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Effects of piclozotan (SUN N4057), a partial serotonin 1A receptor agonist, on motor complications induced by repeated administration of levodopa in parkinsonian rats

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ABSTRACT

Serotonin 1A receptor agonists have attracted much interest recently as potential therapeutic agents for levodopa-induced motor complications, such as dyskinesia and motor fluctuations. The effects of piclozotan (SUN N4057) on a rat model of advanced Parkinson's disease were investigated. Parkinsonian rats, unilaterally 6-hydroxydopamine-lesioned rats, were administered levodopa for 8 to 9 weeks. Based on the results of rotational behavior and forelimb hyperkinesia in Week 5, the rats were allocated to three treatment groups (saline and two dosing rates of piclozotan set at 0.018 and 0.036 mg/kg/h). Piclozotan was administered via continuous subcutaneous infusion using an osmotic pump for 3 to 4 weeks. At Week 7 of repeated levodopa dosing, the effects of piclozotan on levodopa-induced behavior were evaluated. In addition, extracellular levels of levodopa-derived dopamine in the striatum were measured using microdialysis in Weeks 8 to 9 after completion of the respective behavioral studies. Chronic treatment with levodopa-induced forelimb hyperkinesia and shortened the duration of rotational behavior. Piclozotan (0.018 and 0.036 mg/kg/h, plasma concentrations 5.3 ± 0.7 and 14.3 ± 2.9 ng/ml) reduced levodopa-induced forelimb hyperkinesia by 55% and 69%, respectively, at 1 h relative to the control. Piclozotan (0.036 mg/kg/h) significantly lengthened the duration of rotational behavior by 26% versus the control and attenuated the increase in striatal levodopa-derived extracellular dopamine levels. These findings suggest that piclozotan, a serotonin 1A agonist, can improve motor complications in patients with advanced Parkinson's disease.

1. Introduction

Levodopa, the dopamine precursor, compensates for a decrease in the brain dopamine level and is the most effective therapeutic agent for Parkinson's disease (Fahn, 1999); however, long-term treatment with levodopa induces serious motor complications, such as dyskinesia (levodopa-induced involuntary movements), and motor fluctuations, including the wearing-off phenomenon (progressive shortening of the duration of levodopa action) (Ahlskog and Muenter, 2001). Use of dopamine D_2 receptor agonists rather than levodopa in early-stage Parkinson's disease is reported to reduce the risk of developing motor complications in later stages (Montastruc et al., 1994); however, as the disease progresses, dopamine D_2 receptor agonists alone can no longer adequately control symptoms (Ouchchane et al., 2004). Therefore, levodopa-induced motor complications remain a major problem in the management of Parkinson's disease.

Although the mechanisms of motor complications remain obscure, fluctuation of levodopa/dopamine following chronic therapy leads to pulsatile stimulation of receptors (Obeso et al., 2000). Recent data have shown that serotonin innervations of the striatal complex may play a role in handling systemically administered levodopa (Tanaka et al., 1999; Nicholson and Brotchie, 2002). This hypothesis has been strengthened by studies which revealed the existence of levodopa-derived dopamine in serotonergic neurons (Arai et al., 1994, 1995; Yamada et al., 2007), suggesting that peripherally administered levodopa is taken up not only into the remaining dopaminergic neurons but also into serotonergic neurons. Serotonergic neurons, however, are unable to regulate dopamine release, due to the lack of regulatory feedback systems mediated by dopamine transporter and presynaptic autoreceptors. Carta et al. (2007) proposed that such "dysregulated" release of levodopa-derived dopamine from serotonergic terminals may be the main trigger of dyskinesia; therefore, adjunct treatment with drugs that can modulate serotonin release may control striatal levodopa-derived dopamine levels. Serotonin 1A receptor agonists have attracted interest as potential therapeutic agents for the treatment of motor complications in patients with advanced Parkinson's disease. In animal models of Parkinson's disease, several serotonin 1A receptor agonists have shown beneficial effects on levodopa-induced dyskinesia-like abnormal behavior and/or prolong the duration of levodopa action (Bibbiani et al., 2001; Iravani et al., 2006; Matsubara et al., 2006; Ba et al., 2007; Eskow et al., 2007). To date, clinical studies with occasionally conflicting results have revealed that serotonin 1A receptor agonists may be effective to ameliorate motor complications (Kleedorfer et al., 1991; Bonifati et al., 1994; Kannari et al., 2002; Olanow et al., 2004; Bara-Jimenez et al., 2005; Goetz et al., 2007).

