DRUG DEVELOPMENT RESEARCH 29:222–226 (1993)

Research Article

Amantadine Increases Striatal Dopamine Turnover in MPTP-Treated Mice

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ABSTRACT Amantadine is used in the symptomatic treatment of Parkinson's disease to improve the akinesia and rigidity associated with this neurodegenerative disorder. Amantadine acts on the synthesis and release of dopamine (DA). In order to further characterize its mechanism of action, the drug was administered to MPTP-treated mice which were used as a model of the neurochemical deficits associated with Parkinson's disease. The DA turnover in corpus striatum was evaluated. Adult male Swiss albino mice were injected ip with 12.5 mg/kg (82.6 μmol/kg) or 25 mg/kg (165 μmol/kg) of amantadine and 30 min later with MPTP (30 mg/kg, 143 μmol/kg). Both the amantadine and MPTP treatments were repeated for 3 consecutive days. Groups of mice were treated with amantadine or MPTP alone. Seven days after the last injection of drugs, the striatal content of DA and homovanillic acid (HVA) were measured by HPLC-EC analysis. Additional groups of mice were treated with 3 consecutive daily doses of MPTP (30 mg/kg, 143 μmol/kg) and 7 days after the last administration received a single dose of amantadine at 25 mg/kg (165 μmol/kg). Turnover rate was measured by HVA content determination. The results indicate that amantadine induced a significantly increased striatal DA turnover rate (34%) in MPTP-treated animals as compared with those animals treated with only MPTP.

Key Words: amantadine, MPTP, dopamine turnover, Parkinson's disease

INTRODUCTION

The etiology of Parkinson's disease remains unknown. Nonetheless its pathology has been well-described [Jellinger, 1986]. The disease results from the degeneration and ultimate death of the dopaminergic neurons of the nigrostriatal pathway in the brain [Jenner, 1989]. Death of these neurons produces a decrease in the striatal dopamine (DA) content. As a result, bradykinesia, tremor, and rigidity appear [Javoid-Agid et al., 1986]. The disease is routinely treated by administration of the DA precursor, L-DOPA, resulting in a substitution of the neurotransmitter decreased in brain [Quinn, 1990]. L-DOPA treatment has secondary effects, some of them severe, such as the production of uncontrolled movements and dystonia [Kanazawa, 1986]. The search for new therapeutic modalities to treat Parkinson's disease is of considerable interest.

The therapeutic efficacy of new anti-parkinsonian agents is usually assessed in experimental models of the disease [Kaakkola and Teravainen, 1990]. Among them, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is regarded as the best available experimental model of the neurochemical sequelae of Parkinson's disease [Gerlach et al., 1991].

When MPTP is administered to non-human primates and mice it produces hypokinesia and neuronal damage similar to that observed in idiopathic Parkinson's disease [Heikkila et al., 1984a]. This alteration is accompanied by a reduction of striatal dopamine content. The model reproduces the pathological and biochemical characteristics of the human disease [Hall-

Received December 21, 1992; final version accepted February 28, 1993.

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man et al., 1985]. MPTP is metabolized in the organism to the 1-methyl-4-phenylpyridinium ion (MPP+), by monoamine-oxidase B (MAO B) [Heikkila et al., 1984b]. This metabolite is then responsible for the neuronal damage observed in the MPTP model [Sayre, 1989]. MPP+ is introduced into the dopaminergic neurons through the high-affinity DA uptake system [Chiba et al., 1985; Pileblad and Carlsson, 1985]; pharmacological inhibitors of this process are able to prevent MPTP action [Mihatsch et al., 1988].

Amantadine is a drug that has been used widely as an antiviral agent [Davies et al., 1964] and it is relatively free of secondary effects [Kulisevsky and Tolosa, 1990]. Its therapeutic properties in Parkinson's disease were discovered in 1969. Since then it has been only occasionally used, probably because its mechanism of action is not well understood [Kulisevsky and Tolosa, 1990]. Experimental studies have shown that amantadine is able to increase the synthesis and release of DA [Von Viogtlander and Moore, 1973] and to inhibit the uptake of the neurotransmitter [Bailey and Stone, 1975] at the presynaptic level. In the postsynaptic cell, amantadine can function as a DA agonist [Gianutsos et al., 1985]. However, none of the above observations were made in experimental models of the disease, thus its role in the therapy of Parkinson's disease has not yet been clearly defined.

