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Levodopa acts centrally to induce an antinociceptive action against colonic distension through activation of D2 dopamine receptors and the orexinergic system in the brain in conscious rats

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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.