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European Journal of Pharmacology 319 (1997) 197–205

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Anti-tremor activity of talipexole produced by selective dopamine D_2 receptor stimulation in cynomolgus monkeys with unilateral lesions in the ventromedial tegmentum

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Received 28 August 1996; revised 22 October 1996; accepted 25 October 1996

Abstract

The anti-tremor activity of talipexole (6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine dihydrochloride, B-HT 920 CL_2, Domin), a non-ergot dopamine D_2 receptor agonist which possesses α_2-adrenoceptor agonistic and 5-HT_3 receptor antagonistic properties, was examined in monkeys with a unilateral lesion in the ventromedial tegmentum. Talipexole dose dependently suppressed the tremor and had ED_{50} values of 34 μg/kg s.c. and 84 μg/kg p.o. The anti-tremor effect of talipexole occurred at much lower doses than that of an ergot dopamine receptor agonist, bromocriptine (2-bromo-α-ergocryptine mesilate, ED_{50}; 2.5 mg/kg s.c.), and talipexole acted synergistically in combination with L-DOPA (3,4-dihydroxyphenylalanine). In ventromedial tegmentum-lesioned monkeys, anti-tremor doses of talipexole did not cause emetic behavior, but had sedative effects. Conversely, monkeys given bromocriptine exhibited oral movement, salivation and vomiting when anti-tremor effects were observed, but not marked sedative behavior at any of the doses investigated. During repeated administration of talipexole (a daily dose of 50 μg/kg s.c. for 21 days), the extent and duration of the anti-tremor effect did not change, but those of the sedative effect decreased gradually. The anti-tremor effect of talipexole was significantly suppressed by sulpiride, but not by SCH 23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol) or yohimbine, while the sedative effect was inhibited by sulpiride and yohimbine. The main metabolites of talipexole had no anti-tremor or sedative effects. These results indicate that talipexole exerts its anti-tremor activity via selective dopamine D_2 receptor stimulation.

Keywords: Talipexole; Bromocriptine; Dopamine D_2 receptor agonist; Anti-tremor effect; Ventromedial tegmentum lesion; Parkinson's disease; (Cynomolgus monkey)

1. Introduction

It is known that the progressive loss of dopamine-containing cells in the pars compacta of the substantia nigra and disturbance of nigro-striatal dopaminergic functions cause parkinsonisms such as akinesia, rigidity and tremor, and that L-DOPA (3,4-dihydroxyphenylalanine) and dopamine receptor agonists used alone or in combination are effective treatments for Parkinson's disease. Currently various dopamine receptor agonists are used, or are under development, for the treatment of Parkinson's disease. Clinical trials suggest that stimulation of dopamine D_2 receptors is essential; however, the role of dopamine D_1 receptors has not been clearly determined (Uitti and Ahlskog, 1996). Whether a potential therapeutic agent for Parkinson's disease should be a more selective D_2 type agonist or a D_2-type agonist with an additional D_1 agonist, and whether dopamine D_1 or D_2 receptor stimulation improves Parkinson symptoms differently are important theoretical questions. Recently the selective dopamine D_2 receptor gonist, talipexole (6-allyl-2-amino-5,6,7,8,-tetrahydro-4*H*-thiazolo[4,5-*d*]-azepine dihydrochloride, Domin), has become available as an antiparkinsonian agent. Talipexole was originally described as a clonidine-like α_2-adrenoceptor agonist (Kobinger and Pichler, 1980). Thereafter, talipexole has been shown to be mainly a dopamine D_2 receptor agonist which lacks the ability to stimulate dopamine D_1 receptors (Hinzen et al., 1986; Matsumoto et al., 1989; Matsubayashi et al., 1995). In clinical studies, talipexole showed higher efficacy, as as-

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sessed by the global improvement ratings on Parkinson's disease, and fewer gastrointestinal side effects than bromocriptine (2-bromo-α-ergocriptine mesilate), the most widely used dopamine receptor agonist. In the improvement ratings for each symptom, talipexole was significantly superior than bromocriptine on upper limb tremor (Nakanishi et al., 1993). Our electrophysiological findings for rats suggested that talipexole acts as a dopamine D_2 receptor agonist on the striatal caudate neurons receiving input from the substantia nigra pars compacta and increases firing when applied intravenously. Intravenously administered bromocriptine appears to act as both dopamine D_2 and D_1 receptor agonist (Matsubayashi et al., 1995). In experimental Parkinsonian models, talipexole suppressed the motor deficit induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rhesus monkeys, and reserpine-induced akinesia in mice (Hinzen et al., 1986). In MPTP-treated common marmosets, talipexole dose dependently increased motor activity and reversed akinesia and incoordination of movement (Irifune et al., 1993; Irifune et al., 1994). These previous studies with MPTP- or reserpine-treated animal models suggest that talipexole may be effective in the same dose range for the treatment of akinesia and rigidity in Parkinson's disease; however, the effective dose for Parkinsonian-like resting tremors has not yet been identified.

