Pharmacological Characteristics of Hyperambulation Induced by the Sigma Ligand (+)-3-PPP in Rats

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ABSTRACT—(+)-3-(3-Hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP), a sigma ligand, at doses above 3 mg/kg (s.c.) increased the ambulatory activity of rats, while the (−) isomer of 3-PPP with low affinity for sigma receptors, did not significantly modify the ambulatory activity at 10 and 30 mg/kg (s.c.). The ambulation-increasing effect of (+)-3-PPP was prevented by the sigma receptor antagonists BMY 14802 and rimcazole or the sigma/dopamine D_2 antagonist haloperidol. The (+)-3-PPP effect was also attenuated by pretreatment with the monoamine depletor reserpine or the tyrosine hydroxylase inhibitor α-methyltyrosine, but was not affected by the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine. Moreover, the (+)-3-PPP effect was antagonized by the dopamine D_2 antagonist sulpiride, whereas pretreatment with the 5-HT_{1A} agonist 8-OH-DPAT and the α-adrenoceptor antagonist phenoxybenzamine did not exert any significant effect. These results indicate that sigma receptors are involved in the neuronal mechanism(s) of hyperambulation induced by (+)-3-PPP, and the sigma system may exert both a presynaptic action and a dopamine D_2 receptor-mediated action to increase the central dopaminergic function.

Keywords: Sigma receptor, (+)-3-PPP ((+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine), Hyperambulation, Dopaminergic system

On the basis of in vivo behavioral and biochemical studies, it was originally thought that (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine ((+)-3-PPP) activates presynaptic dopamine receptors, autoreceptors, in the central nervous system (1, 2). It has been also proposed that (+)-3-PPP has agonistic and/or antagonistic activity at postsynaptic dopamine receptors (1, 3, 4). However, these suggestions were not supported by in vitro findings, in which (+)-3-PPP failed to activate dopamine autoreceptors, mediating inhibition of dopamine release from the rat striatal slices (4); and it has relatively low binding affinity for dopamine receptors (2, 5-7). Radioreceptor binding assays have shown that (+)-3-PPP exhibits approximately 1000 times greater affinity for sigma receptors than for dopamine D_2 receptors in the central nervous system (8-10). Therefore, (+)-3-PPP is widely used as a sigma ligand. Behavioral studies have shown that low doses of (+)-3-PPP produce inhibited locomotor activity in mice and rats, but at higher doses, it causes hyperactivity (1, 3, 11). The neuronal mechanism(s) of hyperactivity induced by (+)-3-PPP, however, remain unclear. Since pentazocine and (+)-SKF 10,047, other sigma ligands, also stimulate locomotor behavior in rats (12, 13), the central sigma system may be closely involved in the mechanism(s) of hyperlocomotion induced by (+)-3-PPP. In this study, we attempted to find a role for the sigma recognition site in the mediation of hyperactivity induced by (+)-3-PPP.

MATERIALS AND METHODS

Animals

Male Wistar rats were purchased at 5 weeks of age from the Shizuoka Laboratory Animal Center (Shizuoka). Five rats per cage (25 × 35 × 20 cm) were housed and maintained under controlled conditions of room temperature (23±1℃), relative humidity (55±5%) and a 12-hr light, 12-hr dark cycle (light on 07:00 hr) with free access to food and water. The experiment was conducted when the rats reached 6 weeks of age and weighed 130 to 170 g. Each experimental and control group consisted of 5-20 animals, and all the animals were used only once in the experiment. In the oral dosing study, the animals were fasted overnight.

Drugs

Drugs used were as follows: *dl*-α-methyltyrosine methylester hydrochloride (α-MT; Aldrich Chemical, Milwaukee, WI, USA); haloperidol, (+)-3-PPP hydrochloride and (−)-3-PPP hydrochloride (Sigma Chemical, St. Louis, MO, USA); *dl*-*p*-chlorophenylalanine methylester hydrochloride (p-CPA) and phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto); reserpine (Apoplon® Inj; Daiichi Pharm, Tokyo); rimcazole dihydrochloride and sulpiride (Research Biochemicals Inc., Natick, MA, USA). Both BMY 14802 and 8-OH-DPAT hydrobromide were synthesized and supplied by Dr. Toshio Tatsuoka (Laboratory of Medicinal Chemistry, Suntory, Osaka). Haloperidol and sulpiride were suspended in a solution of 0.5% carboxymethylcellulose sodium salt (Wako Pure Chemical, Osaka). The other drugs were dissolved in saline or distilled water.

