Inhibitory Effects of Talipexole and Pramipexole on MPTP-Induced Dopamine Reduction in the Striatum of C57BL/6N Mice

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ABSTRACT—We have investigated the effects of two novel antiparkinsonian drugs, talipexole (Domin®) and pramipexole, on MPTP-induced dopamine (DA) reduction in the striatum of C57BL/6N mice in comparison with those of bromocriptine. Fifteen days after MPTP treatment (25 mg/kg, i.p., given daily for 5 days), the DA content in the striatum was decreased to 40–60% of the control value. Among the three dopamine receptor agonists, talipexole and pramipexole (1 mg/kg, i.p., once a day for 20 days) more significantly suppressed the MPTP-induced DA reduction in the striatum than bromocriptine (10 mg/kg, i.p., once a day for 20 days). Talipexole did not influence [3H]MPP⁺ uptake into striatal synaptosomes. These results suggest that talipexole and pramipexole have a protective effect against MPTP-induced DA reduction in the striatum of C57BL/6N mice.

Keywords: Talipexole, Pramipexole, Antiparkinsonian drug, Neuroprotective effect, MPTP

Systemic injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to reproduce the degeneration of dopaminergic neurons in the substantia nigra pars compacta and the behavioral features of Parkinson's disease in humans and some experimental animals (1). Although the neurodegenerative mechanisms of Parkinson's disease have not yet been elucidated, oxidative stress, excessive free-radical formation, MPTP-like environmental toxins and/or endogenous neurotoxins may be involved in the progressive loss of nigral cells of Parkinson's disease (2, 3). In recent animal studies, dopamine receptor agonists such as bromocriptine and pergolide, which are used for symptomatic therapy of Parkinson's disease, showed possible neuroprotective effects under a variety of neurodegenerative conditions (3–7). Therefore, much attention is being focused on the neuroprotective effects of dopamine receptor agonists for the current and future treatment of Parkinson's disease. However, the following are still unknown: i) Are neuroprotective effects common among dopamine receptor agonists? and ii) Can their neuroprotective effects be expected during the triggering, onset or damage phase of dopaminergic neuron degeneration?

We previously reported in C57BL/6N mice that 5 days of repeated administration of MPTP at a daily dose more than 25 mg/kg, intraperitoneally (i.p.) caused a significant decrease in striatal dopamine (DA) content without changing noradrenaline and 5-hydroxytryptamine metabolisms (8). In the present study, we examined the effects of talipexole and pramipexole, novel azepeine-derivative dopamine D₂-receptor agonists, along with bromocriptine, the most widely used ergot-derivative dopamine receptor agonist, against the MPTP-induced DA reduction in the striatum of C57BL/6N mice.

MATERIALS AND METHODS

Materials
Talipexole (B-HT 920CL₂, 6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo[4,5-d]-azepine dihydrochloride) and pramipexole (SND 919CL₂Y, (−)-2-amino-4,5,6,7-tetrahydro-6-propylamino-benzthiazol dihydrochloride) were obtained from Boehringer Ingelheim (Ingelheim, Germany). Bromocriptine mesilate and MK-801 were kindly donated by Sandoz Pharma A.G. (Basel, Switzerland) and Merck Sharp (Hoddesdon, UK), respectively.
MPTP, 1-methyl-4-phenylpyridinium (MPP⁺), clorgyline, deprenyl and nomifensine were purchased from Research Biochemicals International (Natick, MA, USA). [³H]MPP⁺ from NEN/Dupont (Boston, MA, USA) was used.

Animals and dosing schedule
Three or four male C57BL/6N mice, weighing 18–23 g, were used for each group in the studies. Mice were administered drugs intraperitoneally (i.p.). From days 1 to 5, the mice were given MPTP (25 mg/kg, 0.1 ml/10 g of body weight, once a day for 5 days). We designated period I, period II and period III as the triggering phase, the onset phase and the damage phase of MPTP-induced neurotoxicity, respectively. The neuroprotective effects of the tested drugs against the 5-day MPTP treatment were examined by i.p. administration according to dosing schedule 1 or 2 as described below. Saline was administered in parallel as the corresponding vehicle control. In all experiments, mouse brains were rapidly removed on day 20, and the striatum was immediately dissected out on an ice-cold plastic plate.

