaneous injection, rats received implantation of electrodes and placement of balloon, and were moved to Ballman cages to evaluate the AWR threshold volume as described above. In rats received subcutaneous and intracisternal injections, immediately after injection of chemicals subcutaneously, we injected levodopa intracisternally, and then completed the surgery for electrode implantation and balloon placement. These procedures for each rat were performed within 5 min.

2.6. Statistical analysis

For statistical analysis of the data, data were expressed as means ± S.E. One-way ANOVA followed by Dunnett's multiple comparison test were used. Values of $P < 0.05$ were considered statistically significant.

2.7. Ethical considerations

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

3. Results

Because levodopa is used as systemic administration in clinical setting, we examined first the effect of subcutaneous levodopa on the colonic distension-induced visceral sensation. As demonstrated in Fig. 1, subcutaneously administered levodopa significantly increased the threshold of colonic distension-induced AWR in conscious rats. Next, the effect of intracisternal levodopa on the visceral sensation was examined. As clearly shown in Fig. 2, intracisternal levodopa dose-dependently increased the threshold of colonic distension-induced AWR. The dose difference to induce the antinociceptive action suggests levodopa acts centrally in the brain to exert its antinociceptive action against colonic distension.

Next, the effects of dopamine antagonists on the levodopa-induced antinociceptive action were examined to clarify whether endogenous dopamine signaling may be implicated in the levodopa-induced visceral antinociception. While neither sulpiride, a D2 dopamine receptor antagonist, nor SCH23390, a D1 dopamine receptor antagonist by itself changed the threshold of colonic distension-induced AWR, the levodopa-induced antinociceptive action was significantly blocked by pretreatment with sulpiride but not SCH23390 (Fig. 3), suggesting that the D2 dopamine receptors may mediate the levodopa-induced antinociceptive action against colonic distension.

Finally, an effect of an OX1 antagonist on the levodopa-induced antinociceptive action against colonic distension was investigated to clarify whether endogenous brain orexin may play a role in the levodopa-induced antinociception. Treatment with intracisternal SB334867 (80 μg/10 μl) significantly blocked the subcutaneously administered levodopa-induced antinociceptive action while SB334867 by itself failed to alter the threshold of AWR (Fig. 4), suggesting that endogenous orexin may be involved in the antinociceptive action by levodopa and also the levodopa-induced antinociceptive action is exerted through central OX1 receptors.

4. Discussion

Although studies have suggested that levodopa possesses antinociceptive actions against several somatic pain conditions, we do not know whether levodopa is also effective to visceral pain. The present study was performed to clarify whether levodopa is effective to visceral pain and its mechanisms. Subcutaneous injection of levodopa induced an antinociceptive action against colonic distension, suggesting that levodopa is capable of inducing antinociception for also visceral pain. Wang et al. (18) have demonstrated that peripheral injection of levodopa induced Fos expression in several brain sites such as nigrostriatal system, the paraventricular nucleus of hypothalamus (PVN), the arcuate nucleus (Arc), the central nucleus of amygdala (CeA), lateral parabrachial nucleus (LPB), nucleus tractus solitarius (NTS) and area postrema (AP) in rats. The NTS, the major relay center of visceral afferents (19) is directly connected to the LPB, PVN, and CeA (20–22). The AP receives chemical signals outside the blood-brain-barrier and is reciprocally connected to the NTS (23), suggesting that levodopa injected peripherally may send signals to the NTS via the circulation. These results suggest that peripherally administered levodopa activates a neuronal system in the central nervous system which may be related to visceral pain perception through a direct or indirect manner. To clarify whether levodopa act directly in the brain to induce the antinociceptive action against colonic distension, we next examined the effect of intracisternal injection of levodopa on visceral sensation. Because centrally injected levodopa at much smaller doses when compared with doses injected peripherally could induce the antinociceptive action against colonic distension as seen in this study, the site of action of levodopa is strongly considered to be within the central nervous system.

