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Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms

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Abstract

The role of dopamine D3 receptors was investigated in mediating the neuroprotective effect of the dopamine D2/D3 receptor agonist (*S*)-2-amino-4,5,6,7-tetrahydro-6-propylamine-benzothiazole (pramipexole) in vivo. Pramipexole retained the ability to inhibit 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopamine depletion in mice in which the dopamine D3 receptor had been deleted. However, the neuroprotective efficacy was reduced in the dopamine D3 receptor-deleted mice compared to that in littermates expressing the wildtype receptor. Furthermore, the dopamine D3 receptor selective antagonist 2-{3-[4-(2-*tert*-butyl-6-trifluoromethyl-4-pyrimidinyl)-1-piperazinyl]propylthio}-4-pyrimidinol (A-437203) partially inhibited the neuroprotective effect of pramipexole in dopamine D3 receptor expressing mice but not in receptor-deleted mice. These results indicate that pramipexole protects dopamine neurons from MPTP-induced toxicity by mechanisms that are both dependent and independent of an interaction with dopamine D3 receptors.
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Keywords: Parkinson's disease; MPTP; Pramipexole; Dopamine D_3 receptor

1. Introduction

Parkinson's disease is a syndrome of tremor, rigidity, akinesia, and postural instability first described by James Parkinson in 1817. Landmark studies in the 1960s led to the realization that Parkinson's disease results from the substantial (>70–80%) depletion of the striatal neurotransmitter dopamine, due to the death of the dopamine-producing neurons in the substantia nigra (Wooten, 1997). This discovery prompted clinical experiments to attempt to replace the depleted neurotransmitter. It was found that administering the dopamine precursor, L-dihydroxyphenylalanine (L-DOPA), which is converted to dopamine in situ, alleviates the symptoms of Parkinson's disease. L-DOPA therapy became the standard of care for Parkinson's disease and remains so today. However, for the majority of patients, L-DOPA therapy eventually loses effectiveness and begins to be accompanied by severe side effects including dyskinesias and neuropsychiatric symptoms. Thus, there is a clear need for new, life-long therapies for Parkinson's disease patients, ideally ones that will prevent the loss of dopamine neurons and, thus, halt disease progression.

A potential breakthrough is the recent finding that Parkinson's disease patients treated initially with the dopamine D2/D3 receptor agonist (*S*)-2-amino-4,5,6,7-tetrahydro-6-propylamine-benzothiazole (pramipexole) had a slower rate of loss of striatal dopamine transporter density, as measured in life with the single photon emission computed tomography ligand [^{123}I]β – carbomethoxy-3β-(4-iodophenyltropane) (βCIT, Marek et al., 2002). These data suggest that pramipexole may slow the rate of dopamine neuron loss in Parkinson's disease. Thus, it is of great interest to investigate potential mechanisms for this putative neuroprotective effect, to give insight into the mechanisms of the disease process and to begin to develop more effective disease modifying therapies. Pramipexole has been found to inhibit dopamine neuron toxicity in a variety of experimental paradigms (Anderson et al., 2001; Cassarino et al., 2001; Ferger et al., 2000; Hall et al., 1996; Kitamura et al., 1997; Le et al., 2000; Ling et al., 1999; Sethy et al., 1997; Vu et al., 2000). Several mechanisms for this neuroprotective effect have been proposed. In particular, Carvey et al. have presented evidence from in vitro systems suggesting that an interac-

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tion with the D3 subtype of dopamine receptor is at least partially involved (Ling et al., 1999). In the present study, we undertook to further investigate the role for dopamine D3 receptor interactions in vivo. Specifically, we investigated the effects of a dopamine D3 receptor antagonist 2-{3-[4-(2-*tert*-butyl-6-trifluoromethyl-4-pyrimidinyl)-1-piperazinyl]propylthio}-4-pyrimidinol (A-437203; (Unger et al., 2002; Drescher et al., 2002)) and deletion of the dopamine D3 receptor gene on the neuroprotective effect of pramipexole in a murine model of Parkinson's disease, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced striatal dopamine depletion.