This study evaluated the potential protective role of amantadine in the MPTP-model of Parkinson's disease in mice. The study involved the determination of DA and its main metabolite, homovanillic acid (HVA) in the striatum under conditions that allow the evaluation of neurotransmitter turnover.

MATERIALS AND METHODS

Animals

Adult male Swiss albino mice NIH local strain were used. Animals weighed 25 to 30 g and were ages 11 to 13 weeks. Animals were fed with Purina chow (Purina, Mexico) and drank water freely. The room was kept dark between 7:00 P.M. and 7:00 A.M., temperature was 25°C, and relative humidity 40%.

Reagents

MPTP hydrochloride, DA, HVA, and sodium octylsulfate were obtained from Sigma Chemical Co. (St. Louis, MO). Amantadine hydrochloride and probenecid were synthesized in our laboratory. All other reagents were obtained from E. Merck, Mexico.

Equipment

A Perkin-Elmer LC-250 chromatograph with an electrochemical detector Metrohm-AG (Switzerland) 656 and integrator Hewlett-Packard HP 3396 series II was used for the analysis of DA and HVA.

DA and HVA Determination

Striatal DA and HVA content was measured by HPLC-electrochemical detection analysis, as described previously [Garcia et al., 1992]. Calibration curves were constructed for DA and HVA. Concentrations were obtained by interpolation in the respective standard curve. An Alltech Associates, Inc. (Deerfield, IL), adsorbosphere catecholamine analytical column of 100 × 4.8 mm with 3 μm particle diameter was used. The mobile phase consisted of aqueous phosphate buffer (pH 3.2) which contained 0.2 mM sodium acetyl sulfate, 0.1 mM EDTA, and 15% v/v of methanol. Water and methanol were HPLC-grade reagents. Flow was 1.6 ml/min. The detector potential was adjusted to 0.8 V vs. Ag/AgCl reference electrode. All samples were analyzed in duplicate.

Effect of Amantadine in the MPTP Model of Parkinson's Disease

For these experiments, there were four treatment groups: Group I (n = 5), amantadine and 30 min later MPTP; group II (n = 6), saline solution and 30 min later MPTP; group III (n = 6), amantadine and 30 min later saline solution; group IV (control group, n = 8), saline solution and 30 min later saline solution. Animals of groups I and III received amantadine at two different doses: 12.5 mg/kg (82.6 μmol/kg) and 25 mg/kg (165 μmol/kg). These doses had been used in other reports [Melzacka et al., 1989]. Mice from groups I and II were injected ip with 30 mg/kg (143 μmol/kg) of MPTP. Treatment schedule was repeated for 3 consecutive days. Seven days after the last injection all animals were sacrificed by decapitation and the striatal DA and HVA contents were obtained by HPLC as described above.

Extraction of DA and HVA From Samples

At the end of treatments all animals were sacrificed by decapitation. The brains were removed rapidly and the striata dissected out on ice, as described by Glowinski and Iversen [1966]. An aliquot (300 μl) of cold (5°C) perchloric acid-sodium metabisulfite solution was added to the tissue which was then sonified with a Lab-line ultratip labsonic system (Lab-line Instruments, Melrose Park, IL). Samples were centrifuged at 4,000g for 10 min and the supernatants were kept at −70°C until analyzed.

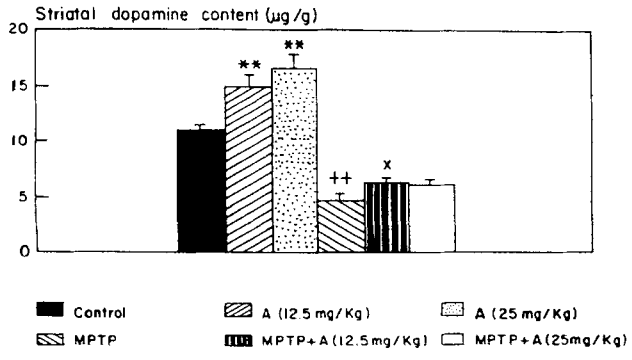

Figure 1. Striatal dopamine content. Results are expressed as mean ± s.e.m. of n = 5–8 independent experiments. ** = statistically different from control, $P < 0.01$, Tukey's test; + + = statistically different from control, $P < 0.01$, Tukey's test; x = statistically different from MPTP, $P < 0.05$, Tukey's test; A = amantadine; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