Measurement of ambulatory activity

The rat's ambulatory activity was automatically recorded with a tilting-type ambulometer (ACTY-303; Bio Medica, Ltd., Osaka), consisting of a plastic activity cage (30 cm in diameter, 30 cm in height). The ambulatory activities of 10 rats were measured simultaneously using

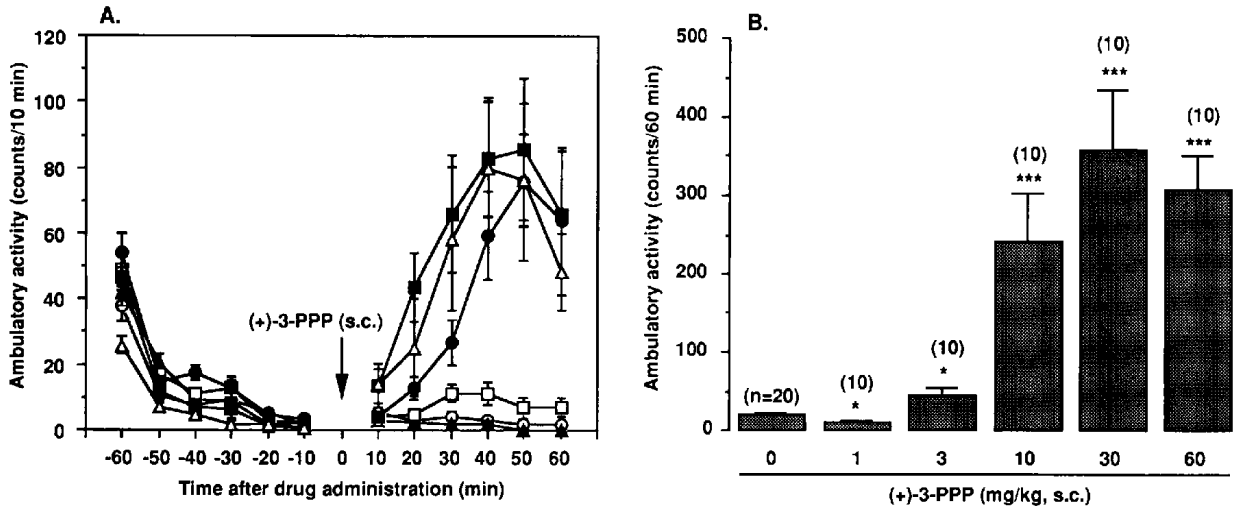

Fig. 1. Time-course changes (A) and 1-hr overall counts (B) of the rat ambulatory activity after a subcutaneous administration of (+)-3-PPP (1–60 mg/kg). The data represent the means±S.E.M. of 10–20 rats. ○: control, ▲: 1 mg/kg, □: 3 mg/kg, ●: 10 mg/kg, ■: 30 mg/kg, △: 60 mg/kg. *P<0.05 and ***P<0.001 vs. control.