Unilateral lesion in the ventromedial tegmentum of cynomolgus monkeys causes persistent Parkinsonian signs such as resting tremors and akinesia (Ohye et al., 1979; Poirier et al., 1966). In this study, the anti-tremor activity of talipexole and bromocriptine after a single administration, and the behavioral changes generally associated with dopamine D_2 receptor agonists were investigated in the ventromedial tegmentum-lesioned cynomolgus monkeys. As patients with Parkinson's disease are treated with the dopamine receptor agonist alone or in combination with L-DOPA, the effects of talipexole were investigated under both conditions. Then we studied the effects of talipexole during repeated administration and elucidated the mechanisms underlying the anti-tremor effect of talipexole. Their implications in relation to the potential clinical profile are discussed.

2. Materials and methods

2.1. Animals

Healthy male cynomolgus monkeys (*Macaca fascicularis*, P.T. SNBL Indonesia), weighing 3.6–5.8 kg, were used in this study. Animals were housed individually in stainless steel cages (70 cm in width, 68 cm in depth, and 77 cm high) at a constant temperature (26 ± 2°C) and humidity (50 ± 10%), and under a 12-h automatic light cycle (6:00 to 18:00). Food and water were available ad libitum.

2.2. Ventromedial tegmentum lesions and the production of spontaneous tremors

Animals were anesthetized with ketamine hydrochloride and their heads were fixed into the standard stereotaxic apparatus. Referring to the stereotaxic atlas of the monkey brain of Szabo and Cowan (1984), a unilateral lesion was made by electrocoagulation (3 mA, 30 s) on the right side of the ventromedial tegmentum (A: 1.6, L: 6.0, H: −8.0 mm). The lesion was verified by the appearance of tremor in animals afterwards. We previously used this ventromedial tegmentum-lesion technique and confirmed that only monkeys who had the top of the electrode inserted into the right side of the ventromedial tegmentum exhibit persistent tremor; otherwise monkeys not exhibit the tremor.

Immediately after coagulation, dilated pupils and contralateral hypokinesia were observed. Two to three days after coagulation, the subjects had a characteristic postural tremor in their contralateral upper and lower limbs. They were allowed to recover for 2 weeks to eliminate the influence of surgery. The individual responses of ventromedial tegmentum-lesioned animals differed. Therefore, monkeys showing tremors with an average score of approximately 2, a score used for evaluating the effects of drugs, were selected prior to the experiments as described below.

Animals were placed in a monkey chair, and their spontaneous tremors were evaluated after they had adapted to the circumstances. The tremor score was assessed according to the following scale: 0 (no tremor), 1 (the observer cannot feel vibrations when handling the limbs of the animals, but upon close observation there is a small but visible continuous tremor), 2 (the observer feels slight vibrations and can easily observe small tremors), 3 (the observer feels obvious vibrations and can observe obvious limb tremors).

The average score from 2 examinations (not blinded) was taken. Each examination lasted 10 s, and was performed after a 30-s interval. Finally 12 ventromedial tegmentum-lesioned animals were selected as appropriate animal models for this study.

The tremor score after drug administration was expressed as a percentage of the pretreatment score. Usually 4 animals were incorporated per experiment. If the ventromedial tegmentum-lesioned animals were re-examined, they were allowed to recover for at least 6 days prior to re-examination (on average the animals were re-examined 9 times in this study).

2.3. General behavior

Observations were made while the animals were placed in monkey chairs. The observer performed the examinations as quietly as possible. As a measure of emetic behavior, the incidence of oral movement, salivation or vomiting was recorded for each animal. The sedative effect

was measured by grading behavior as a reflection of central nervous system (CNS) depression. Animals were judged to be sedated if their eyes were closed and if they did not move, but still reacted to noises or movements made by the observer. If the animal did not react to the movements of the observer, the animal was judged as being either asleep or drowsy. The distinction between asleep and drowsy was made by lightly touching the animals. If the animal woke up when touched, it was judged to be drowsy. If the animal did not react to the touch it was judged as being asleep. The depression score of the CNS (CNS depression score) was then calculated based on the following scale; 1 (point for sedation), 2 (for drowsiness) and 4 (for asleep).

2.4. Drugs

Talipexole (B-HT 920 CL_2, 6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine dihydrochloride, Boehringer-Ingelheim) was dissolved in a 0.9% saline solution for subcutaneous administration and in distilled water for oral administration. The major metabolites of talipexole, BX-OX 213 and B-HT 938 CL_2 (6-allyl-2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine oxide and 2-amino-5,6,7,8-tetrahydro-4*H*-thiazolo[4,5-*d*]azepine dihydrochloride, Boehringer-Ingelheim), were also dissolved in a 0.9% saline solution. Bromocriptine (2-bromo-α-ergocryptine mesilate, Sandoz) was dissolved in cremophor and sodium hydrochloride, and diluted with a 0.9% saline solution. L-DOPA (Sigma) was suspended in a 0.5% carboxymethyl cellulose sodium salt solution. Clonidine (Boehringer-Ingelheim), SCH 23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol, RBI) and yohimbine (Wako) were dissolved in 0.9% saline solutions. (−)-Sulpiride (Sigma) was dissolved in 0.5 M hydrochloric acid, adjusted to pH 6.3 with 0.5 M sodium hydroxide, and diluted in 0.9% saline solution. All doses refer to the bases.