This work has been presented in preliminary form.[1]

2. Materials and methods

2.1. Chemicals

Pramipexole (Hammer et al., 2001) and A-437203 (Hoger et al., 2002) were synthesized as described. MPTP was purchased from Aldrich Sigma (St. Louis, MO).

2.2. Animals

Male C57BL/6 mice weighing 25 g ± 1 were obtained from Charles River Laboratories (Raleigh, NC). Male and female C57BL/6 mice in which the dopamine D3 receptor gene has been deleted (Accili et al., 1996) were obtained from Jackson Laboratories (Bar Harbor, ME) and bred in house. Male mice homozygous for the gene deletion and littermates expressing the wildtype gene were used. Animals were housed five to six/cage with ad libitum access to food and water and maintained at a constant temperature (21–23 °C) and humidity (45–50%) with lights on 0700–1900 h. The Pfizer Institutional Animal Care and Use Committee approved all experimental protocols.

2.3. MPTP and compound administrations

MPTP (HCl salt dissolved in 0.9% saline) was administered by intraperitoneal (i.p.) injection at a dose of 30 mg/kg. Pramipexole or pramipexole plus A-437203 (dissolved in 0.9% saline) were administered by subcutaneous (s.c.) injection at doses indicated in Results. These compounds were administered 30 min prior to MPTP and again after 24 and 48 h. Animals were euthanized 24 h after the final treatment for determination of striatal dopamine content.

2.4. Determination of striatal dopamine content

After animals were euthanized, brains were rapidly removed and cooled on ice. Striatal tissue was dissected using the atlas of Paxinos and Franklin (2001) as a guide, homogenized in 500 μl of 0.1 M $HClO_4$, and centrifuged at 10,000 × *g* for 30 min. The supernatants were analyzed for dopamine content by high performance liquid chromatography with electrochemical detection. Dopamine was separated from its metabolites and other monoamines using a C-18 column (BDS hypersil, 3 μm, 150 × 3 mm, Keystone Scientific, Waltham, MA). The mobile phase consisted of 0.1 M sodium acetate trihydrate, 6% methanol, 84 μM *n*-octylsodium sulfate, 10–15 mg/l disodium EDTA, in filtered distilled water (pH 4.1, adjusted with glacial acetic acid). The mobile phase was delivered at a flow rate of 0.3 ml/min with an ESA pump model 580. Dopamine was quantified using a DECADE Digital Electrochemical Amperometric Detector (Antec Leyden, Zoeterwoude, the Netherlands) at an electrode potential of 0.55 V with respect to the reference electrode. The protein content in the pellet was determined by the Pierce bincinchononic acid protein assay method. Dopamine content is expressed as ng/mg protein.

2.5. Data analysis

Differences in striatal dopamine content between differently treated groups were analyzed using one-way analysis of variance (ANOVA) followed by pair wise comparisons with Fisher's probability of least significant difference (PLSD) test. $*P < 0.05$ was considered statistically significant. Because of differences in basal levels of striatal dopamine content in the dopamine D3 receptor knock out mice and littermate controls, we did not perform ANOVA to analyze differences in dopamine contents after MPTP treatments across these two types of animals. The descriptive statistic of the *percentage depletion* of striatal dopamine content in the MPTP-treated groups was calculated as: 100 − ((mean striatal dopamine content of MPTP-treated group/striatal dopamine content of non-MPTP-treated control group) × 100). The *percentage inhibition* is the descriptive statistic of the extent to which pramipexole treatment inhibited the MPTP-induced dopamine depletion. This value was calculated as: (% depletion of MPTP only group − % depletion of MPTP/pramipexole group)/% depletion of MPTP only group) × 100.