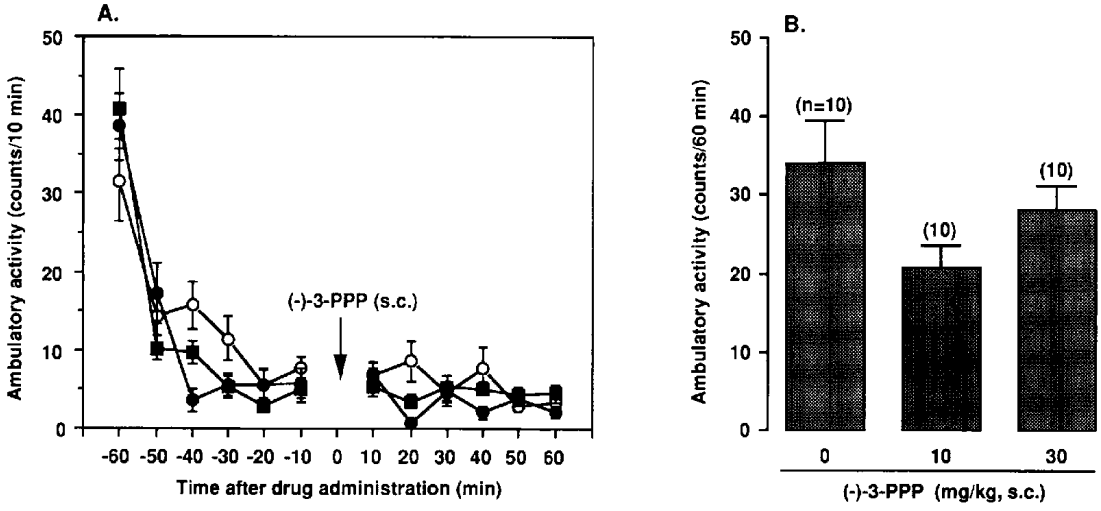

Fig. 2. Time-course changes (A) and 1-hr overall counts (B) of the rat ambulatory activity after a subcutaneous administration of (−)-3-PPP (10 and 30 mg/kg). The data represent means±S.E.M. of 10 rats. ○: control, ●: 10 mg/kg, ■: 30 mg/kg.

10 ambulometers. Rats were individually placed in the cages, and the ambulatory activity of each animal was measured. Experiments were performed between 10:00 hr to 17:00 hr.

Drug treatments

In the single administration study, the ambulatory activity of each animal was measured at 10-min intervals for 60 min before and after subcutaneous administration of 3-PPP enantiomers. In the coadministration study, a dose of 30 mg/kg of (+)-3-PPP, a sigma ligand, was injected subcutaneously 30 or 60 min after intraperitoneal or oral administration of central-acting drugs. The ambulatory activity for 30 or 60 min before and for 60 min after the (+)-3-PPP injection was measured. The sigma/dopamine D_2 antagonist haloperidol (0.1 and 1 mg/kg), the sigma antagonist BMY 14802 (3, 10 and 30 mg/kg) and the dopamine D_2 antagonist sulpiride (10 and 30 mg/kg) were administered orally 60 min prior to the (+)-3-PPP injection. The 5-HT_{1A} agonist 8-OH-DPAT (0.3 and 1 mg/kg), the α-adrenoceptor antagonist phenoxybenzamine (10 mg/kg) and the sigma antagonist rimcazole (3, 10 and 30 mg/kg) were injected intraperitoneally 30 min before (+)-3-PPP administration. α-MT (250 mg/kg, i.p.), a tyrosine hydroxylase inhibitor, and reserpine (2 mg/kg, s.c.), a monoamine depletor, were given 4 and 6 hr before the (+)-3-PPP injection, respectively. *p*-CPA (200 mg/kg), a tryptophan hydroxylase inhibitor, was administered intraperitoneally once a day for 3 consecutive days, and (+)-3-PPP was injected 24 hr after the final administration of *p*-CPA. Controls received equivalent volumes of the vehicle by the same route.

Statistics

The results are presented as the means±S.E.M. The statistical evaluation was performed by the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA), followed by the Mann-Whitney *U*-test. Differences were considered significant if the probability of error is less than 5%.

RESULTS

Effects of 3-PPP enantiomers on ambulatory activity

As shown in Fig. 1, (+)-3-PPP had a biphasic effect on ambulatory activities in rats. Namely, (+)-3-PPP, at a subcutaneous dose of 1 mg/kg, decreased the ambulatory activity as compared with the vehicle-treated control group, but at doses of 3, 10 and 30 mg/kg, it increased the ambulation in a dose-dependent fashion, except that the effect at the highest dose used, 60 mg/kg, was less potent than that at 30 mg/kg. The peak time of the ambulation-increasing effect of (+)-3-PPP was 40–50 min after