Dosing schedule 1 (see Fig. 1): During days 1–5 (period I), a test drug from the group comprising deprenyl (10 mg/kg), clorgyline (10 mg/kg), nomifensine (10 mg/kg) and MK-801 (5 mg/kg) was injected daily 1 hr before each MPTP administration. During days 6–10 (period II), the same dose of the test drug was further injected. Subsequently, these mice were kept for 10 days without drug administration (during days 11–20, period III). On the other hand, a test drug from the group comprising talipexole (1 mg/kg), bromocriptine (10 mg/kg) and pramipexole (1 mg/kg) was injected 1 hr before each MPTP administration. During days 6–20 (periods II and III), the same dose of the test drug was further injected. On the day 20, the striatum was dissected out 40 min (for talipexole and pramipexole) or 4 hr (for bromocriptine) after the final administration.

Dosing schedule 2 (see Fig. 2): During days 1–5 (period I), talipexole (1 mg/kg) was injected 1 hr before each MPTP administration. During days 6–12 (period II) and/or days 13–19 (period III), the same dose of talipexole was injected. One day after the final administration in period III (on day 20), the striatum was dissected out.

Measurement of DA and its metabolites
The content of DA, as well as those of 3,4-dihydroxyphenylacetate (DOPAC) and homovanillate (HVA), metabolites of DA, in the striatum were measured by a high-performance liquid chromatography apparatus with an electrochemical detector (HPLC-ECD system) (Eicom, Kyoto), as described previously (8). In brief, the analytical column was an Eicompak MK-5 ODS column (4.6 × 150 mm). The mobile phase was 100 mM citrate–100 mM sodium acetate buffer containing 17% methanol. Each striatum was homogenized and then sonicated with 10 vol. of 200 mM perchloric acid containing 200 ng isoproterenol as the internal standard. The homogenate was placed in an ice bath for 30 min. Subsequently, the sample was centrifuged at 15,000 × g, for 15 min at 4°C. The supernatant (150 μl) was adjusted to pH 3 with 1 M sodium acetate (62.5 μl) and then filtered through Columnguard-LCR₄ pre-column (Millipore, Bedford, MA, USA). The sample (20 μl) thus obtained was injected into the HPLC-ECD system.

Results are given as means ± standard error (S.E.) values. Statistical differences were determined by the analysis of variance (ANOVA). Further statistical analysis for post hoc comparisons was done with the Dunnett multiple comparisons test.

Measurement of synaptosomal uptake of [³H]MPP⁺
The crude synaptosomal fractions (P₂ fractions) obtained from the striatum of the C57BL/6N mice were incubated for 30 min at 30°C with 20 nM [³H]MPP⁺ and the test drug (e.g., talipexole, nomifensine, DA or MPP⁺) in Krebs-Ringer buffer of the following composition: 1.1 mM CaCl₂, 0.83 mM MgCl₂, 126 mM NaCl, 2.4 mM KCl, 27.5 mM NaHCO₃, 0.5 mM KH₂PO₄ and 5.9 mM glucose, pH 7.4, bubbled with O₂/CO₂ (95 : 5, v/v). After incubation, the mixture was filtered under reduced pressure through a Whatman GF/C filter (pre-soaked in 0.30% polyethyleneimine) and rinsed three times with 3 ml ice-cold buffer. The radioactivity was measured by a liquid scintillation counter (model 300C; Packard, Downer Grove, IL, USA). Specific uptake was determined as the radioactivity after subtraction of the non-specific uptake (in the presence of 100 μM MPP⁺) from the total uptake. Protein concentration was determined by Lowry’s method (9) with bovine serum albumin as the standard.

RESULTS

Effects of test drugs on MPTP-induced DA reduction with dosing schedule 1
In the present study, the administration of neither MPTP (at 25 mg/kg) nor other drugs caused death in mice. As shown in Fig. 1 (A and B) and Fig. 2A, MPTP treatments decreased the striatal DA content to 40–60% of the control value. For dosing schedule 1, the administration of deprenyl and nomifensine, inhibitors of monoamine oxidase-B (MAO-B) and DA transporter respectively, appeared to protect the neurons from MPTP-induced DA reduction (Fig. 1A). On the other
hand, the administration of clorgyline and MK-801, inhibitors of MAO-A and N-methyl-D-aspartate (NMDA) receptor, respectively, did not protect against DA reduction.
Treatments with talipexole, bromocriptine and pramipexole with dosing schedule 1 significantly inhibited the MPTP-induced DA reduction in the striatum (Fig. 1B). The inhibiting potency was talipexole > pramipexole > bromocriptine. In addition, DA content in the striatum after treatment with talipexole and pramipexole were significantly higher than that in the control group (Fig. 1B).