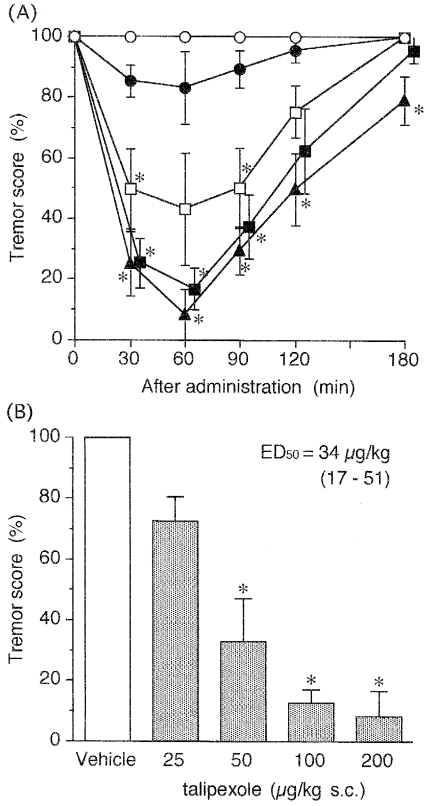

Fig. 1. Acute effects of subcutaneous administration of talipexole on tremor in ventromedial tegmentum-lesioned monkeys. Talipexole or vehicle (saline) was administered subcutaneously. Tremor scores were judged by observation ratings, and are presented as percentages of the pretreatment values. Tremors were assessed 30, 60, 90, 120 and 180 min after the administration of talipexole or the vehicle. Results are shown as the means ± S.E.M. for 4 animals. * $P < 0.05$ compared to the vehicle-treated control (Dunnett's multiple comparison test). (A) Time-course data (○) vehicle control; (●) 25 μg/kg; (□) 50 μg/kg; (■) 100 μg/kg; (▲) 200 μg/kg. (B) Dose–response data. The tremor score was calculated using the lowest computed score for each animal during the 180-min observation period.

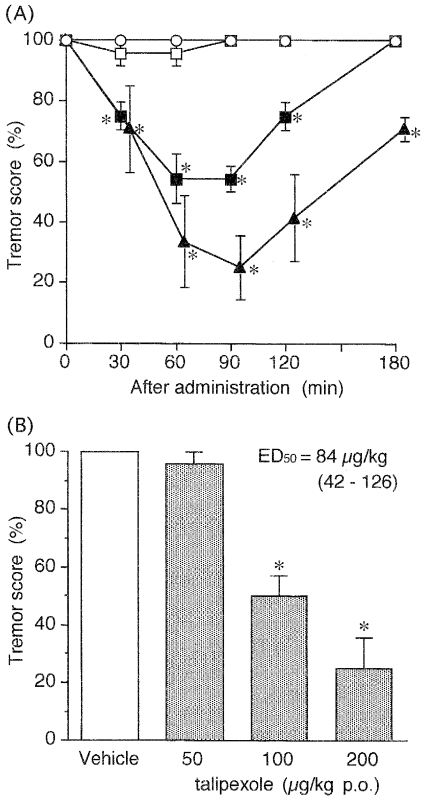

Fig. 2. Acute effects of oral administration of talipexole on tremor in ventromedial tegmentum-lesioned monkeys. Talipexole or vehicle (distilled water) was administered orally, otherwise this figure legend is the same as in Fig. 1. (A) Time-course data. (○) Vehicle control; (□) 50 μg/kg; (■) 100 μg/kg; (▲) 200 μg/kg. (B) Dose–response data.

2.5. Statistical analysis

The data were analyzed by the Mann-Whitney U-test (for independent data), and the Dunnett's multiple compar-

ison test (for dependent data). The results were considered significant when P values were less than 0.05. The ED_{50} value was calculated by the method of Miller and Tainter, which uses an effective 50% decrease compared to the pretreatment level.

3. Results

3.1. Evaluation of the anti-tremor activities of the test drugs in ventromedial tegmentum-lesioned monkeys

3.1.1. Single administration of talipexole

In monkeys with ventromedial tegmentum lesions, vehicle did not influence the spontaneous tremor at all. Subcutaneous administration of talipexole (25–200 μg/kg) suppressed the tremors in a dose-dependent manner. The effects of talipexole were noted within 30 min, and the peak effect appeared approximately 60 min after injection.