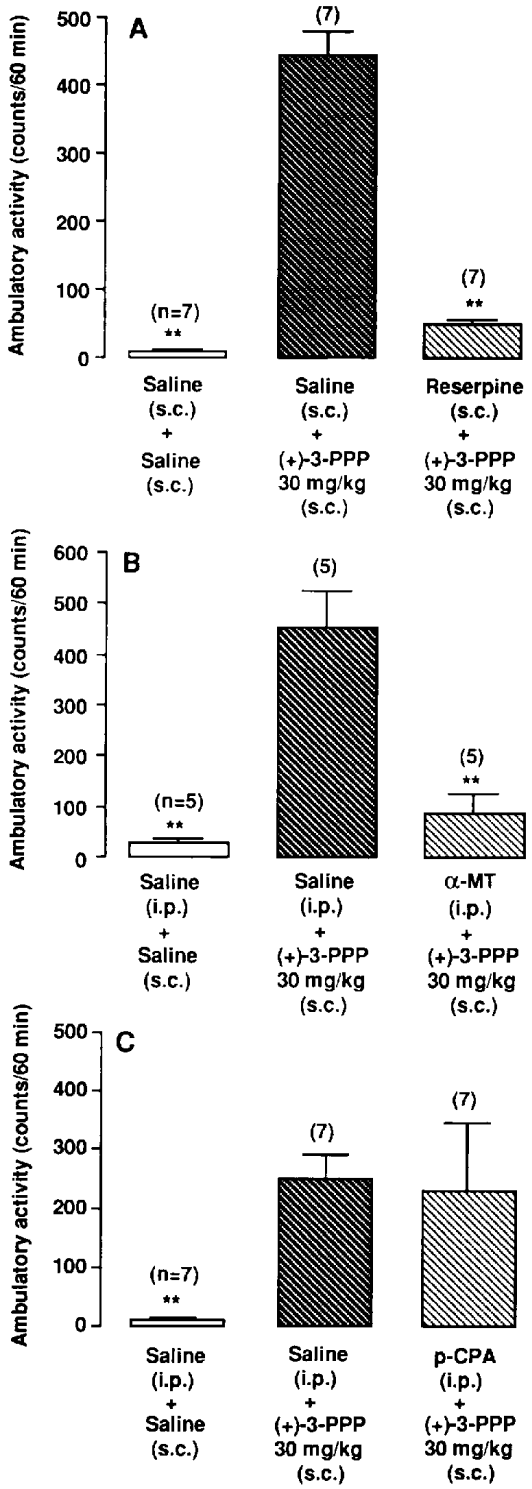

Fig. 3. Effects of reserpine (A), α-methyltyrosine (α-MT) (B) and *p*-chlorophenylalanine (p-CPA) (C) on (+)-3-PPP-induced hyperambulation in rats. α-MT (250 mg/kg, i.p.) and reserpine (2 mg/kg, s.c.) was admnistered 4 and 6 hr before a subcutaneous injection of 30 mg/kg of (+)-3-PPP, respectively. p-CPA (200 mg/kg) was administered intraperitoneally once a day for 3 consecutive days, and 24 hr after the last p-CPA injection, (+)-3-PPP was given. Ambulatory activity was measured for 60 min thereafter. The data represent means±S.E.M. of 5–7 rats. **$P<0.01$ vs. (+)-3-PPP-treated control.

the administration (Fig. 1A), and the dose of (+)-3-PPP that induced the maximal response in overall activity for 60 min after administration was 30 mg/kg (Fig. 1B). On the other hand, subcutaneous doses of 10 and 30 mg/kg of (−)-3-PPP slightly inhibited the ambulatory activities as compared with the control. (Fig. 2, A and B).

Effects of various central-acting drugs on (+)-3-PPP-induced hyperambulation

The subcutaneous dose of 30 mg/kg of (+)-3-PPP, which produced the maximal behavioral effect, was adopted in this experiment. As shown in Fig. 3, the ambulation-increasing effect of (+)-3-PPP was markedly inhibit-