![Graphs showing DA, DOPAC, and HVA content](image)

Fig. 2. Investigation of the neuroprotective phase of talipexole against MPTP-induced DA reduction. C57BL/6N mice were given MPTP (25 mg/kg) for 5 days during period I, and talipexole (1 mg/kg, once a day) was administered repeatedly according to dosing schedule 2. Each group consisted of 4 mice. On day 20, the contents of DA (A), DOPAC (B) and HVA (C) in the striatum were measured by the HPLC-ECD system. Each point shows the mean ± S.E. of 4 mice. F values by ANOVA were $F(6,21)=7.279$ (P=0.0003), $F(6,21)=2.145$ (P=0.0906) and $F(6,21)=7.641$ (P=0.0002) in panels A, B and C, respectively. Subsequently, the Dunnett multiple comparisons test was done. *P<0.05, **P<0.01: statistically significant compared to the control group. *P<0.05, **P<0.01: statistically significant compared to the vehicle group with MPTP treatments (Dunnett multiple comparisons).
Effects of talipexole on MPTP-induced DA reduction with dosing schedule 2

We further examined when the inhibiting effect of talipexole against MPTP-induced DA reduction might be expected: during the triggering phase (period I), the onset phase of damage (period II) and the damage phase (period III). With dosing schedule 2, MPTP treatment caused a marked decrease in the contents of DA, DOPAC and HVA (Fig. 2). The administration of talipexole throughout periods I – III significantly inhibited this decrease in DA, DOPAC and HVA, but the administration of talipexole for the same period did not cause changes in mice in the absence of MPTP treatment. On the other hand, the administration of talipexole during only period I or II tended to inhibit the reduction of DA content (Fig. 2). The administration of talipexole during period III in no way inhibited these changes.

Effects of talipexole on the uptake of [3H]MPP+ into striatal synaptosomes

The uptake of [3H]MPP+ into striatal synaptosomes of C57BL/6N mice was highest compared to the uptake into synaptosomes of the cerebral cortex, hippocampus, midbrain, hypothalamus, cerebellum and medulla oblongata (data not shown); the [3H]MPP+ uptake into these brain regions significantly correlated with [3H]DA uptake (r=0.971, P<0.001) but not with [3H]choline uptake (r=0.170, no significance).

[3H]MPP+ uptake into striatal synaptosomes was completely inhibited by DA and nomifensine, but not by talipexole (Fig. 3).

DISCUSSION

In agreement with our previous finding (8), MPTP treatment (25 mg/kg, i.p., once a day for 5 days) decreased the striatal DA content to 40-60% of the control value on day 20. With dosing schedule 1, the MPTP-induced DA reduction was significantly suppressed by deprenyl and nomifensine but not by clorgyline and MK-801. Recent studies have determined the detailed mechanism of MPTP neurotoxicity (1, 10). In brief, i) MPTP easily penetrates the blood-brain barrier even with peripheral administration; ii) MPTP is catalyzed by MAO-B in astrocytes but not by MAO-A in vivo in the brain and is converted into a metabolite MPP+, and iii) MPP+ is rapidly taken up into dopaminergic neurons containing neuromelanin through DA transporters after which it induces neurotoxicity. Taking these mechanisms of MPTP neurotoxicity into consideration, the inhibitory effects of deprenyl and nomifensine observed in the present experiment seem to be caused by inhibition of the triggering and onset phases of MPTP neurotoxicity. On the other hand, a recent paper reported that MK-801 protected neurons in the substantia nigra of Wistar rat from MPP+ neurotoxicity (11). In the present study, however, MK-801 did not inhibit MPTP-induced DA reduction in the striatum of C57BL/6N mice.

