68. Dopaminergic Neurotoxins, MPTP and MPP⁺, Inhibit Activity of Mitochondrial NADH-Ubiquinone Oxidoreductase

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Despite recent progress in biochemical findings on Parkinson's disease, exact cause is not known. Recently, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to induce marked loss of striatal dopamine in humans as well as in animals (Davis *et al.*, 1979; Burns *et al.* 1983; Langston *et al.* 1983; Heikkila *et al.* 1984), and has been considered as the best model available at present of Parkinson's disease. It was shown that MPTP was first metabolized to 1-methyl-4-phenylpyridinium ion (MPP⁺) by mitochondrial monomamine oxidase before it exerted dopaminergic toxicity (Langston *et al.* 1984a). Although it has been shown that MPTP toxicity has marked selectivity against dopaminergic neurons, exact mechanism of neuronal degeneration is not known. Elucidation of mechanism of MPTP-induced cytotoxicity appears to be important not only for the study of MPTP per se but also for the investigation on pathogenesis of Parkinson's disease. We have been studying the effects of MPTP on the respiration of mitochondria where the initial step of metabolism of MPTP takes place, and we found that MPTP and MPP⁺ dose-dependently inhibited NADH-ubiquinone oxidoreductase activity.

Methods. Male C57BL/6J mice of 2- to 4-month-old (20–30 g) were used. Animals were sacrificed by cervical dislocation, and brains were immediately taken out into a medium containing 0.3 M mannitol and 0.1 mM EDTA pH 7.4. Mitochondria were prepared from whole brains according to the method of Ozawa *et al.* (1966). P₀ pellets were suspended in the medium of Voss *et al.* (1961), and one cycle freeze-thawing was performed. Activity of the NADH-ubiquinone oxidoreductase in the isolated mitochondrial preparation was assayed according to the method of Hatefi (1978). Reduction in absorbance of NADH at 340 nm was followed by a Hitachi 139 spectrophotometer equipped with an isothermal cell holder. Reaction was performed at 37°C. Extinction coefficient of NADH of 6270 was used for calculation of the activity. MPTP-HCl and MPP⁺ were purchased from Research Biochemicals Inc. (Wayland, U.S.A.), and other reagents used from Sigma (St. Louis, U.S.A.) or Wako (Osaka, Japan). Ubiquinone 1 was a generous gift from Eisai Pharmaceutical Company (Tokyo, Japan).

Results. Effects of MPTP in varying concentrations on the activity of NADH-ubiquinone oxidoreductase are shown in Fig. 1. At 5 mM concentration, the activity was close to but slightly above the rotenone resistant activity. Rotenone sensitive activity represents the activity of NADH-ubiquinone oxidoreductase. The concentration which induced 50% inhibition of the rotenone

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sensitive activity was 0.61 mM. Higher concentration was required for MPP^+ to induced similar degree of inhibition, in that the concentration of 50% inhibition was 3.4 mM. Both pargyline, a monoamine oxidase inhibitor, and superoxide dismutase were of little effects on the inhibition by MPTP or MPP^+ .

Fig. 1. Effect of MPTP on the activity of NADH-ubiquinone oxidoreductase. The ordinate indicates activity of NADH-ubiquinone oxidoreductase, and the abscissa concentration of MPTP expressed as millimole per liter. The enzyme activity is expressed as per cent of the activity of the control. The activity of the control was 11.4 micromole NADH oxidized per hour per mg mitochondrial protein. I_{50} of MPTP was 0.61 mM. All assays were done on duplicate samples. Assay mixtures did not contain pargyline.

Discussion. Most of the rotenone sensitive mitochondrial NADH oxidation was inhibited by MPTP at 5 mM concentration, indicating that MPTP inhibited the activity of NADH-ubiquinone oxidoreductase, the complex I of the respiratory chain. MPP^+ was a less effective inhibitor of the same complex. I_{50} of MPTP is 0.61 mM which is reasonably low, although it is another question whether that concentration is achieved in the nigrostriatal neurons of animals treated with MPTP or not.

MPTP per se has been said to be non-toxic to dopaminergic neurons, and to have to be metabolized to MPP^+ before it exerts neurotoxicity. However, these conclusions were made based mainly on acute experiments, in which pre-treatment with monoamine oxidase inhibitor prior to the injection of MPTP was shown to prevent development of Parkinsonism (Langston *et al.* 1984b). However, effects of long-term exposure of animals to MPTP have not been studied well. Therefore, inhibition of NADH-ubiquinone oxidoreductase by MPTP may become important in long-term exposure to MPTP.

Present study was done using mitochondrial suspension prepared from whole brains. Neurotoxicity of MPTP is fairly selective against nigrostriatal

dopaminergic neurons. Therefore, an experiment using whole brain mitochondria may not be appropriate for the study of mechanism of cytotoxicity of MPTP. However, selectivity against dopaminergic neurons depends mainly on the accumulation of MPP⁺ within nigral neurons through the specific dopamine uptake system (Irwin and Langston 1985), and on the affinity of MPP⁺ to neuromelanin (Snyder and D'Amato 1986), and apparently not on the specificity of nigral mitochondria. The presence of specific uptake system for MPP⁺ enables achievement of high concentration of the substance within nigral neurons (Johannessen *et al.* 1985). Therefore, studies using the whole brain mitochondria seem to be an appropriate approach for the investigation on the mechanism of MPTP or MPP⁺ cytotoxicity. Further studies on the effects of MPTP and MPP⁺ on mitochondrial metabolism appear to be important for the investigation of MPTP cytotoxicity, elucidation of which in turn will give an insight into the research on pathogenesis of Parkinson's disease.

Summary. Effects of MPTP and MPP⁺, potent dopaminergic neurotoxins, on the activity of NADH-ubiquinone oxidoreductase were studied using isolated mitochondrial preparation from C57BL/6J mouse brains. Both MPTP and MPP⁺ dose-dependently inhibited activity of NADH-ubiquinone oxidoreductase, however, MPTP found to be a more potent inhibitor compared with MPP⁺. Significance of these findings was discussed with regard to the mechanism of MPTP-induced neuronal degeneration.

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