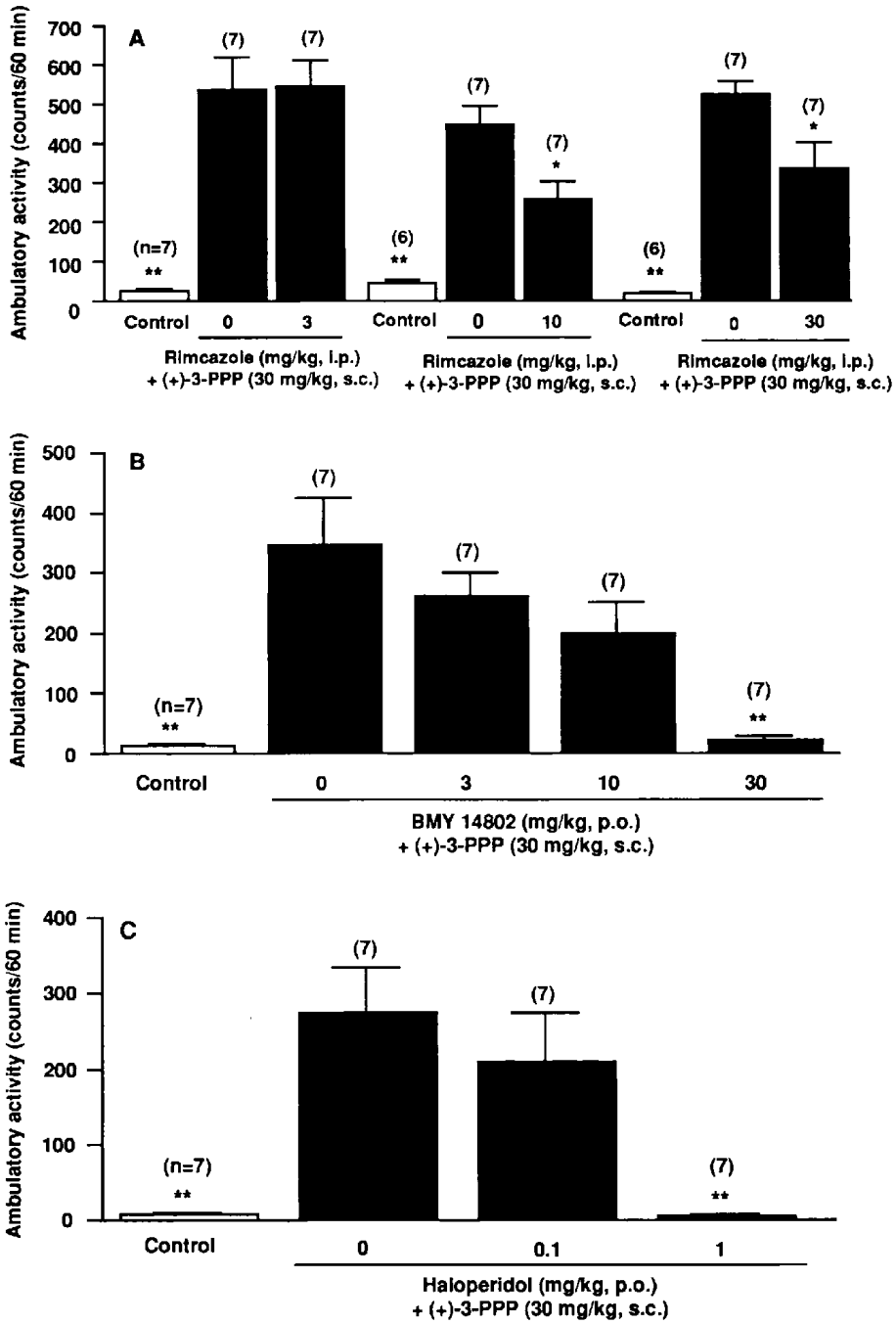

Fig. 4. Antagonistic actions of rimcazole (A), BMY 14802 (B) and haloperidol (C) on (+)-3-PPP-induced hyperambulation in rats. Intraperitoneal doses of rimcazole (3, 10 and 30 mg/kg) or oral doses of BMY 14802 (3, 10 and 30 mg/kg) and haloperidol (0.1 and 1 mg/kg) were administered 30 or 60 min before a subcutaneous injection of 30 mg/kg of (+)-3-PPP, respectively. Ambulatory activity was measured for 60 min thereafter. The data represent means±S.E.M. of 6–7 rats. *$P<0.05$, **$P<0.01$ vs. (+)-3-PPP-treated control.