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Piclozotan (SUN N4057) is a 1,4-benzoxazepine derivative that exhibits sub-nanomolar affinity at serotonin 1A receptor with good selectivity over dopamine D_2 and α1-adrenoceptors (Kamei et al., 2001, 2005). In the present study, we examined the effects of piclozotan on motor complications by repeated levodopa treatment, and on levodopa-derived excessive swings of striatal dopamine release in 6-hydroxydopamine-lesioned rats treated with levodopa repeatedly.

2. Materials and methods

2.1. Animals

Male SD rats (6 to 7 weeks of age) purchased from Charles River Japan Inc. were used. They were allowed free access to food (CRF-1; Charles River Japan Inc.) and drinking water (tap water: using an automatic water supply system) and housed at a temperature of 23 ± 1 °C with a humidity of 55 ± 5% and a 12-hour light-dark cycle. All animal experiments were approved by the Ethics Committee for Animal Experiments of Biomedical Research Laboratories, Asubio Pharma Co., Ltd. and were performed in accordance with the Guideline for Animal Experiments of the laboratories. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Piclozotan (SUN N4057) (3-chloro-4,5-dihydro-4-{4-[4-(2-pyridyl)-1,2,3,6-tetrahydropyridin-1-yl] butyl}-1,4-benzoxazepin-5-one dihydrochloride dihydrate) was synthesized by Daiichi Asubio Pharma Co., Ltd. (currently Asubio Pharma Co., Ltd.). The subcutaneous dosing rates of piclozotan were set at 0.018 and 0.036 mg/kg/h, respectively, using an osmotic pump (Alzet Osmotic Pump: 2ML4, volume: 2 ml, flow rate: 2.5 μl/h). The following drugs were used: pentobarbital (Nembutal; Dainippon Pharmaceutical Co., Ltd.), penicillin (Crystalline Penicillin G Potassium Meiji; Meiji Seika Kaisha Ltd.), 6-hydroxydopamine with ascorbic acid hydrobromide (Sigma), benserazide hydrochloride (Sigma), L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (Sigma), apomorphine hydrochloride (Sigma), and desipramine hydrochloride (Sigma). All drugs were dissolved in saline at the time of use in a volume of 0.1 ml/100 g body weight. All doses were calculated as free base.

2.3. 6-Hydroxydopamine lesion surgery

Surgery for microinjection of 6-hydroxydopamine into the right medial forebrain bundle (MFB) was conducted in rats at 8 weeks of age. A norepinephrine reuptake inhibitor, desipramine (25 mg/kg), was intraperitoneally administered 30 min before the injection of 6-hydroxydopamine. The head of the rat was fixed in a stereotaxic apparatus under anesthesia with pentobarbital (40 mg/kg i.p.). 6-hydroxydopamine (8 μg/4 μl each site) with ascorbic acid dissolved in saline was injected into 2 sites in the right medial forebrain bundle according to the brain map (Swanson, 1992) (at 1.8 mm posterior to the bregma, 2.0 mm right-lateral to the median line, and 8.3 mm ventral to the surface of the skull, and at 4.5 mm posterior to the bregma, 1.4 mm right-lateral to the median line, and 8.5 mm ventral to the surface of the skull) at a flow rate of 1.0 μl/min for 4 minutes through an injection needle for microinjection, and then the needle was retained for 5 min. The injection needle for microinjection was prepared in-house using a Terumo dental injection needle (31G). Penicillin was intramuscularly injected into the hindlimb at a dose of 5000 units to prevent infection.