3. Results

3.1. Pramipexole inhibits MPTP-induced dopamine depletion

The dose–response relationship for the neuroprotective effect of pramipexole was first investigated in C57BL/6 mice obtained from Charles River. Mice were administered

[1] A. D. Ramirez, F. S. Menniti, S. K. F. Wong. 2002. Pramipexole inhibits MPTP toxicity in male mice: Reversal by the D3-selective antagonist LU-201640. *Society for Neuroscience 32nd Annual Meeting.* Program No. 690.16.

0.1–3.2 mg/kg pramipexole s.c. or vehicle 30 min prior to a single 30 mg/kg i.p. administration of MPTP. Animals received the same dose of pramipexole again after 24 and 48 h. An additional group of animals received vehicle injections and no MPTP to serve as a control. Striatal dopamine content was determined at 24 h after the last compound administration.

In the non-MPTP-treated group, the striatal dopamine content was 146 ± 6 ng/mg protein. MPTP reduced striatal dopamine content by 85%, to 22 ± 6 ng/mg protein (Fig. 1). This effect of MPTP was inhibited by pramipexole in a dose-dependent fashion. The striatal dopamine contents were significantly ($P < 0.05$) higher in animals treated with 0.32, 1.0 and 3.2 mg/kg of pramipexole compared to MPTP/vehicle treated animals. At the maximally effective dose of 1.0 mg/kg, striatal dopamine content was reduced by MPTP by only 47% (to 78 ± 15 ng/mg protein) relative to the non-MPTP-treated group. Thus, the percentage inhibition of MPTP-induced dopamine depletion by this dose of pramipexole was 55%. The minimum effective dose of pramipexole was 0.32 mg/kg, which produced a 33% inhibition of MPTP-induced dopamine depletion.

3.2. A-437203 inhibits the effect of pramipexole on MPTP-induced dopamine depletion

We next investigated whether the dopamine D3 receptor antagonist A-437203 inhibited the efficacy of pramipexole

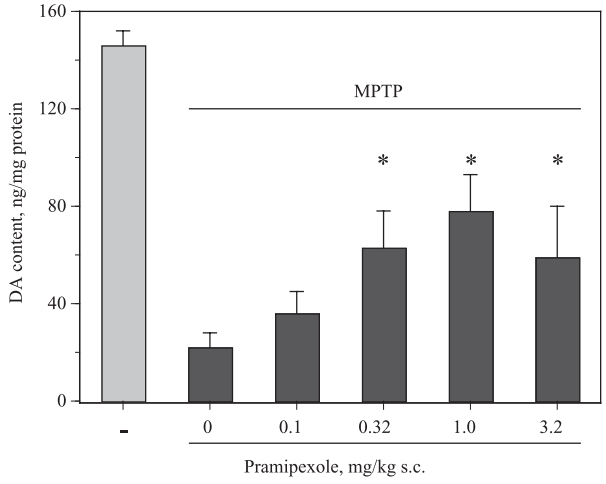

Fig. 1. Effect of pramipexole on MPTP-induced striatal dopamine depletion. Mice (C57BL/6 obtained from Charles River) received no MPTP (light gray bar) or MPTP and the indicated doses of pramipexole (dark gray bars) and striatal dopamine content was determined as described in Materials and methods. ANOVA followed by pair wise comparisons with Fisher's probability of least significant difference (PLSD) test indicates that dopamine content is significantly reduced in each group receiving MPTP compared to the no MPTP group ($P < 0.05$). * indicates that the dopamine content was significantly higher in the groups treated with 0.32, 1.0 and 3.2 mg/kg pramipexole compared to the group that received 0 mg/kg pramipexole ($P < 0.05$). Each bar represents the mean ± S.E.M., $n = 4–6$/group.