ed by pretreatment with a subcutaneous dose of 2 mg/kg of reserpine or an intraperitoneal dose of 250 mg/kg of α-MT. However, the (+)-3-PPP-induced hyperactivity was negligibly modified by p-CPA (3 × 200 mg/kg, i.p.) (Fig. 3C). Moreover, the effects of (+)-3-PPP were prevented in a dose-dependent manner by pretreatment with oral doses of BMY 14802 (3, 10 and 30 mg/kg), haloperidol (0.1 and 1 mg/kg) and sulpiride (10 and 30 mg/kg), and with intraperitoneal doses of rimcazole (3 and 10 mg/kg) (Figs. 4 and 5B). Especially, a dose of 30 mg/kg of BMY 14802 and 1 mg/kg of haloperidol blocked (+)-3-PPP-induced hyperactivity almost completely. An increased dose of 30 mg/kg of rimcazole significantly and partially reduced (+)-3-PPP-induced hyperambulation, and its inhibitory activity was similar to that of 10 mg/kg. All doses used of BMY 14802, haloperidol, sulpiride and rimcazole did not exert any effect as compared with the control group on 30-min or 1-hr overall counts of ambulatory activity prior to the (+)-3-PPP treatment (data are not shown). On the other hand, 8-OH-DPAT (0.3 and 1 mg/kg, i.p.) tended to potentiate the hyperambulation induced by (+)-3-PPP (Fig. 5A). Phenoxybenzamine (10 mg/kg, i.p.) had no effect on the (+)-3-PPP effect (Fig. 5C).

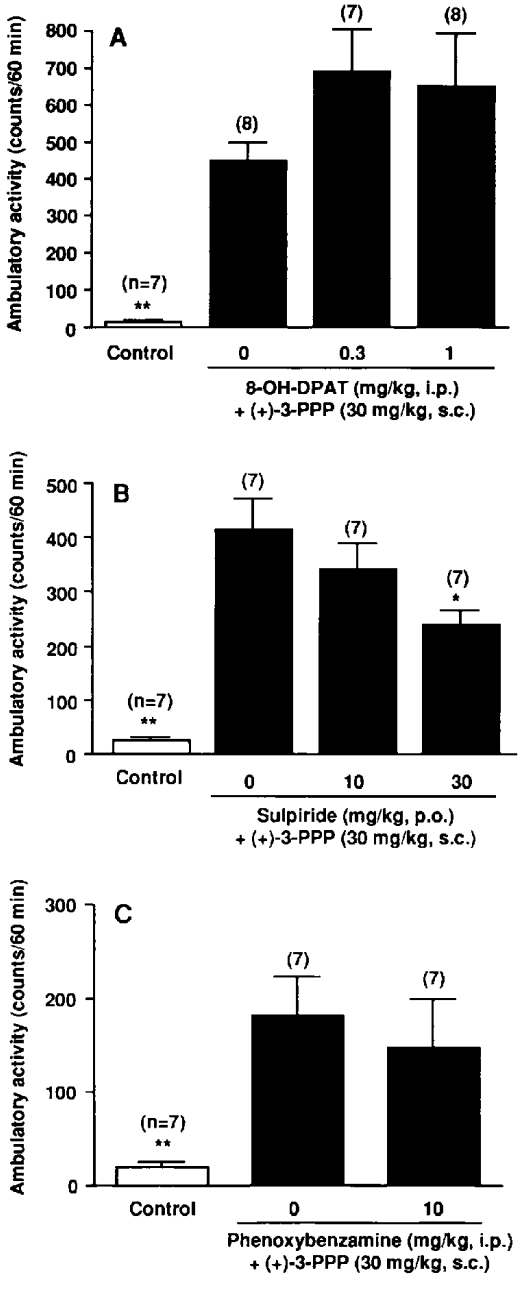

Fig. 5. Effects of 8-OH-DPAT (A), sulpiride (B) and phenoxybenzamine (C) on (+)-3-PPP-induced hyperambulation in rats. Intraperitoneal doses of 8-OH-DPAT (0.3 and 1 mg/kg) and phenoxybenzamine (10 mg/kg) or oral doses of sulpiride (10 and 30 mg/kg, p.o.) were administered 30 or 60 min before a subcutaneous injection of 30 mg/kg of (+)-3-PPP, respectively. Ambulatory activity was measured for 60 min thereafter. The data represent means±S.E.M. of 7–8 rats. *$P<0.05$ and **$P<0.01$ vs. (+)-3-PPP-treated control.