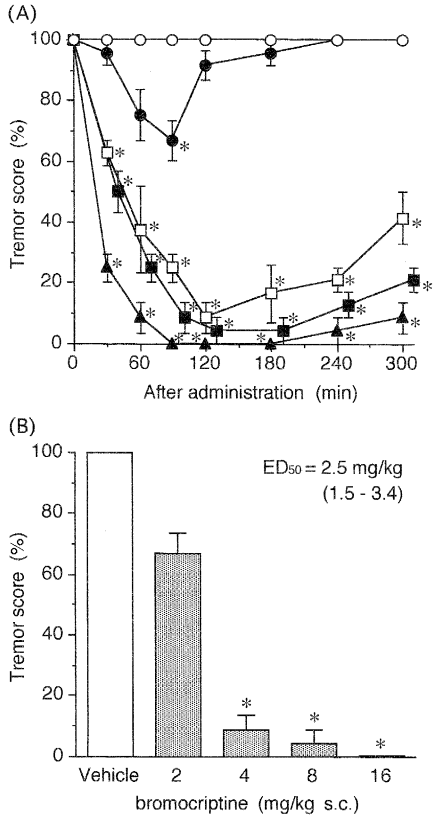

Fig. 3. Acute effects of subcutaneous administration of bromocriptine on tremor in ventromedial tegmentum-lesioned monkeys. Bromocriptine or vehicle was administered subcutaneously. Tremor scores were judged as described in the legend to Fig. 1. Tremors were assessed 30, 60, 90, 120, 180, 240 and 300 min after the administration of bromocriptine or the vehicle. Results are presented as the means ± S.E.M. for 4 animals. * $P < 0.05$ compared to vehicle-treated control (Dunnett's multiple comparison test). (A) Time-course data. (○) Vehicle control; (●) 2 mg/kg; (□) 4 mg/kg; (■) 8 mg/kg; (▲) 16 mg/kg. (B) Dose–response data. The tremor score was calculated using the lowest score of each animal during the 300-min observation period.

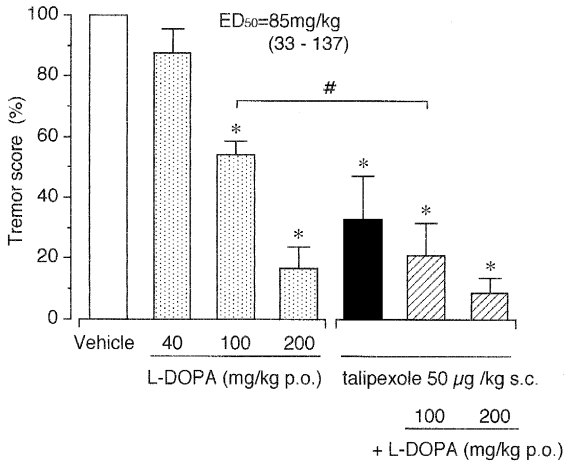

Fig. 4. Acute effects of oral administration of L-DOPA alone, and in combination with talipexole in ventromedial tegmentum-lesioned monkeys. L-DOPA or vehicle (distilled water 2 ml/kg) was administered orally. Tremor scores were judged as described in the legend to Fig. 1, and the lowest score for each animal during the 180-min observation period was calculated. Results are presented as the means ± S.E.M. for 4 animals. * $P < 0.05$ compared to vehicle-treated control (Dunnett's multiple comparison test) # $P < 0.05$ between 2 groups (Mann-Whitney U-test).

The potency and duration of the anti-tremor action increased dose dependently (Fig. 1A). When a tremor score was calculated using the lowest score for each animal during 180-min observation period, talipexole had significant effects at doses between 50 to 200 μg/kg s.c. compared with the control. The ED_{50} and its 95% confidence limit was 34 (17–51) μg/kg s.c. (Fig. 1B). Talipexole was found to induce sedative effects in a dose-dependent manner (Table 1). Interestingly, no emetic behavior such as oral movement, salivation or vomiting, was observed at any dose in the animals tested.

After the oral administration of talipexole (50–200 μg/kg), anti-tremor activity was also noted, and its potency and duration increased dose dependently (Fig. 2A). Compared with subcutaneous administration, the onset of talipexole's effects was slightly delayed, reaching a peak effect between 60–90 min after administration. In the dose–response study, a significant effect appeared at 100 μg/kg p.o.; the ED_{50} and its 95% confidence limit was 84 (42–126) μg/kg p.o. (Fig. 2B). Oral administration of talipexole caused only a slight sedative effect compared to that caused by subcutaneous administration and did not induce emetic behavior in any animal (Table 1).

3.1.2. Single administration of bromocriptine

Bromocriptine (2–16 mg/kg s.c.) dose dependently suppressed tremors in ventromedial tegmentum-lesioned monkeys with peak effects between 90–180 min after the subcutaneous injection, depending on the dose (Fig. 3A). Compared to the control group, bromocriptine had a significant anti-tremor effect at 4 mg/kg s.c. The ED_{50} and its 95% confidence limit was 2.5 (1.5–3.4) mg/kg s.c. (Fig.