on MPTP-induced striatal dopamine depletion. A-437203 binds with high affinity to human recombinant dopamine D3 receptors ($K_i = 1.3$ nM) and has significantly lower affinity for the human recombinant dopamine D2 receptor ($K_i = 443$ nM) (Schmidt, personal communication). A-437203 was used at a dose of 10 mg/kg s.c. This dose in rat failed to block alkylation of dopamine D2 receptors by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in brain in vivo (Drescher et al., 2002), and also failed to cause dopamine D2 receptor inhibition-induced elevation in serum prolactin levels (Tingley and Schmidt, personal communication), suggesting that exposure was below the level at which significant interaction with dopamine D2 receptors occurs. Since A-437203 is competitive with pramipexole for binding to dopamine D3 receptors, we used pramipexole at the minimum effective neuroprotective dose established above.

Mice (Charles River) were administered vehicle, 0.32 mg/kg pramipexole or 0.32 mg/kg pramipexole plus 10 mg/kg A-437203 followed after 30 min by a single dose of MPTP (30 mg/kg). Animals received the pramipexole ± A-437203 again after 24 and 48 h. An additional group of animals received vehicle injections and no MPTP. Striatal dopamine content was determined at 24 h after the last compound administration.

The striatal dopamine content was 134 ± 9 ng/mg protein in the non-MPTP-treated group and was reduced by 82% to 24 ± 4 ng/mg protein by MPTP administration (Fig. 2). Administration of 0.32 mg/kg pramipexole inhibited the depletion of striatal dopamine, as in the previous experiment. The striatal dopamine content of the group receiving 0.32 mg/kg pramipexole prior to MPTP was 51 ± 9 ng/mg protein, which is significantly ($P < 0.05$) higher than the striatal dopamine content of the MPTP only group. Thus, this dose of pramipexole inhibited the effect of MPTP by 24%. In the group receiving pramipexole in combination with A-437203, the striatal dopamine content was depleted by 74% to 35 ± 9 ng/mg protein protein. This dopamine level is significantly lower than that of the group receiving pramipexole alone. Thus, A-437203 inhibited the efficacy of pramipexole against MPTP-induced dopamine depletion by 60%.

3.3. Effect of pramipexole in dopamine D3 receptor knockout mice

In a second approach to investigating the role of dopamine D3 receptors in the neuroprotective efficacy of pramipexole, we utilized C57BL/6 mice obtained from Jackson Laboratories in which the gene for the dopamine D3 receptor was deleted (D3 −/− mice). D3 −/− mice and littermates homozygous for the wildtype gene (D3 +/+ mice) were obtained by crossing mice heterozygous for the gene deletion. Groups of D3 −/− and D3 +/+ mice were administered vehicle or 0.32 mg/kg pramipexole followed after 30 min by a single dose of MPTP (30 mg/kg).