DISCUSSION

In the present study, we confirmed the reports of Hjorth et al. (1), Arnt et al. (3) and Imperato et al. (11) that a sigma ligand (+)-3-PPP at above 3 mg/kg (s.c.) produced hyperambulation. In addition, these authors have reported that both lower doses of (+)-3-PPP and the (−) isomer of 3-PPP inhibit locomotor activities in mice and rats. The present results with a low dose (1 mg/kg, s.c.) of (+)-3-PPP and subcutaneous doses of 10 and 30 mg/kg of (−)-3-PPP are also in support of them, although to clearly detect the inhibitory effect of drugs on ambulatory activity may be difficult because the present study was performed under habituated conditions.

We demonstrated that the ambulation-increasing effect of (+)-3-PPP is prevented by both BMY 14802 (14, 15) and rimcazole (16, 17), sigma receptor antagonists with negligible affinity for dopamine D_1 and D_2 receptors, and the sigma/dopamine D_2 antagonist haloperidol (15). The anti-(+)-3-PPP effect of BMY 14802 was much more potent than that of rimcazole. The finding might be explained by the biochemical data (15) that the sigma binding affinity of BMY 14802 ($IC_{50}=74$ nM) is about 20 times greater than that of rimcazole ($IC_{50}=1460$ nM). BMY 14802 also binds to $5\text{-}HT_{1A}$ receptors and exerts agonistic action (18, 19). The anti-(+)-3-PPP action of BMY 14802 seems not to be due to its $5\text{-}HT_{1A}$ effects, since 8-OH-DPAT, a selective $5\text{-}HT_{1A}$ agonist, failed to attenuate the (+)-3-PPP-induced hyperambulation. Accordingly, these results indicate the involvement of sigma receptors in the neuronal mechanisms of (+)-3-PPP-induced hyperactivity. Sigma receptors are generally enantioselective for (+) isomers (10). (+)-3-PPP has high

affinity for sigma receptors, while the (−)-enantiomer has low affinity (8, 20). As described above, induction of hyperactivity by 3-PPP was confirmed to be enantioselective for the (+) isomer of 3-PPP, as well as for the sigma binding affinities of 3-PPP enantiomers. Recently, subtypes of sigma receptors, termed sigma-1 and sigma-2, have been postulated on the basis of ligand binding and biochemical studies (21). It has been demonstrated that (+)-SKF 10,047 and (+)-pentazocine selectively recognize sigma-1 sites, and (+)-3-PPP binds to both sigma-1 and -2 sites. Sigma-1 ligands such as pentazocine and (+)-SKF 10,047 stimulate locomotor behavior in rodents (12, 13). Thus, hyperambulation by (+)-3-PPP is likely to be produced through action on the sigma-1 sites.

(+)-3-PPP-induced hyperambulation was inhibited by pretreatment with a monoamine depletor (reserpine), a catecholamine synthesis inhibitor (α-MT) and a selective dopamine D_2 antagonist (sulpiride), but was not prevented by a serotonin synthesis inhibitor (p-CPA) and an α-adrenoceptor antagonist (phenoxybenzamine). These findings may indicate that the (+)-3-PPP effect results from its presynaptic and dopamine D_2 receptor-mediated actions on the central dopaminergic system rather than on the noradrenergic and serotonergic systems. The modulation of dopaminergic neuronal activity by (+)-3-PPP may be mediated through sigma, but not dopamine receptors because of the relatively poor affinity of (+)-3-PPP for dopamine receptors in vitro (2, 5–7). This viewpoint is supported by several lines of evidence that sigma receptors interact closely with the dopaminergic system. Namely, anatomical studies have shown that sigma receptors are distributed in the substantia nigra, striatum, nucleus accumbens and the other areas where dopamine is plentiful (20, 22, 23) and that sigma receptors may be located on both dopaminergic neurons in the substantia nigra pars compacta and presynaptic dopaminergic terminals in the striatum (24). Moreover, electrophysiological studies have shown that sigma ligands modulate the activity of dopaminergic neurons in areas A9 and A10 (17, 25).

In conclusion, we suggest that sigma receptors are involved in the neuronal mechanism(s) of hyperambulation induced by high doses of (+)-3-PPP and the sigma system may exert both a presynaptic action and a dopamine D_2 receptor-mediated action to increase the central dopaminergic function.

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