Treatment with talipexole (1 mg/kg, i.p.), bromocriptine (10 mg/kg, i.p.) and pramipexole (1 mg/kg, i.p.) with dosing schedule 1 also inhibited the MPTP-induced DA reduction in the striatum on day 20. Among the three dopamine receptor agonists, talipexole and pramipexole more significantly protected against the MPTP-induced DA reduction than bromocriptine. As shown in Fig. 2, repeated administration of talipexole did not cause changes in DA, DOPAC and HVA content in normal mice in the absence of MPTP treatment. In another experiment, we found that a single administration of talipexole increased the DA content in the striatum of normal rats after 40 min, and DA content recovered to the normal level after 24 hr (12). It was also known that single administration of talipexole after 40 min or of bromocriptine after 4 hr inhibited DA metabolism and resulted in a slightly increased DA content (13, 14). Taking together these findings, the inhibitory effect of talipexole on MPTP-induced DA reduction seems not to be exerted via its influence on DA metabolism.

Both talipexole and pramipexole are azepine-derivatives that are D2-receptor agonists lacking the ability of D1-receptor stimulation, and they showed similar effects on [3H]spiperone binding (12) or MPTP-induced parkinsonian symptoms (15, 16). Therefore, further studies
were performed with talipexole. With dosing schedule 2, the administration of talipexole throughout periods I–III significantly inhibited the MPTP-induced decrease in DA, DOPAC and HVA. The administration of talipexole during only period I or II tended to inhibit the reduction of DA, and the administration of talipexole during period III lacked the inhibitory effect. These results suggest that the inhibitory effects of talipexole is exhibited by its repeated administrations at least in periods I and II (the triggering and onset phases of MPTP-induced neurotoxicity).

In the present study, [3H]MPP+ uptake into striatal synaptosomes of C57BL/6N mice was completely inhibited by DA and nomifensine, but not by talipexole. These results supported the idea that MPP+ was taken up into dopaminergic neurons by means of the DA transport system. However, talipexole does not inhibit the uptake of MPP+ into dopaminergic neurons. One possible effect of neuroprotective agents such as deprenyl and nomifensine could be mediated via inhibition of MAO-B or MPP+ uptake into dopaminergic neurons. This seems not to be the case with the effect of talipexole, as talipexole does not inhibit the MPP+ uptake in the present study and also does not inhibit MAO-B activity (W.D. Bechtel et al., unpublished observation, Boehringer Ingelheim in Germany). Talipexole may exert its inhibitory effect on MPTP-induced DA reduction by other mechanisms. On the neuroprotective effect of DA-receptor agonists, recent papers indicate some possibilities that bromocriptine and pergolide have properties of an antioxidant and radical scavenger (17–19) and that bromocriptine inhibits glutamate uptake into synaptic vesicles (20). We previously reported that MPP+ -induced cell death in PC12 cells was accompanied by a decrease in mRNA expression of tyrosine hydroxylase and DA content (21) and that nerve growth factor protected against this cell death (22). MPP+ induces cell death partly through an apoptotic process (23, 24) and the apoptotic process of neurons occurred in brains of Parkinson's disease (25). The neuronal apoptosis is also inhibited by overexpression of protooncogene Bcl-2 protein (26, 27). Thus, intracellular mechanisms of the neuroprotective effects of talipexole such as antioxidant action and induction of Bcl-2 protein remain for further investigation.

In conclusion, a 20-day repeated administration of talipexole, pramipexole and bromocriptine exerted an inhibitory effect on MPTP-induced DA reduction in the striatum of young C57BL/6N mice. The effects of talipexole and pramipexole (1 mg/kg, i.p.) were more pronounced than those of bromocriptine (10 mg/kg, i.p.) and were comparable to the effects of deprenyl or nomifensine, although their mechanisms seem to differ. In addition, the neuroprotective effect of talipexole might be exhibited at the triggering and onset phases of the striatal degeneration following MPTP injection.

Unfortunately, therapeutic possibilities for the neuroprotective effect of talipexole were not identified in the present study. Its protective dose, 1 mg/kg, was higher than those improving parkinsonian symptoms in controlled clinical trials (maximum daily dose was set at 3.6 or 4.8 mg/day) (28, 29), in MPTP-treated monkeys (0.01–0.2 mg/kg) (15, 30), or in cynomolgus monkeys with unilateral lesion in the ventromedial tegmentum (0.025–0.2 mg/kg) (31). The clinical significance of the neuroprotective effects of talipexole should be further examined by employing lower doses.

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