Table 1
Time-course changes in general behavior after the administration of talipexole and bromocriptine in ventromedial tegmentum-lesioned monkeys

	Sedative effects					Emetic behavior [a]
	30 min	60 min	90 min	120 min	180 min	30–180 min
Talipexole						
0 μg/kg s.c.	0	0	0	0	0	0/4
25	1.5	0.5	0	0	0	0/4
50	2.0	1.5	0.75	0.5	0	0/4
100	2.0	2.0	1.5	1.25	0.75	0/4
200	4.0	4.0	3.0	2.25	1.25	0/4
Talipexole						
0 μg/kg p.o.	0	0	0	0	0	0/4
50	0.25	0.25	0	0	0	0/4
100	1.0	1.0	0.75	0.25	0	0/4
200	1.25	1.25	1.0	0.75	0	0/4

	Sedative effects		Emetic behavior, 30–300 min		
	30–60	90–300 min	Oral movement	Salivation	Vomiting
Bromocriptine					
0 mg/kg s.c.	0	0	0/4	0/4	0/4
2	0	0	0/4	0/4	0/4
4	0	0	0/4	0/4	0/4
8	0.25	0	4/4	2/4	1/4
16	0.25	0	4/4	2/4	2/4

Talipexole or vehicle was administered subcutaneously or orally. The sedative effects were judged by calculating CNS depression scores at each time point after administration as described in Methods, and are given as the mean scores from 4 animals. [a] Talipexole did not induce emetic behavior such as oral movement, salivation or vomiting during the behavioral observations. Bromocriptine was administered subcutaneously. The incidence of emetic behavior is given as the number of animals showing each behavior. Time-course changes in emetic behavior were not evident during the observation period.

3B). Bromocriptine at doses of 8 and 16 mg/kg s.c. caused emetic responses such as oral movement, salivation and vomiting in all animals tested, but sedative effects were only observed in a few animals (Table 1).

3.1.3. Single administration of L-DOPA and evaluation of anti-tremor effects of talipexole in combination with L-DOPA

Following administration of L-DOPA (40–200 mg/kg p.o.), anti-tremor effects were observed in ventromedial tegmentum-lesioned monkeys. The ED_{50} and its 95% confidence limit was 85 (33–137) mg/kg p.o. (Fig. 4). The peak effects of L-DOPA appeared 30 min after oral administration. No abnormal behavior was observed after the administration of L-DOPA.

A combination of talipexole (50 μg/kg s.c.), the minimum effective dose noted in Fig. 1B, and L-DOPA (100 and 200 mg/kg p.o.) more significantly suppressed tremors than did either talipexole or L-DOPA alone. The anti-tremor effects of talipexole (50 μg/kg s.c.) and L-DOPA (100 and 200 mg/kg p.o.) were synergistic (Fig. 4). However the sedative effect caused by talipexole remained unchanged (data not shown).

3.1.4. Repeated administration of talipexole

During repeated administration of talipexole (50 μg/kg s.c., once a day for 21 days), its anti-tremor activity remained unchanged with respect to potency and duration (Fig. 5A); however, the degree and duration of the sedative effect decreased gradually in the ventromedial tegmentum-lesioned monkeys (Fig. 5B).

3.2. The mechanisms of the anti-tremor activity of talipexole

3.2.1. Effects of receptor antagonists on talipexole-induced anti-tremor activity and the sedative effect

In addition to disturbance of the nigro-striatal dopamine system, there is a close relationship between parkinsonisms and dysfunction of the noradrenergic neurons of the brain. The mechanisms of the anti-tremor activity of talipexole, therefore, were studied using antagonists acting on dopamine D_1/D_2 receptors or α_2-adrenoceptors in the brain.

SCH 23390 (0.1 mg/kg s.c., a specific dopamine D_1 receptor antagonist), sulpiride (10 mg/kg s.c., a specific dopamine D_2 receptor antagonist) or yohimbine (0.4 mg/kg s.c., a specific α_2-adrenoceptor antagonist) was administered to ventromedial tegmentum-lesioned monkeys 30 min prior to the administration of 50 or 200 μg/kg s.c. of talipexole. Both the lower and higher doses of talipexole had significant anti-tremor activity (Fig. 1B). SCH 23390 as well as yohimbine had no significant effect on the anti-tremor action of talipexole. Conversely, sulpiride significantly suppressed the anti-tremor action of talipexole at both doses (Fig. 6). Sulpiride and yohimbine

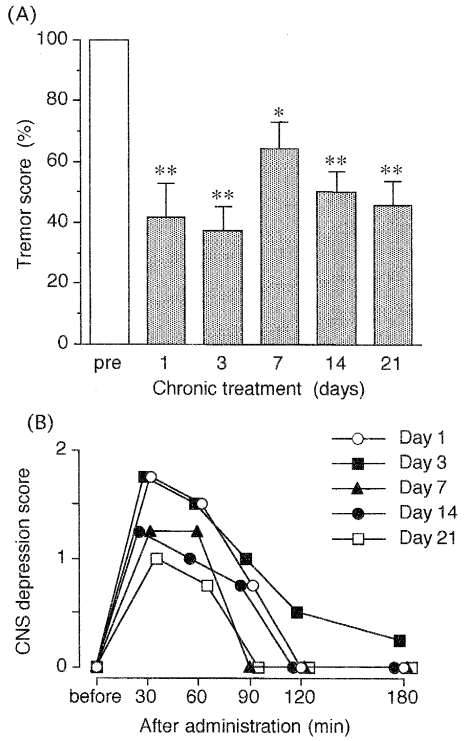

Fig. 5. Anti-tremor activity and the sedative effects of talipexole during repeated administration in ventromedial tegmentum-lesioned monkeys. Talipexole (50 μg/kg) was injected subcutaneously daily for 21 days. Tremors and sedative effects were assessed 30, 60, 90, 120 and 180 min after the injection of talipexole. (A) Anti-tremor activity. The tremor score was calculated using the computed lowest score for each animal during the 180-min observation period. Results are presented as the means ± S.E.M. ($n = 4$) * $P < 0.05$; ** $P < 0.01$ compared to their pre-treatment values (Dunnett's multiple comparison test). No significant differences were found between the effect on day 1 and the effect on days 3–21. (B) Sedative effect. The sedative effect was evaluated by using the CNS depression score as described in Methods. Results are presented as the means for 4 animals.

