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Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium on aromatic L-amino acid decarboxylase in rat brain

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The compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been reported to cause a Parkinson-like syndrome in humans following intravenous administration [1]. Since then, the neurotoxic effects of MPTP have been widely studied in monkeys [2, 3] and several rodent species [3, 4]. In addition to the reported effects on the dopaminergic nigrostriatal system, MPTP treatment has been reported to affect some non-nigrostriatal catecholamine systems in the mouse [5, 6]. Recently, much attention has been focused on elucidating the mechanism of action of MPTP causing cell death. It has been shown that MPTP is oxidized *in vitro* [7, 8] and *in vivo* [9–11] to 1-methyl-4-phenylpyridinium ion (MPP^+) by monoamine oxidase (MAO) and that this conversion of MPTP to MPP^+ can be blocked by deprenyl and pargyline, two MAO inhibitors [7, 11]. Studies also indicate that MPTP reduces dopamine concentration in rat brain [3, 12]. However, the effect of MPTP on the enzyme catalyzing the formation of dopamine and serotonin, aromatic L-amino acid decarboxylase (AADC, EC 4.1.1.28), has not been reported. The present study was undertaken to determine the *in vitro* effect of MPTP and its metabolite, MPP^+, on AADC using both 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) as substrates.

Materials and methods

L-3,4-Dihydroxyphenyl[3-^{14}C]alanine (sp. act. 10.9 mCi/mmole) was purchased from the Amersham Corp. (Arlington Heights, IL, U.S.A.) while DL-5-[3-^{14}C]hydroxytryptophan (sp. act. 59.0 mCi/mmole) was obtained from the New England Nuclear Corp. (Boston, MA, U.S.A.). MPTP hydrochloride and MPP^+ were from Research Biochemicals Inc. (Wayland, MA, U.S.A.). All other chemicals were of reagent grade.

Sprague–Dawley rats (150–200 g) were killed by decapitation, and their brains were rapidly removed, frozen on dry ice, and stored at −70°. DOPA decarboxylase and 5-HTP decarboxylase activities were assayed in whole brain homogenates as described previously [13], except that pargyline was excluded in the 5-HTP decarboxylase assay.

Protein concentration was measured using the Bio-Rad Protein Assay which is based on the method of Bradford [14], with bovine serum albumin as a reference standard.

Results and discussion

The *in vitro* effects of MPTP and MPP^+ on the activities of DOPA decarboxylase and 5-HTP decarboxylase in rat brain are shown in Table 1. In the presence of 5 or 10 μM MPTP or MPP^+, the activity of 5-HTP decarboxylase increased by about 71–107%. In contrast, the activity of the DOPA decarboxylase was relatively unchanged. The increase in 5-HTP decarboxylase activity seen here is in accordance with the increase in the brain level of serotonin following *in vivo* administration of MPTP [3, 5, 12, 15]. The low concentrations of MPTP and MPP^+ used in this investigation are in the range expected following intravenous administration of MPTP and have been used in organotypic culture of embryonic rat mesencephalon [2, 16].

It has been reported that MPTP and MPP^+ are potent inhibitors of MAO [17]. It is possible that the increase in 5-HTP decarboxylase activity observed *in vitro* by MPTP or MPP^+ addition might be due to the inhibition of MAO. This would cause an accumulation of serotonin. Thus, the increase in decarboxylation of 5-HTP observed might be an artifact of the assay conditions. If this were so, there should be an accumulation of both serotonin and dopamine as well as an apparent increase in the activities of both DOPA and 5-HTP decarboxylases under the assay conditions. However, this was not so. The selective inhibition of the two forms of MAO by MPTP or MPP^+ [17] should not come into question since both dopamine and serotonin can be deaminated by rat brain MAO-A [18–20]. Deprenyl, a specific MAO-B inhibitor, has been shown to prevent the

Table 1. Effects of MPTP and MPP^+ on whole brain DOPA decarboxylase and 5-HTP decarboxylase activities in the rat

Compound	Conc (μM)	DOPA decarboxylase activity*†	5-HTP decarboxylase activity†‡
None		986 ± 100	16.0 ± 3.4
MPTP	5	1089 ± 102 (+11%)	27.3 ± 6.0 (+71%)
	10	1097 ± 101 (+12%)	29.6 ± 6.0 (+86%)
MPP^+	5	880 ± 57 (−9%)	31.6 ± 8.0 (+96%)
	10	909 ± 53 (−6%)	33.2 ± 4.6 (+107%)

* Brain homogenate was incubated at 37° for 15 min in a medium (1-ml vol.) containing: 80 μmoles sodium phosphate buffer (pH 6.7), 0.125 μmole pyridoxal 5′-phosphate, 10 μmoles 2-mercaptoethanol, 1 μmole DOPA (containing 0.1 μCi L-[3-^{14}C]DOPA) with and without MPTP or MPP^+.

† Values represent mean ±S.E.M. for five separate experiments, each performed in duplicate. Enzyme activities are expressed in pmoles/min/mg protein. Numbers in parentheses show percent changes from control.

‡ Brain homogenate was incubated at 37° for 60 min in a medium (1-ml vol.) containing: 75 μmoles Tris buffer (pH 8.3), 0.3 μmole pyridoxal 5′-phosphate, 10 μmoles 2-mercaptoethanol, 0.6 μmole 5-HTP (containing 0.1 μCi DL-[3-^{14}C]5-HTP) with and without MPTP or MPP^+.

oxidation of MPTP to MPP^+ [7, 11]. When it was included in the assay for 5-HTP decarboxylase, the increase in decarboxylase caused by MPTP and MPP^+ remained unchanged. Hence, the oxidative metabolism of MPTP to MPP^+ or the dihydropyridinium intermediate [21] is not required for the stimulation of 5-HTP decarboxylase.

The enzyme AADC has generally been referred to as a single enzyme capable of decarboxylating both DOPA and 5-HTP [22]. However, earlier work from our laboratory [13] has suggested the possible existence of different forms of AADC with different substrate specificities for DOPA and 5-HTP, respectively, being present in varying amounts in different brain regions. This would explain the differential effects of MPTP and MPP^+ on the activity of AADC using DOPA and 5-HTP as substrates in the rat brain. The increased serotonin level [3, 12, 15] seen in rats administered MPTP could be explained by the action of MPTP and MPP^+ or their metabolites on 5-HTP decarboxylase. However, the mechanism of this action has yet to be investigated. It should also be noted that it is possible that this action of MPTP and MPP^+ on 5-HTP decarboxylase has no bearing on the nigral cell death induced by MPTP in mice [4–6] since DOPA decarboxylase was not affected. Furthermore when MPTP is administered in low concentrations to rats, it does not cause selective destruction of dopaminergic neurons [3, 12, 15]. Since primates and rodents exhibit marked differences in their sensitivity to the neurotoxic effects of MPTP [23], it would be of interest to examine the effects of MPTP and MPP^+ on AADC in the primate brain.

In summary, our results show that MPTP and MPP^+ have differential effects on the activity of AADC, using DOPA and 5-HTP, respectively, as substrates in the rat brain. The oxidative metabolism of MPTP to MPP^+ is not essential for the stimulation of 5-HTP decarboxylase activity.

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