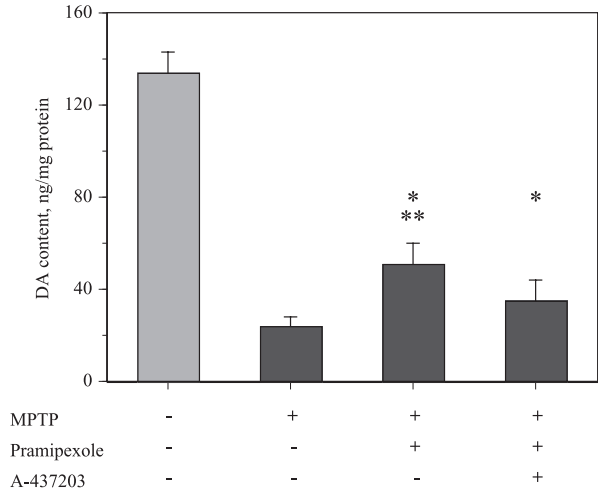

Fig. 2. Effect of A-437203 on the inhibition of MPTP-induced striatal dopamine depletion by pramipexole. Mice (C57BL/6 obtained from Charles River) received no MPTP (light gray bar) or MPTP alone, with 0.32 mg/kg pramipexole, or with 0.32 mg/kg pramipexole plus 10 mg/kg A-437203 (dark gray bars) and striatal dopamine content was determined as described in Materials and methods. ANOVA followed by pair wise comparisons with Fisher's probability of least significant difference (PLSD) test indicates that dopamine content is significantly reduced in each group receiving MPTP compared to the no MPTP group ($P<0.05$). * Indicates that the dopamine content was significantly higher in the groups treated with pramipexole and pramipexole plus A-437203 compared to the group that received no pramipexole ($P<0.05$). ** Indicates that the dopamine content was significantly higher in the group treated with pramipexole compared to the group that received pramipexole plus A-437203 ($P<0.05$). Each bar represents the mean ± S.E.M., $n=4-6$/group.

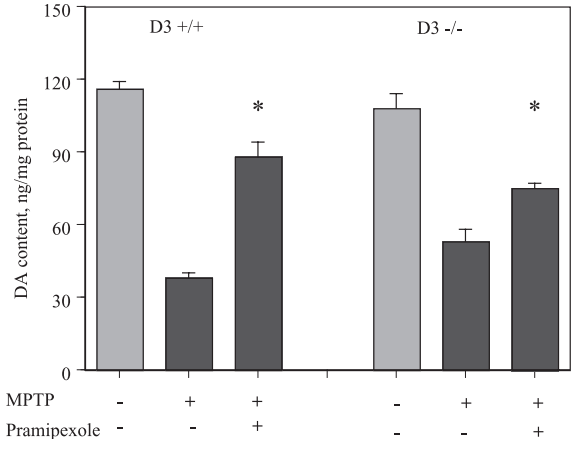

Fig. 3. Effect of pramipexole on MPTP-induced striatal dopamine depletion in dopamine D3 receptor knock out mice. D3 +/+ or D3 −/− mice (C57BL/6 obtained from Jackson Laboratories) received no MPTP (light gray bars) or MPTP alone or with 0.32 mg/kg pramipexole (dark gray bars) and striatal dopamine content was determined as described in Materials and methods. ANOVA followed by pair wise comparisons with Fisher's probability of least significant difference (PLSD) test was performed separately for the D3 +/+ and D3 −/− groups. In both the D3 +/+ and D3 −/− mice, dopamine content is significantly lower in each group receiving MPTP than in the no MPTP group ($P<0.05$). * Indicates that the dopamine content is significantly higher in the groups treated with pramipexole than in the groups that received no pramipexole ($P<0.05$). Each bar represents the mean ± S.E.M., $n=4-6$/group.

Animals received the same dose of pramipexole again after 24 and 48 h. A group of D3 −/− and of D3 +/+ mice also received vehicle injections and no MPTP. Striatal dopamine content was determined at 24 h after the last compound administration.

In the absence of MPTP treatment, the striatal dopamine content was slightly higher in the D3 +/+ compared to the D3 −/− mice (Fig. 3, see also Fig. 4). In the D3 +/+ mice, MPTP caused a 67% depletion of striatal dopamine content (Fig. 3, Table 1). In contrast, when these mice received pramipexole with MPTP, the striatal dopamine content was depleted by only 24%. This represents a 64% inhibition of the effect of MPTP.

In the D3 −/− group, MPTP was less effective in reducing striatal dopamine content (Fig. 3). In these mice, MPTP induced a 51% depletion of striatal dopamine content (Table 1, Experiment 1). Administration of pramipexole with MPTP reduced the extent of MPTP-induced striatal