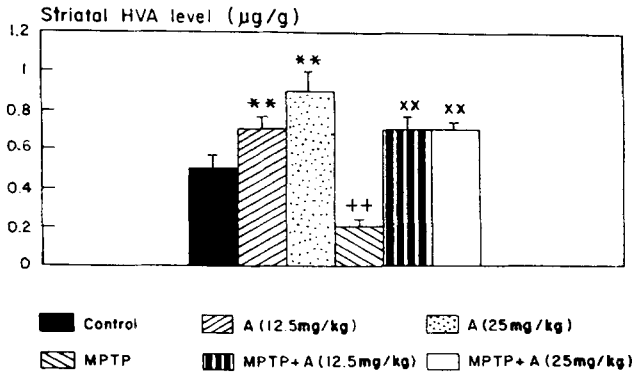

Figure 2. Striatal HVA content. Results are expressed as mean ± s.e.m. of n = 5–8 independent experiments. ** = statistically different from control, $P < 0.01$, Tukey's test; + + = statistically different from control, Tukey's test; xx = statistically different from MPTP-treated group, $P < 0.01$, Tukey's test; A = amantadine; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Amantadine Effect on DA Turnover

The DA turnover was estimated according to the technique described by Diggory and Buckett [1984] which consists of the administration of probenecid, a drug that blocks the transport of HVA and other brain organic acids from cerebrospinal fluid to blood [Tamarkin et al., 1970]. In this way, the brain content of HVA increases proportionally to DA turnover.

In these experiments, there were three treatment groups: Group A (n = 5) animals received 3 daily doses of MPTP (30 mg/kg, 143 µmol/kg, ip); 7 days after the last administration probenecid (280 mg/kg, 981 µmol/kg) was injected sc and 5 min later amantadine (25 mg/kg, 165 µmol/kg) was injected ip Group B (n = 5) animals were injected ip with 3 daily doses of MPTP (30 mg/kg, 143 µmol/kg); 7 days after the last administration probenecid (280 mg/kg, 981 µmol/kg) was injected sc and 5 min later saline solution was injected ip. Group C (control, n = 5) animals were injected with 3 daily doses of saline solution ip; 7 days after the last administration probenecid (280 mg/kg, 981 µmol/kg) was injected sc and 5 min later saline solution was injected ip. Two hours after the probenecid administration all the mice were sacrificed and HVA content determined by HPLC as described above.

RESULTS

Effect of Amantadine in the MPTP Model of Parkinson's Disease

Amantadine administration to control mice results in a significant dose-dependent increase in striatal DA content (Fig. 1). This increase was 34% and 50% for the 12.5 mg/kg (82.6 µmol/kg) and 25 mg/kg (165 µmol/kg) doses, respectively, when compared with control animals (without MPTP and without amantadine treatment). MPTP-treated animals presented markedly reduced DA levels as a result of the neurotoxic action of the compound (Fig. 1). Administration of amantadine to MPTP-treated animals partially protected the neurotoxic effect of MPTP (Fig. 1). This protective effect was not dose-dependent.

The HVA content in the striatum was increased in both the MPTP-treated (groups I, II) and MPTP-untreated animals (groups III, IV) (Fig. 2). Amantadine protection in this case was complete, returning the HVA levels to those observed in the MPTP-untreated groups (III, IV). Again, the dose-dependence of amantadine effect was observed only in the MPTP-untreated groups (Fig. 2).

Figure 3 shows the results expressed as the HVA/DA ratio in order to clarify the effect of amantadine on striatal dopaminergic systems. As can be seen, amantadine increased the HVA/DA ratio in both the MPTP treated and untreated groups, suggesting that the main effect of amantadine was to induce an increment in the DA turnover rate in the striatum. This effect was more pronounced in those animals with MPTP-induced dopamine depletion (groups I, II) (Fig. 3).