With regard to the mechanisms by which levodopa exerts its antinociceptive action, central dopaminergic signaling may be involved in the process because levodopa is the precursor to the neurotransmitter dopamine and exogenously administered levodopa increases dopaminergic concentrations (1). In addition, we have very recently demonstrated that the dopamine signaling in the brain induced an antinociceptive action against colonic distension through D1 and D2 dopamine receptors (16). We therefore examined the involvement of dopamine receptors in the levodopa-induced antinociception against colonic distension. As clearly demonstrated in this study, levodopa specifically induced the

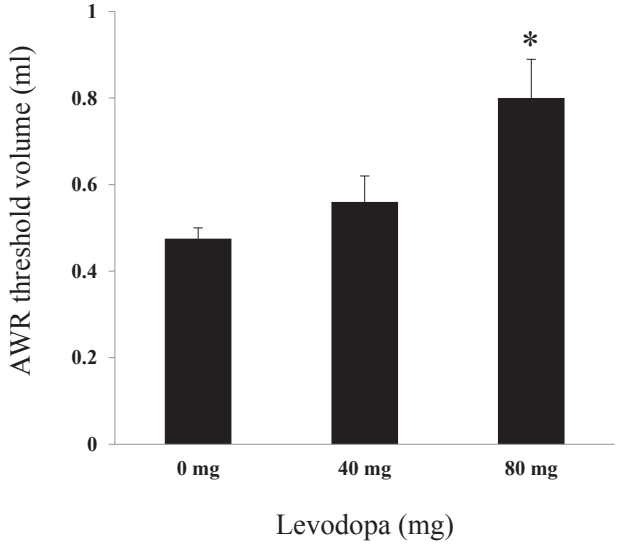

Fig. 1. Effect of subcutaneous injection of levodopa on the colonic distension-induced abdominal withdrawal reflex (AWR) threshold volume in conscious rat. Each column represents the mean ± S.E. Number of rats was 5 in each group. *P < 0.01, when compared with control.

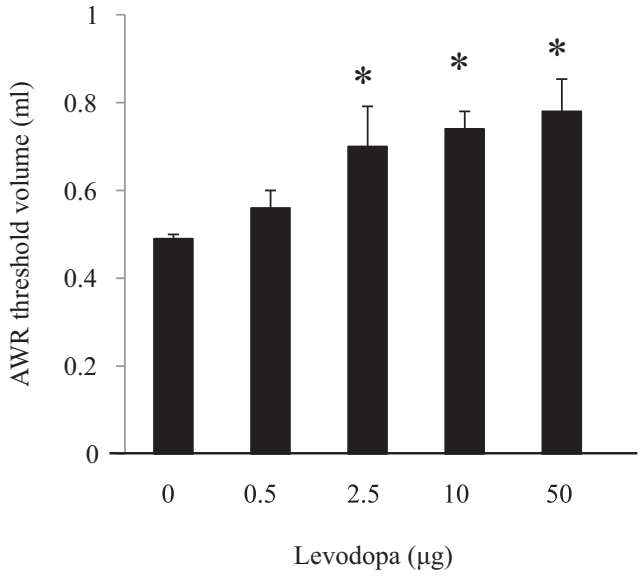

Fig. 2. Effect of intracisternal injection of levodopa on the colonic distension-induced abdominal withdrawal reflex (AWR) threshold volume in conscious rat. Each column represents the mean ± S.E. Number of rats was 5 in each group. *P < 0.01, when compared with control.

