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## III. 2. Neuroprotective Effects of Monoamine Oxidase Inhibitor and Glutamate Receptor Inhibitor on MPTP-induced Dopamine and DOPAC Depletion in Mice

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### Introduction

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is well known to produce clinical, biochemical and neuropathological changes analogous to those observed in idiopathic Parkinson's disease. This neurotoxin also leads to a decrease of dopamine content in the striatum and loss in the number of the nigrostriatal dopaminergic neurons in several species including monkeys<sup>1,2</sup>), dogs<sup>3,4</sup>), cats<sup>5</sup>) and mice<sup>6,7</sup>). The neurotoxic effects of MPTP are thought to be initiated by MPP<sup>+</sup>, which is a major metabolite formed by the monoamine oxidase (MAO) B-mediated oxidation of MPTP<sup>8</sup>). MPP<sup>+</sup> is relatively taken up by high affinity dopamine and noradrenaline uptake systems and is subsequently accumulated within mitochondria of nigrostriatal dopaminergic cells. There it disrupts oxidative phosphorylation by inhibiting complex I of the electron transport chain<sup>9</sup>). This can lead to a number of deleterious effects on cellular functions, resulting in neuronal cell death. Therefore, MPTP is widely used as a rodent model of Parkinson's disease.

To examine whether N-methyl-D-aspartate (NMDA) receptors or MAO are related to the neurotoxicity induced by MPTP, we investigated possible effects of NMDA receptor antagonist MK-801 and MAO inhibitor pargyline in the striatum of MPTP-treated mice.

### Materials and Methods

Male C57BL/6 mice (22-28 g) were used in this study. The mice received intraperitoneal four injections of MPTP (10 mg/kg) at 1 h intervals, the total dose per mice being 40 mg/kg. In the present study, there were no died animals after MPTP treatments. The mice were sacrificed by cervical dislocation at 1, 3 and 7 days after the last MPTP injection for biochemical study as described below.

### *Measurement of dopamine and its metabolite*

The mice were killed by cervical dislocation at 1, 3 and 7 days after MPTP treatments. After decapitation, brains were quickly removed and the two striata were rapidly dissected out freehand on an ice-cold glass Petri dish. Samples were immediately weighted, then frozen and stored at  $-80^{\circ}\text{C}$  until assay. The dissection procedure was performed in less than 2 min. Striata were sonicated ice-cold 0.2 M perchloric acid containing 100 ng/ml isoproterenol as internal standard. Homogenates were centrifuged at 2500 rpm for 15 min at  $4^{\circ}\text{C}$ . The supernatant was filtered (pore size 0.45  $\mu\text{m}$ , Millipore filter) and a 30  $\mu\text{l}$  aliquot of the supernatant was used for determination of the content of the dopamine, 3,4-dihydroxyphenyl acetic acid (DOPAC) and isoproterenol by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD) (Eicom, Japan). The mobile phase consisted of 0.1 M sodium citrate-0.1 M sodium acetate solution (pH 3.5) including 1.064 M octane sulfonic acid and 0.013 mM EDTA 2Na and 15% (v/v) methanol. The recoveries of dopamine, DOPAC and isoproterenol through the present procedures were  $> 93\%$ . Levels of dopamine and its metabolite were calculated from the comparison of simple peak area with internal standard peak region and are expressed as  $\mu\text{g/g}$  tissue weight. Each group contained 5-9 mice.

### **Experimental design**

The animals were divided into 7 groups; (1) Vehicle (saline)-treated group; (2) Pargyline (10 mg/kg)-treated group; (3) MK-801 (3 mg/kg)-treated group; (4) MPTP- and saline-treated group; (5) MPTP- and pargyline (10 mg/kg)-treated group; (6) MPTP- and MK-801 (1 mg/kg)-treated group; (7) MPTP- and MK-801 (3 mg/kg)-treated group. The mice were injected intraperitoneally (i.p.) with pargyline, MK-801 or saline 30 min before and 90 min after the first administration of MPTP (Groups 4, 5, 6 and 7). For groups 1, 2 and 3, pargyline (10 mg/kg)-, MK-801 (3 mg/kg)- or saline-treated mice were injected i.p. in the same manner with saline treatments instead of MPTP. In addition, pargyline hydrochloride (Sigma) and MK-801 maleate (Research Biochemicals Int.) were dissolved in saline. The mice were killed by cervical dislocation at 3 days after the last MPTP treatment to measure dopamine and its metabolite in the striatum.

All values were expressed as means  $\pm$  S.E. and statistical significance was evaluated using an analysis of variance (ANOVA) followed by Williams multiple range test.

### **Results**

The depletion in dopamine and DOPAC content of the striatum induced by MPTP is shown in Fig. 1. Effects of pargyline and MK-801 on the striatal dopamine and DOPAC depletion in MPTP-treated mice are presented in Tables 1 and 2.