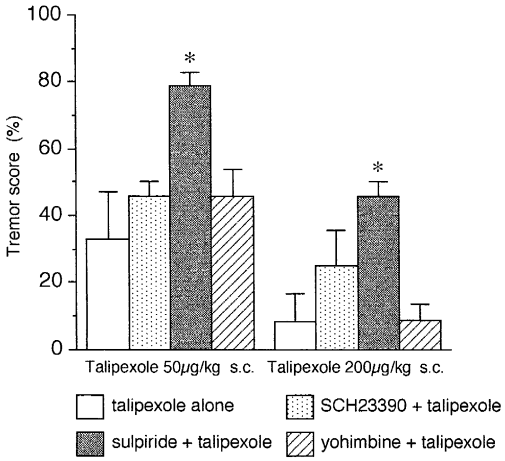

Fig. 6. Effects of receptor antagonists on talipexole-induced anti-tremor activity in ventromedial tegmentum-lesioned monkeys. SCH 23390 (0.1 mg/kg s.c.), sulpiride (10 mg/kg s.c.), or yohimbine (0.4 mg/kg s.c.) was administered to VMT-lesioned monkeys 30 min prior to a subcutaneous injection of talipexole. (A) Talipexole 50 μg/kg s.c. (B) Talipexole 200 μg/kg s.c. Tremor scores were judged as described in the legend to Fig. 1, and the lowest score of each animal during the 180-min observation period was calculated. Results are presented as the means ± S.E.M. for 4 animals. * $P < 0.05$ compared to talipexole alone (Dunnett's multiple comparison test).

reduced the sedative effects of talipexole in all animals tested, but SCH 23390 had no influence (Fig. 7).

3.2.2. Effects of clonidine on tremor in ventromedial tegmentum-lesioned monkeys

The significance of the α_2-adrenoceptor agonistic properties of talipexole in relation to its anti-tremor activity was further evaluated by examining the effects of clonidine, a specific α_2-adrenoceptor agonist. Subcutaneous administration of clonidine (20 μg/kg) had no effect on the tremors in ventromedial tegmentum-lesioned monkeys (Fig. 8), but caused sedation in 2 of 4 animals, 30 min after administration (data not shown).

3.2.3. Evaluation of the effects of B-HT 938 CL_2 and BX-OX 213, the main metabolites of talipexole

Subcutaneous administration of 100 μg/kg of B-HT 938 CL_2 or BX-OX 213, the main metabolites of talipexole, did not cause changes in the tremor (Fig. 8) or the

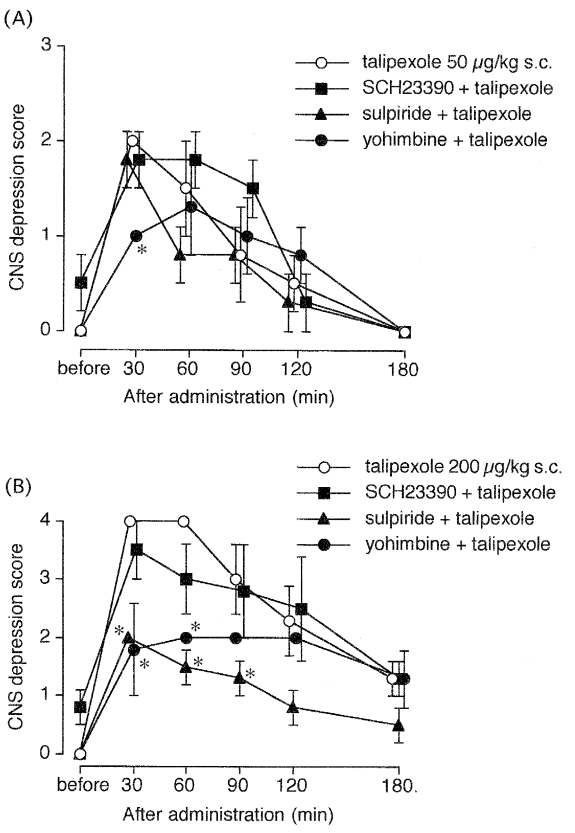

Fig. 7. Effects of receptor antagonists on the talipexole-induced sedative effects in ventromedial tegmentum-lesioned monkeys. Sedative effects were evaluated by using the CNS depression score, as described in Methods. Further legends are as in Fig. 6.