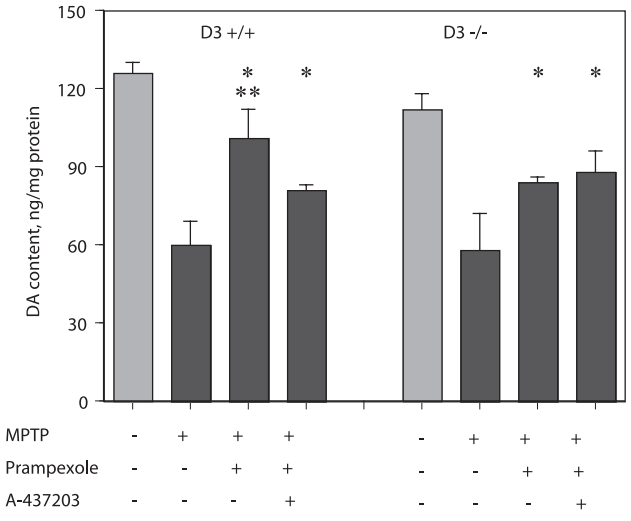

Fig. 4. Effect of A-437203 on the inhibition of MPTP-induced striatal dopamine depletion by pramipexole in dopamine D3 receptor knock out mice. D3 +/+ or D3 −/− mice (C57BL/6 obtained from Jackson Laboratories) received no MPTP (light gray bar) or MPTP alone, with 0.32 mg/kg pramipexole, or with 0.32 mg/kg pramipexole plus 10 mg/kg A-437203 (dark gray bars) and striatal dopamine content was determined as described in Materials and methods. ANOVA followed by pair wise comparisons with Fisher's probability of least significant difference (PLSD) test was performed separately for the D3 +/+ and D3 −/− groups. In both the D3 +/+ and D3 −/− mice, dopamine content is significantly lower in each group receiving MPTP than in the no MPTP group ($P<0.05$). In the D3 +/+ mice, * indicates that the dopamine content was significantly higher in the groups treated with pramipexole and pramipexole plus A-437203 compared to the group that received no pramipexole ($P<0.05$) and ** indicates that the dopamine content was significantly higher in the group treated with pramipexole compared to the group that received pramipexole plus A-437203 ($P<0.05$). In the D3 −/− mice, * indicates that the dopamine contents were also significantly higher in the groups treated with pramipexole or pramipexole plus A-437203 compared to the group that received no pramipexole ($P<0.05$). However, the dopamine content was not statistically different between the group treated with pramipexole compared to the group that received pramipexole plus A-437203 ($P<0.05$). Each bar represents the mean ± S.E.M., $n=4-6$/group.

Table 1
Descriptive statistics for effects of pramipexole and A-437203 on MPTP-induced striatal dopamine depletion in dopamine D3 receptor knock out mice

	D3 +/+		D3 −/−	
	% Depletion	% Inhibition	% Depletion	% Inhibition
From Fig. 3				
MPTP only	67		51	
Pramipexole/ MPTP	24	64	31	39
From Fig. 4				
MPTP	52	–	48	–
Pramipexole/ MPTP	20	63	25	48
Pramipexole/ A-437203/ MPTP	36	31	21	56

The % Depletion of striatal dopamine content caused by MPTP and the % Inhibition of the effect of MPTP by pramipexole in the absence or presence of co-administration of A-437203 was calculated as described in Materials and methods. The data from which these descriptive statistics were derived is presented in Figs. 3 and 4.

dopamine depletion to 31%. However, the efficacy of pramipexole in the D3 −/− mice, 39% inhibition of the effect of MPTP, was less than that the 64% inhibition in the D3 +/+ mice.

3.4. Effect of A-437203 in dopamine D3 receptor knockout mice

In the experiment shown in Fig. 2, A-437203 was found to partially inhibit the efficacy of pramipexole against MPTP-induced striatal dopamine depletion. In order to determine if this was due to competitive inhibition of pramipexole binding to the D3 receptor, we examined the effect of A-437203 in the D3 −/− mice. Groups of D3 −/− and D3 +/+ animals were administered vehicle, 0.32 mg/kg pramipexole, or 0.32 mg/kg pramipexole plus 10 mg/kg A-437203 followed after 30 min by a single dose of MPTP (30 mg/kg). Animals received the same compounds again after 24 and 48 h. A group of D3 −/− and of D3 +/+ mice also received vehicle injections and no MPTP. Striatal dopamine content was determined at 24 h after the last compound administration.