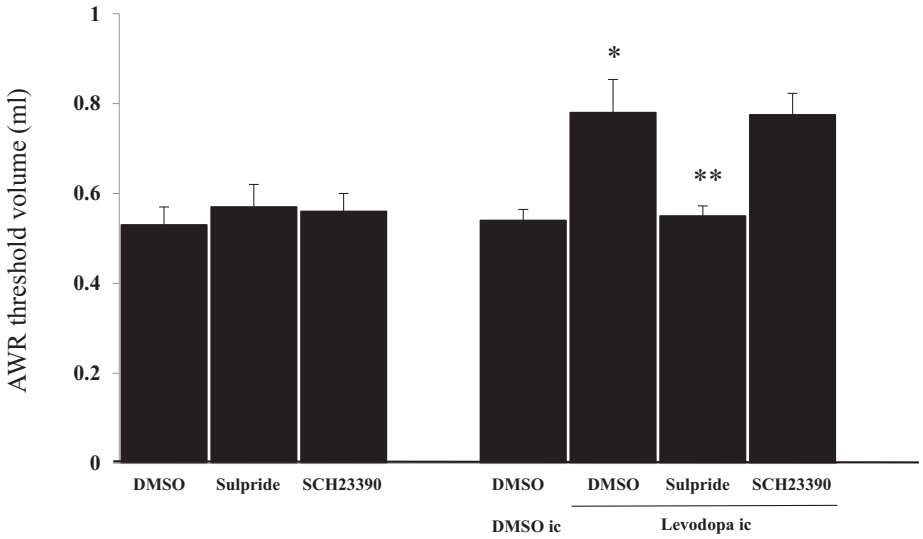

Fig. 3. Effect of subcutaneous injection of D1 dopamine receptor antagonist (SCH23390) or D2 dopamine receptor antagonist (sulpiride) on the intracisternally (ic) administered levodopa-induced antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± S.E. Number of rats was 5–6 in each group. *P < 0.01, when compared with DMSO sc + DMSO ic. **P < 0.01, when compared with DMSO sc + levodopa ic.

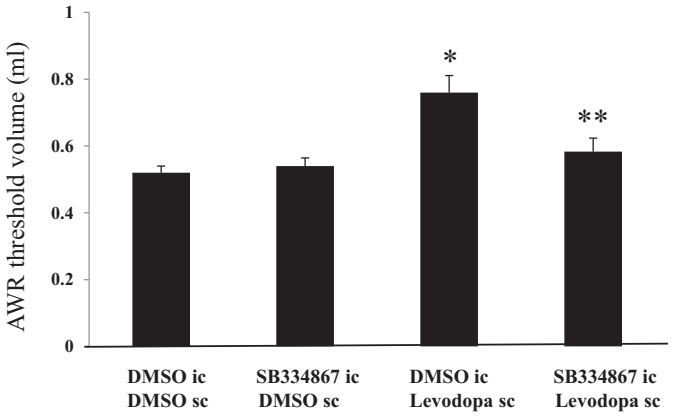

Fig. 4. Effect of intracisternal injection of SB334867, an OX1 receptor antagonist, on the levodopa-induced antinociceptive action against colonic distension in conscious rats. Each column represents the mean ± S.E. Number of rats was 6 in each group. *P < 0.01, when compared with DMSO ic + DMSO sc. **P < 0.01, when compared with DMSO ic + levodopa sc.

antinociception through D2 dopamine receptors because sulpiride, a D2 dopamine receptor antagonist but not SCH23390, a D1 dopamine receptor antagonist, potently blocked the levodopa-induced visceral antinociception. These results suggest that levodopa acts centrally in the brain to induce antinociceptive action against colonic distension through activation of dopamine D2 receptors.

Decreased dopamine signaling plays a key role in the pathophysiology of PD (24). Long-term levodopa use is associated with the "End of Dose Wearing Off" (EODWO) phenomenon wherein Parkinsonian symptoms return before a patient's next scheduled dose of levodopa. Abdominal pain is an important wearing off symptom as an early indicator of the development of EODWO in PD patients (25), suggesting that reduced dopamine signaling might induce visceral hypersensitivity. The present finding that levodopa evoked a visceral antinociceptive action via the dopamine signaling may explain the mechanism by which abdominal pain is considered as EODWO phenomenon in PD patients.