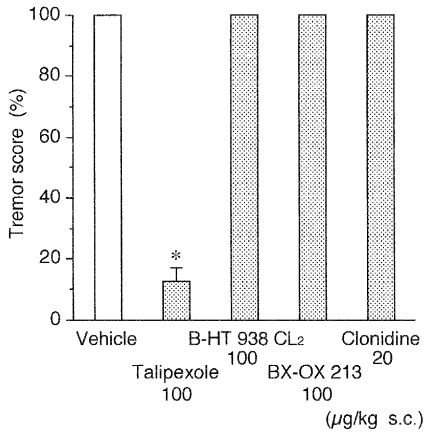

Fig. 8. Studies on the anti-tremor activities of B-HT 938CL2 and BX-OX 213, the main metabolites of talipexole, and clonidine in ventromedial tegmentum-lesioned monkeys. Talipexole, B-HT 938 CL_2, BX-OX 213, clonidine or vehicle (saline) was administered to ventromedial tegmentum-lesioned monkeys. Tremor scores were judged as described in the legend to Fig. 1, and the lowest score of each animal during the 180-min observation period was calculated. Results are presented as the means ± S.E.M. for 4 animals. * $P < 0.05$ compared to the vehicle-treated control (Dunnett's multiple comparison test).

general behavior of ventromedial tegmentum-lesioned monkeys (data not shown).

4. Discussion

Animal models of Parkinsonian-like tremors are produced by electric lesion of the unilateral ventromedial tegmentum and treatment with MPTP, harmaline or tremorine. The persistent resting tremor of the contralateral limbs of ventromedial tegmentum-lesioned monkeys is reported to closely resemble the tremor seen in patients with Parkinson's disease (Ohye et al., 1979; Poirier et al., 1966). As anti-Parkinson's disease drugs suppress these tremors in ventromedial tegmentum-lesioned cynomolgus monkeys, the model can be used to demonstrate the actions of drugs considered for use in humans (Goldstein et al., 1973; Miyamoto et al., 1974). For these reasons, using ventromedial tegmentum-lesioned animal models placed in a monkey-chair, we evaluated the anti-tremor activity of talipexole, an azepine derivative D_2 agonist, and the behavioral changes generally associated with dopamine receptor agonists in comparison with bromocriptine, an ergot derivative dopamine receptor agonist.

Our experiments demonstrated that both subcutaneous and oral administration of talipexole had dose-dependent anti-tremor activity in ventromedial tegmentum-lesioned monkeys. The potency of the anti-tremor activity of talipexole given by oral administration was approximately 40% of that caused by subcutaneous administration of talipexole, as shown by ED_{50} values (34 μg/kg s.c. vs. 84 μg/kg p.o.), even though 94.6% of the orally administered talipexole is absorbed in rats (Kurakazu et al., 1994a). The peak effects appeared approximately 60 min after the subcutaneous injection, and 60–90 min after oral administration, and the duration and the potency of the anti-tremor activity increased dose dependently when talipexole was given by both routes. Talipexole was also found to have anti-tremor activity when used in combination with L-DOPA. In ventromedial tegmentum-lesioned monkeys, anti-tremor doses of talipexole did not cause emetic behavior, such as oral movement, salivation and vomiting, but had sedative effects. During the repeated administration of talipexole (a daily dose of 50 μg/kg s.c. for 21 days), the degree and duration of the anti-tremor activity did not change, although the potency and duration of its sedative effects gradually decreased.

The anti-tremor effect of talipexole does not arise from its sedative effects because of the following results: the monkeys could be aroused when they were observed for assessment of their tremor score (except 30–60 min after administration of 200 μg/kg s.c. of the drug) and the anti-tremor activity of the drug was maintained but the sedative effect weakened during repeated administration. In electroencephalographic (EEG) studies with rats, talipexole (32–100 μg/kg s.c.) caused a drowsy pattern in the frontal cortex and disappearance of the hippocampal theta rhythm, but it did not influence the EEG arousal responses induced by electrical stimulation of the mesencephalic reticular formation, suggesting that its sedative effects are relatively weak and quite different from those caused by sleep inducers (Kohno et al., 1996). Furthermore, talipexole even showed anti-tremor activity in halmarine- and tremorine-treated animals, although the effective doses were much higher than those needed for ventromedial tegmentum-lesioned monkeys (unpublished observations).

For the treatment of Parkinson's disease, dopamine receptor agonists and L-DOPA are usually administered orally. In this study, oral administration of talipexole or L-DOPA had anti-tremor activity with a peak effect 60–90 min, or 30 min, after their administration, respectively. However, following oral administration of bromocriptine, the onset and the time at which the peak effect was observed differed depending on the test doses used in the preliminary experiment. Thus the effects of the subcutaneous administration of talipexole and bromocriptine were compared. Subcutaneous administration of bromocriptine also suppressed tremor in a dose-dependent manner in ventromedial tegmentum-lesioned monkeys, and the ED_{50} of its effect was 2.5 mg/kg s.c. However, talipexole had several properties different from those of bromocriptine. First, it was 70 times more potent than bromocriptine (ED_{50} values were talipexole 34 μg/kg s.c. vs. bromocriptine 2.5 mg/kg s.c.). Secondly, it had a rapid onset of anti-tremor activity compared with bromocriptine (peak effect times were talipexole 60 min vs. bromocriptine 90–180 min). Thirdly, the drug effects on general behavior were quite different. Anti-tremor doses of talipexole did

not induce emetic behavior but did have a sedative effect, while bromocriptine induced oral movement, salivation, and vomiting without having a marked sedative effect when anti-tremor effects were observed.