In the absence of MPTP treatment, the striatal dopamine content was again slightly higher in the D3 +/+ compared to the D3 −/− mice (Fig. 4). In the D3 +/+ mice, the efficacy of pramipexole against MPTP-induced striatal dopamine depletion was attenuated by co-administration of A-437203 (Fig. 4). MPTP caused a 52% depletion of striatal dopamine content (Table 1, Experiment 2). Administration of pramipexole with MPTP significantly reduced striatal dopamine depletion to only 20%. Co-administration of A-437203 significantly reduced the efficacy of pramipexole. The extent of depletion was 36% when A-437203 was administered with pramipexole. Thus, A-437203 inhibited the protective effect of pramipexole by 50%, similar to the efficacy in the experiment in Fig. 2.

In the D3 −/− mice, pramipexole also attenuated the MPTP-induced dopamine depletion. In the absence of pramipexole, MPTP caused a 48% depletion of striatal dopamine content. In D3 −/− mice which received pramipexole, the MPTP-induced reduction in striatal dopamine content (25% depletion) was inhibited by 48% (Table 1, Experiment 2). However, in the D3 −/− mice, A-437203 did not inhibit the efficacy of pramipexole. In fact, the % inhibition of MPTP-induced dopamine depletion was slightly greater in the group that received pramipexole plus A-437203 (56%) than in the group receiving pramipexole alone (48%).

4. Discussion

A growing body of evidence in experimental models suggests that the dopamine D2/D3 receptor agonist pramipexole may protect dopamine neurons from toxic insult. The aim of the present study was to investigate the role of dopamine D3 receptor interactions in mediating this neuroprotective effect in vivo, using the murine MPTP model of Parkinson's disease. MPTP was discovered to be a dopamine neurotoxin after addicts taking a synthetic heroin contaminated with the compound developed classical signs of Parkinson's disease (Langston et al., 1987). Subsequent studies demonstrated that MPTP causes dopamine neuron death in mice and nonhuman primates and, in primates, produces many of the neurological symptoms of idiopathic Parkinson's disease. MPTP is metabolized by monoamine oxidase to 1-methyl-4-phenylpyridinium, which is then selectively taken up by dopamine neurons where it accumulates in mitochondria and inhibits complex I of the electron transport system. Significantly, defects in mitochondrial electron transport have now been identified in Parkinson's disease brain (Greenamyre et al., 2001). Thus, the parallels between MPTP poisoning and Parkinson's disease has lead to the acceptance of MPTP toxicity as a model of Parkinson's disease and to the use of such models to investigate novel approaches to halting disease progression, such as the studies described here.

In the present study, pramipexole was found to inhibit the depletion of striatal dopamine content caused by MPTP in mice. This effect was dependent on dose, with a minimum efficacious dose of 0.32 mg/kg. In the two experiments in which this dose was used in C57BL/6 mice obtained from Charles River Laboratories, inhibition of MPTP-induced dopamine depletion was 24% and 33%. In C57BL/6 mice from Jackson Laboratories (D3 +/+ expressing dopamine D3 receptors, see below), the efficacy of pramipexole was higher, inhibiting of MPTP-induced dopamine depletion by 63% and 64% in two experiments. Furthermore, MPTP appeared to be slightly more effective in the mice obtained from Charles River. These data suggest subtle differences in

the dopaminergic system between mice from these different vendors. Nonetheless, the dose–effect relationship and magnitude of effects of pramipexole observed here are comparable to that observed previously by others for inhibition of MPTP- or amphetamine-induced striatal dopamine depletion in mice (Anderson et al., 2001; Kitamura et al., 1997; Hall et al., 1996), 6-hydroxydopamine-induced dopamine neuron loss in rats (Vu et al., 2000), and ischemia-induced dopamine neuron loss in gerbils (Hall et al., 1996).