The striatal dopamine and DOPAC levels were significantly decreased from 1 day after MPTP treatments. Thereafter, the striatal dopamine and DOPAC levels were markedly

reduced at 3 and 7 days after MPTP treatments as shown in Fig. 1. In addition, the depletion in striatal dopamine and DOPAC concentrations reached maximal levels at 3 days after MPTP treatments. Although four injections of MPTP caused a marked decrease in dopamine and DOPAC content of the mouse striatum after 3 days, pargyline was completely protective against MPTP-induced dopamine depletion in the striatum of mice (Table 1). However, this compound did not show a significant effect on the striatal DOPAC levels in mice 3 days after MPTP treatments (Table 1). In contrast, MK-801 failed to protect against MPTP-induced dopamine depletion in the mouse striatum. However, this drug prevented a significant reduction in the striatal DOPAC content of mice 3 days after MPTP treatments (Table 2).

## Discussion

In the present study, we investigated the neuroprotective effects of pargyline and MK-801 on MPTP-induced decrease in dopamine and its metabolite in the mouse striatum. MPTP caused a significant reduction in dopamine and DOPAC levels from 1 day. Thereafter, the depletion in the striatal dopamine and DOPAC concentrations reached maximal levels at 3 days after MPTP treatments. Therefore, we evaluated the effects of two drugs on the striatal dopamine and DOPAC levels at 3 days after MPTP treatments.

MPTP is known to convert by the action of MAO-B to MPP<sup>+</sup>, which is the neurotoxic metabolite<sup>10</sup>. It has been shown that MAO inhibitors, especially MAO-B inhibitors, can protect against MPTP-induced dopamine depletion and nigrostriatal dopaminergic cell death by preventing the conversion of MPTP to MPP<sup>+</sup><sup>6,11</sup>. In the presented study, pargyline (a relatively selective inhibitor of MAO-B) was completely protective against MPTP-induced striatal dopamine depletion in mice. Furthermore, mice given pargyline and saline had the striatal dopamine levels higher than saline values. The findings suggest that pargyline can inhibit the conversion of dopamine to DOPAC.

MK-801 is one of the most potent and selective NMDA receptor antagonist available and has been used as a neuroprotectant to decrease ischemic injury in experimental animals. The role of NMDA receptors had also been extensively discussed in epilepsy, stroke and neurodegenerative disorders such as Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis<sup>12</sup>. Interestingly, some previous evidences demonstrate that MPP<sup>+</sup> toxicity is mediated by NMDA receptors<sup>13,14</sup>. In contrast, other reports suggest that MK-801 failed to protect against the nigral neurotoxicity caused by systemic administration of MPTP in mice or after local MPP<sup>+</sup> injections in rats<sup>15,16</sup>. Thus, much evidence for the efficacy of MK-801 against MPTP-induced parkinsonian animals is contradictory. In the present study, MK-801 showed no significant change on MPTP-induced dopamine depletion of the mouse striatum. Therefore, our findings seem to suggest that NMDA receptors are not mainly involved in mediating MPTP- or MPP<sup>+</sup>-induced neurodegeneration.

In the present study, of interest is that MK-801 dose-dependently protected against MPTP-induced DOPAC depletion in the striatum of mice. The reason for this phenomenon is presently unclear. However, for both dopamine and DOPAC concentrations in MPTP-treated mice, MK-801 showed a tendency to increase these concentrations. Therefore, the findings seem to suggest that NMDA receptor antagonist can partially protect against MPTP-induced neurodegeneration.

In conclusion, our results show that NMDA receptors are not mainly involved in mediating MPTP-induced neurodegeneration, whereas MAO-B plays a crucial role in MPTP-induced degeneration of the nigrostriatal dopaminergic neuronal pathway.

## References

- 1) Burns R. S., Chiuen C. C., Markey S. P., Ebert M. H., Jacobowitz D. M. and Kopin I. J., *Proc. Natl. Acad. Sci. USA* **80** (1983) 4546.
- 2) Ferrante R. J., Hantraye P., Brouillet E. and Beal M. F., *Brain Res.* **823** (1999) 177.
- 3) Rapisardi S. C., Warrington V. O. and Willson J. S., *Brain Res.* **512** (1990) 147.
- 4) Johannessen J. N., Sobotka T. J., Weise V. K. and Markey S. P., *J. Neurochem.* **57** (1991) 981.
- 5) Schneider J. S. and Markhan C. H., *Brain Res.* **373** (1986) 258.
- 6) Heikkila R. E., Manzino L., Cabbat F. S. and Duvoisin R. C., *Nature* **311** (1984) 467.
- 7) Arai N., Misugi K., Goshima Y. and Misu Y., *Brain Res.* **515** (1990) 57.
- 8) Tipton K. F. and Singer T. P., *J. Neurochem.* **61** (1993) 1191.
- 9) Gluck M. R., Krueger M. J., Ramsey R. R., Sabin S. O., Singer T. P. and Nicklas W. J., *J. Biol. Chem.* **269** (1994) 3167.
- 10) Markey S. P., Johannessen J. N., Chiuen C. C., Burns R. S. and Herkenham M. A., *Nature* **311** (1984) 464.
- 11) Heikkila R. E., Hess A., Duvoisin R. C., *Science* **224** (1984) 1451.
- 12) Olney J. W., *Biol. Psychiatr.* **26** (1989) 505.
- 13) Turski L., Bressler K., Rettig K. J., Löshmann P. A. and Wachel H., *Nature* **349** (1991) 414.
- 14) Storey E., Hyman B. T., Jenkins B., Brouillet E., Miller J. M., Rosen B. R. and Beal M. F., *J. Neurochem.* **58** (1992) 1975.
- 15) Kupsh A., Löshmann P. A., Sauer H., Arnold G., Renner P., Puffer D., Burg M., Wachtel H., ten Bruggencate G. and Oertel W. H., *Brain Res.* **592** (1992) 74.
- 16) Sonsalla P. K., Zeevalk G. D., Manzino L., Giovanni A. and Nicklas W. J., *J. Neurochem.* **58** (1992) 1979.