Orexins are neuropeptides that are localized to neurons in the lateral hypothalamus (26,27). Despite their highly restricted origin, orexin nerve fibers have been identified throughout the central nervous system (28,29), suggesting that activation of orexin signaling modulates various biological systems. Increasing evidence has demonstrated that orexin-A acts centrally to regulate gastrointestinal functions such as gastric secretion and gastrointestinal motility (30–34). In addition, we have very recently demonstrated that not only exogenously administered but also endogenously released orexin in the brain induces an antinociceptive action against colonic distension in conscious rats (13), indicating a role of brain orexin in the regulation of visceral sensation. We therefore made a hypothesis that endogenous orexin in the brain may mediate the levodopa-induced antinociceptive action against colonic distension. To clarify the hypothesis, the effect of intracisternal SB334867, a specific OX1 receptor antagonist on the subcutaneous levodopa-induced antinociceptive action against colonic distension was examined. As clearly shown in this study, SB334867 significantly blocked the levodopa-induced antinociceptive action against colonic distension, suggesting that endogenous orexin in the brain mediates the levodopa-induced antinociceptive action through OX1 receptors.

SB334867 should be injected centrally to block the brain orexin action as reported previously (13). On the other hand, the dopamine antagonists used in the present study could be injected peripherally to block the brain dopaminergic signaling as reported previously (17). Since we considered that two chemicals should be administered via different routes (intracisternal and subcutaneous) to rule out unexpected reactions when injected via a same intracisternal route, we selected the injection routes as shown in this study. Not only intracisternal but also subcutaneous levodopa is capable of inducing an antinociceptive action as shown in the present study, we planned the experimental protocol to examine the effects of dopamine antagonists or the orexin antagonist on the levodopa-induced visceral antinociception. Levodopa was therefore injected intracisternally in Fig. 3 while levodopa was administered subcutaneously in Fig. 4.

We have recently demonstrated that not only dopamine D2 receptor agonist but also dopamine D1 agonist could induce an antinociceptive action against colonic distension in rats, suggesting that dopamine is capable of inducing antinociception through dopamine D1 and D2 receptors (17). We have furthermore reported that centrally injected orexin-A induces antinociceptive action against colonic tension is blocked by either dopamine D1 or D2

receptor antagonist, suggesting that both dopamine D1 and D2 receptors mediate the centrally injected orexin-A-induced visceral nociception (17). On the other hand, the present results demonstrated that the antinociceptive action by levodopa was significantly blocked by SB334867, an orexin 1 receptor antagonist, or dopamine D2 receptor antagonist, but not D1 antagonist. The discrepancy may be explained by following speculation. Endogenously released orexin in the brain induced by levodopa may preferentially activate the D2 dopamine signaling while exogenously administered orexin may activate equally both dopamine D1 and D2 receptors signaling. Further studies should be needed to clarify the speculation.

We have very recently demonstrated that the dopamine signaling in the brain induced an antinociceptive action against colonic distension and the dopamine signaling may mediate the brain orexin-induced antinociception (16). In turn, the present findings indicate that endogenous orexin may mediate the levodopa-induced antinociception. Levodopa is the precursor to the neurotransmitter dopamine and exogenously administered levodopa increases dopaminergic concentrations (1). In addition, the present study clearly showed that the dopamine D2 receptor antagonist blocked the levodopa-induced antinociception, suggesting that the endogenous dopamine signaling is involved in the antinociceptive process by levodopa. Based on these findings, we would propose a hypothesis that dopamine and orexin may act bidirectionally in each other in the brain to induce an antinociceptive action against colonic distension. It would be furthermore speculated that levodopa is capable of inducing a visceral antinociceptive action through activation of the bidirectional dopamine and orexinergic signaling circuit. The above speculation could explain the present findings that the dopamine receptor antagonist or the orexin receptor antagonist significantly blocked the levodopa-induced visceral antinociception.

In conclusion, the present study clearly demonstrated that levodopa acts centrally to induce a visceral antinociception through activation of D2 dopamine receptors and the orexinergic system in the brain.

Conflict of interest

The authors declare that they have no conflict of interest.

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