Several groups have presented evidence indicating that the neuroprotective effects of pramipexole are the result of the antioxidant properties of the compound (Ferger et al., 2000; Le et al., 2000; Vincenzi and Hinds, 1998; Zou et al., 1999, 2000). Pramipexole also inhibits the activation of apoptotic pathways (Abramova et al., 2002; Cassarino et al., 2001; Kitamura et al., 1998). These neuroprotective effects of pramipexole are apparently independent of dopamine receptor interactions. However, studies by Carvey et al. in in vitro systems suggest that the neuroprotective effect of pramipexole may also be mediated, at least in part, by interaction with the dopamine D3 receptor (Ling et al., 1999). We further investigated this latter hypothesis, using two complimentary approaches in an in vivo system. We determined whether the dopamine D3 receptor selective antagonist, A-437203, inhibits the efficacy of pramipexole for reducing MPTP-induced striatal dopamine depletion. We also investigated MPTP-induced striatal dopamine depletion in mice in which the dopamine D3 receptor was deleted (Accili et al., 1996). For this latter approach, we predicted that if the neuroprotective activity of pramipexole or the ability of A-437203 to inhibit this activity was mediated by an interaction with the dopamine D3 receptor, then these effects would be attenuated in the D3 $-/-$ mice.

Pramipexole was found to retain the ability to inhibit MPTP-induced dopamine depletion in mice in which the dopamine D3 receptor was deleted. In two experiments in the D3 $-/-$ mice, pramipexole inhibited MPTP-induced striatal dopamine depletion by 39% and 43%. Thus, dopamine D3 receptors are not requisite for the ability of pramipexole to reduce MPTP toxicity. However, pramipexole was apparently less effective in the D3 knockout mice than in mice expressing dopamine D3 receptors. Specifically, in parallel experiments in wildtype littermate mice, pramipexole inhibited MPTP-induced striatal dopamine depletion by 63% and 64%. These data suggested that interaction of pramipexole with dopamine D3 receptors may contribute to the neuroprotective effects of the compound.

The partial involvement of a dopamine D3 receptor interaction in the neuroprotective effect of pramipexole is more firmly supported by results of studies using the dopamine D3 receptor antagonist A-437203. A-437203 is a highly selective antagonist of the dopamine D3 receptor (Drescher et al., 2002; Unger et al., 2002). In the present study, this compound was found to partially inhibit the protective effect of pramipexole against MPTP-induced striatal dopamine depletion. In the two experiments in mice expressing dopamine D3 receptors, A-437203 inhibited the efficacy of pramipexole by 50% and 60%. Significantly, the inhibitory effect of A-437203 was lost in the D3 $-/-$ mice. This indicates that this effect of A-437203 is mediated by an interaction with the dopamine D3 receptor, presumably by displacing pramipexole from binding to this receptor.

The conclusion from our studies is that the neuroprotective effect of pramipexole in vivo is partially mediated by an interaction with dopamine D3 receptors. This conclusion is similar to that of Ling et al. (1999), based on in vitro studies in primary cultures of mesencephalic neurons. A role for interaction with the dopamine D3 receptor is also consistent with the finding of Hall et al. (1996) on the neuroprotective effect of pramipexole in a gerbil global ischemia model. In this latter study, pramipexole reduced dopamine neuron loss but not loss of CA1 hippocampal pyramidal neurons. This is consistent with the distribution of dopamine D3 receptors in these neuronal populations (Levant, 1998; Stanwood et al., 2000). Ling et al. (1998) have suggested that the mechanism for the dopamine D3 receptor-dependent neuroprotective effect of pramipexole may result from a receptor-induced release of a trophic factor. It will be important to further investigate this and other possible receptor-mediated mechanisms, as an approach to developing new therapeutics to treat Parkinson's disease and other neurodegenerative conditions.

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