The pharmacological differences between talipexole and bromocriptine are consistent with previous findings obtained with MPTP-treated common marmosets (Irifune et al., 1993) and in clinical studies (Nakanishi et al., 1993). Talipexole (40–160 μg/kg i.p.) dose dependently increased motor activity, reversed akinesia and incoordination of movement, and was more than 25 times more potent than bromocriptine. An active dose of talipexole (80 μg/kg i.p.) did not induce emesis as strongly as an inactive (against tremor) dose of bromocriptine (0.5 mg/kg i.p.). In clinical studies, talipexole more effectively reduces the symptoms of Parkinson's disease, particularly the upper limb tremor, has few gastrointestinal side effects, but causes greater sleepiness than bromocriptine (Nakanishi et al., 1993). Emesis is induced by most dopamine receptor agonists and L-DOPA. A possible reason why emesis was reduced during treatment with talipexole without a reduction in its antiparkinsonian efficacy, compared with bromocriptine, might be that talipexole possesses a 5-HT_3 receptor blocking action (Nishio et al., 1996) and a higher absorption rate (94.6%, Kurakazu et al., 1994a). However, the precise nature of the mechanism remains to be elucidated.

A single administration of talipexole (50 μg/kg s.c.) in combination with L-DOPA (100 mg/kg p.o.) synergistically suppressed tremor in ventromedial tegmentum-lesioned monkeys. Administration of talipexole alone or with L-DOPA also had antiparkinson effects in MPTP-treated common marmosets (Irifune et al., 1993, 1994), and in clinical trials (Nakanishi et al., 1993).

In order to determine the mechanisms underlying the anti-tremor activity and the sedative effect of talipexole in ventromedial tegmentum-lesioned monkeys, the animals were pretreated with antagonists acting on dopamine D_1/D_2 receptors or on α_2-adrenoceptors. The anti-tremor activity of talipexole was significantly suppressed by sulpiride, but not by SCH 23390 or yohimbine, while its sedative effect was inhibited by sulpiride and yohimbine. These results suggest that talipexole, irrespective of its dose, exerts its anti-tremor activity via selective dopamine D_2 receptor stimulation. This is also supported by the results of previous behavioral, biochemical and electrophysiological studies on talipexole, which demonstrated selective dopamine D_2 receptor stimulation and a lack of dopamine D_1 receptor agonistic properties (Hinzen et al., 1986; Matsubayashi et al., 1995). The α_2 effect of talipexole did not seem to be involved in its anti-tremor activity, as pretreatment with yohimbine did not prevent the anti-tremor effects of talipexole, and also because clonidine did not have anti-tremor activity in ventromedial tegmentum-lesioned monkeys. The dose of clonidine tested (20 μg/kg) corresponded to more than 100 μg/kg of talipexole, as the α_2 effect of clonidine was 6–7 times more potent than that of talipexole when compared with their IC_{50} values for [^{3}H]clonidine binding in rat brain membranes, or with the ID_{50} values for the spontaneous firing of locus coeruleus neurons (Kobinger and Pichler, 1980). Conversely, the sedative effects of low and high doses of talipexole seemed to result from the stimulation of dopamine D_2 and α_2-autoreceptors.

Anticholinergic agents are generally used to treat the tremor that occurs in Parkinson's disease. Anticholinergic mechanism are not involved in the anti-tremor effects of talipexole, since talipexole had no binding affinity for muscarinic ($M_1 + M_2 + M_3$) receptors in rat brain membranes (unpublished observation). The involvement of 5-HT_3 receptor antagonist activity seemed negligible, because serotonergic mechanisms have not been reported in relation to anti-tremor activity or antiparkinsonian activity.

Administration of BX-OX 213 CL_2 and B-HT 938, the main metabolites of talipexole in rats and monkeys (Kurakazu et al., 1994b; Sakai et al., 1994), did not cause anti-tremor effects or sedative effects. These results indicate that talipexole itself produced these effects.

The doses of talipexole showing anti-tremor activity in ventromedial tegmentum-lesioned monkeys (50–100 μg/kg) are quite similar to those that improve akinesia and rigidity in MPTP- or reserpine-treated animals (Hinzen et al., 1986; Irifune et al., 1993, 1994), and also correlate reasonably well with the clinical doses (maintenance doses of talipexole in the clinical studies were 1.2–3.6 mg/day, Nakanishi et al., 1993).

In conclusion, this study demonstrates that talipexole improves tremor as well as akinesia and rigidity via the selective stimulation of dopamine D_2 receptors, and that talipexole exhibits anti-tremor effects alone or in combination with L-DOPA. These properties of talipexole seem favorable for the treatment of Parkinson's disease.

Acknowledgements

We wish to thank Prof. Tatsuo Furukawa of Fukuoka University and Prof. Masashi Sasa of Hiroshima University for their continuous support and active discussion throughout this study.

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