2.4. Experimental design

Time schedule of the experiment is shown in Fig. 1. At approximately 3 weeks after microinjection of 6-hydroxydopamine (11 weeks of age), rotational behavior was measured for 1 h after subcutaneous administration of a dopamine receptor agonist, apomorphine (0.05 mg/kg), in order to determine the success or failure of dopaminergic neuron destruction. Destruction by microinjection of 6-hydroxydopamine was judged to be insufficient when the number of rotations in the direction opposite to the microinjection side was 100 or below, but no rats fell under this criterion in the study.

Levodopa (25 mg/kg) was administered intraperitoneally (twice daily, from Monday to Friday) for 8 to 9 weeks, starting from the week after the administration of apomorphine. A peripherally-acting dopa decarboxylase inhibitor, benserazide (10 mg/kg), was administered intraperitoneally 30 min before the administration of levodopa. Observation of rotational behavior and forelimb hyperkinesia was conducted on Day 1 and in Week 3 and Week 5 of levodopa dosing. In the behavior observation of rats conducted in Week 5 of levodopa dosing, any rats whose expression time of forelimb hyperkinesia was less than 10 s at 1 h after levodopa administration were judged to be in the forelimb hyperkinesia-insufficient group, and were not used in the subsequent study (7 of 32 rats fell under this criterion).

Animals were allocated to 3 groups (8 to 9 animals per group: saline continuous subcutaneous infusion group and piclozotan continuous subcutaneous infusion groups with dosing rates of 0018 and 0.036 mg/kg/h) based on the duration of rotational behavior and forelimb hyperkinesia in Week 5 of repeated dosing. The following week (Week 6 of repeated levodopa dosing), an osmotic pump filled with piclozotan or saline was subcutaneously implanted in the rat, and behavioral examination of levodopa-induced rotational behavior and forelimb hyperkinesia was conducted in Week 7 of repeated levodopa dosing (Week 2 of continuous subcutaneous infusion).

Blood samples were collected from the lateral tarsal vein of rats in Week 8 of repeated levodopa dosing. During Weeks 8 to 9 of repeated levodopa dosing, extracellular levels of dopamine in the striatum were measured using brain microdialysis.

2.5. Behavioral experiments

Rotational behavior, the number of rotations during every 5 min, was automatically measured using rotational behavior-measuring equipment (Rotameter 6ch System; Muromachi Kikai Co., Ltd.) and forelimb hyperkinesia was visually observed after levodopa administration in the same rats. The number of rotations was measured for 4 h after levodopa administration. Based on the number of rotations, the time during which at least 20% of the maximum number of rotations was observed in each rat was calculated as the duration of rotational behavior according to the report of Bibbiani et al. (2001). Associated with repeated treatment with levodopa, 6-hydroxydopamine-lesioned parkinsonian rats showed abnormal behavior (forelimb hyperkinesia), such as involuntary extending of the forelimb or opening and closing of the hand, up-and-down movement of the wrist, and choreiform tremor in the forelimb on the side opposite the microinjection of 6-hydroxydopamine (Steece-Collier et al., 2003). Forelimb hyperkinesia was visually observed for 2 min at 30 min, 1, 2 and 3 h after levodopa administration. In addition, the presence or absence of rotational behavior of the rat was recorded approximately 1 h after levodopa administration during the repeated dosing period.

2.6. Microdialysis

Brain microdialysis was performed according to the procedure in our laboratory (Tani et al., 1998). The head of the rat was fixed in a stereotaxic apparatus under anesthesia with pentobarbital (40 mg/kg i.p.), and then a guide cannula for brain microdialysis (PC12 guide cannula; Carnegie Medicin) was inserted into the right striatum according to the brain map (Swanson, 1992) (0.5 mm anterior to the bregma, 3.0 mm right-lateral to the median line, and 4.0 mm ventral to the surface of the skull) and fixed to the skull with dental cement. The following day, a probe for brain microdialysis (PC12: outer diameter: 0.5 mm, length of the dialysis