Table 1. Effects of pargyline on the striatal dopamine and DOPAC content in MPTP-treated mice

Treatments	Dopamine ( $\mu\text{g/g}$ tissue)	DOPAC ( $\mu\text{g/g}$ tissue)
Vehicle(saline)	$9.69 \pm 0.70^{**}$	$4.60 \pm 0.58^{**}$
Pargyline (10 mg/kg)	$14.83 \pm 1.32^{**}$	$2.40 \pm 0.32$
MPTP+saline	$1.97 \pm 0.30$	$2.01 \pm 0.21$
MPTP+pargyline (10 mg/kg)	$12.82 \pm 0.42^{**}$	$2.35 \pm 0.13$

Values are expressed as mean  $\pm$  S.E.  $^{**}p < 0.01$ , compared with MPTP+saline group (Williams multiple range test).  $n=5-6$ . Drug treatments schedules were expressed in experimental design section.

Table 2. Effects of MK-801 on the striatal dopamine and DOPAC content in MPTP-treated mice

Treatments	Dopamine ( $\mu\text{g/g}$ tissue)	DOPAC ( $\mu\text{g/g}$ tissue)
Vehicle(saline)	$9.69 \pm 0.70^{**}$	$4.60 \pm 0.58^*$
MK-801 (10 mg/kg)	$11.11 \pm 0.46^{**}$	$4.91 \pm 0.35^*$
MPTP+saline	$1.97 \pm 0.30$	$2.01 \pm 0.21$
MPTP+MK-801 (3 mg/kg)	$3.22 \pm 0.69$	$5.32 \pm 1.37^{**}$
MPTP+MK-801 (10 mg/kg)	$1.83 \pm 0.19$	$6.31 \pm 0.75^{**}$

Values are expressed as mean  $\pm$  S.E.  $^*p < 0.05$ ,  $^{**}p < 0.01$ , compared with MPTP+saline group (Williams multiple range test).  $n=5$ . Drug treatments schedules were expressed in experimental design section.

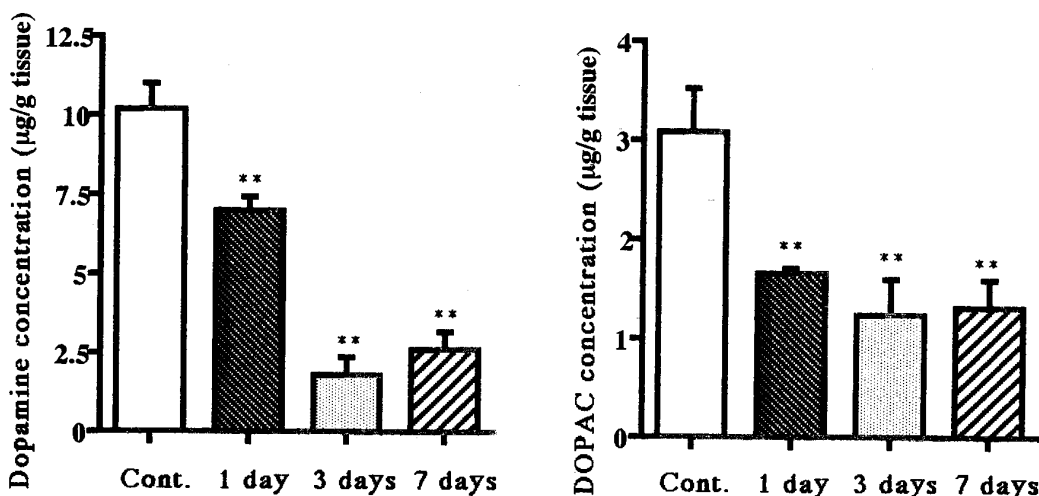


Figure 1. Levels of dopamine and DOPAC in the striatum after MPTP treatments. Cont. ; saline-treated mice, 1 day; mice 1 day after MPTP treatments, 3 days; mice 3 days after MPTP treatments, 7 days; mice 7 days after MPTP treatments. Left half: dopamine levels. Right half; DOPAC levels. Values are expressed as mean  $\pm$  S.E.  $^{**}p < 0.01$ , compared with (Cont.) saline-treated group (Williams multiple range test).  $n=6-9$ .