Amantadine Effect on DA Turnover

Figure 4 shows the effect of amantadine on the DA turnover in the striatum. A single dose of amantadine (25 mg/kg, 165 µmol/kg) in MPTP-treated animals (group B) increased DA turnover by 34% in comparison with those animals treated only with

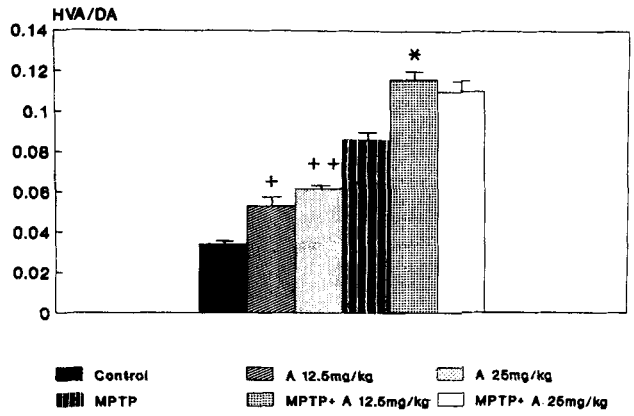

Figure 3. HVA/DA ratio. Results are expressed as mean ± s.e.m. of n = 5–8 independent experiments. Statistically different from control: + = $P < 0.05$; + + = $P < 0.01$, Tukey's test; * = statistically different from MPTP-treated group, $P < 0.05$, Tukey's test; A = amantadine; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

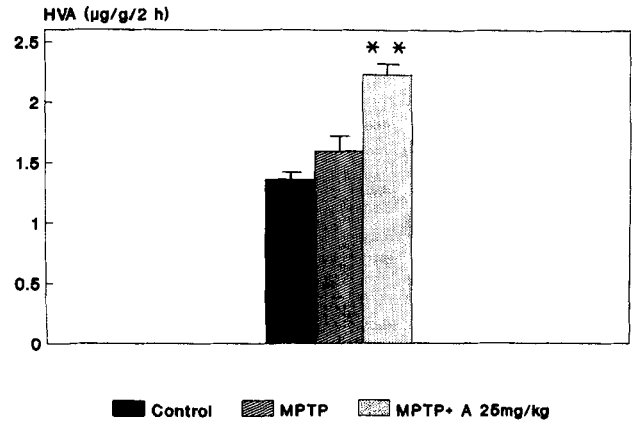

Figure 4. Dopamine turnover as striatal HVA content. Results are expressed as mean ± s.e.m. of n = 5 independent experiments. ** = statistically different from MPTP-treated group, $P < 0.01$, Tukey's test; A = amantadine; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

MPTP, suggesting that the main effect of amantadine is to accelerate the neurotransmitter turnover in order to balance the lack of DA which in turn is due to the death of neurons produced by MPTP.

DISCUSSION AND CONCLUSIONS

As a result of its effects at the synaptic level, amantadine was able to prevent the neurotoxic action of MPTP in mice. This protective effect could be achieved by two different hypothetical mechanisms: 1) inhibition of high affinity MPTP uptake into dopaminergic cells by amantadine and 2) enhancement of DA turnover by amantadine. As the increase in the DA content was observed both in the control and the MPTP-treated mice (Fig. 1), it is difficult to conclude that the only effect of amantadine was to prevent MPTP uptake by dopaminergic cells in vivo. The increased DA content in control animals is better explained by an enhancement in the DA turnover after amantadine administration. This enhancement could be more pronounced in MPTP-treated mice as suggested by the complete recovery of HVA after amantadine in the MPTP-treated mice (Fig. 2). This is further supported by the results of the DA turnover measurements (Fig. 3) which indicate a DA turnover enhancement in MPTP-treated mice.

Recent speculations have postulated the existence of an endogenous or exogenous neurotoxin similar to MPTP as the etiological cause of idiopathic Parkinson's disease [Jenner, 1989]. If that proves to be true, amantadine could prevent the neurotoxic action of this hypothetical compound, probably by inhibiting its uptake in the presynaptic dopaminergic terminals. However, the results of our experiments do not support such an idea as the main effect of amantadine in vivo seems to be an enhancement of DA turnover rate. This effect was more pronounced in MPTP-treated animals, suggesting that the effect of amantadine is more potent in the presence of a decreased number of dopaminergic neurons. To our knowledge, this is the first report on the effects of amantadine in the striata of mice treated with MPTP.

The present study provides new information regarding the mechanism of action of amantadine at the molecular level in a model of Parkinson's disease that supports its use as an anti-parkinsonian agent in clinical therapeutics.

ACKNOWLEDGMENT

We thank Dr. Julio Sotelo for his valuable comments.

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