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Acute Peripheral Catecholaminergic Changes in Rat after MPTP and MPP^+ Treatment

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The effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its main metabolite 1-methyl-4-phenylpyridinium ion (MPP^+) on the peripheral catecholaminergic system of the rat were investigated. MPTP and MPP^+ injections (20 mg/kg i.p.) caused a marked acute depletion of heart noradrenaline, up to 75 % twelve hours after the administration, and a decrease of adrenal gland adrenaline. The time-course of the effect of MPTP and MPP^+ is reported, together with a decrease in the tyrosine hydroxylase activity after MPTP treatment, more evident in the adrenal glands. Pargyline (50 mg/kg i.p.) is not able to prevent such a neurotoxic peripheral effect.

Key words: MPTP, MPP^+, Heart, Noradrenaline, Adrenal catecholamines, Pargyline, Tyrosine hydroxylase.

The neurotoxic effect of MPTP is now well established (5). This neurotoxicity seems to be both species-specific i.e. the rat is more resistant than the mouse (17), and selective for the nigro-striatal dopaminergic system, since other central monoamines are less affected (15). Previous reports (10, 11) have shown a marked depletion of noradrenaline (NA) in the heart and mesenteric arteries of rats and mice at the doses of MPTP currently used in experimental practice. It is not yet clear whether this peripheral effect is similar to the central one. The experiments described in the present work were conducted to find out whether, as in the CNS, the effect of MPTP is due to its conversion to MPP^+, the main MPTP metabolite (2), and whether it is specific for NA of sympathetic nerve terminals. The effect of both MPTP and MPP^+ were compared on the catecholamine (CA) concentration in the heart, an organ rich in noradrenergic innervation, and in the adrenal medulla, where catecholamines are present in non-neuronal cells. In order to make a comparison with another largely sympathetically innervated organ, the NA content of the spleen was also determined. The effect of MPTP on the peripheral tyrosine hydroxylase (TH) activity was examined to

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establish its possible direct inhibitory role as it has been suggested in previous reports (16). In order to establish a possible comparison between central and peripheral effects of MPTP on the catecholaminergic system, its effect on the peripheral TH activity of both organs as well as the possible protective effect of pargyline on the treatment (6) was examined.

Materials and Methods

Groups of five male Sprague Dawley rats (200 g) were injected i.p. with 20 mg/kg MPTP-HCl (Research Biochemicals, Wayland, USA), 20 mg/kg MPP^+ (28 mg/kg MPP^+I^-, Research Biochemicals) and saline solution (0.9 % NaCl), in a volume of 0.2 ml/100 g. Animals were decapitated 30 min, 2, 6 and 12 h after injection. Controls receiving saline were killed at the same time.

Heart and adrenal medullary glands were quickly removed, frozen in dry ice and weighed. Hearts and spleens were homogenized in 3 ml of 0.1 N $HClO_4$ with a Branson Sonifier. All catecholamines were extracted with alumina (1) and determined by high-performance liquid chromatography (HPLC) and electrochemical detection (ED) (Bioanalytical System) at a fixed potential of 0.7 V. A phosphate/citrate buffer (pH 4.0) with octanesulfonic acid (2 mM) and 8 % of methanol, filtered and degassed, was used as a mobile with a reverse-phase column (Nucleosil-C18 10 µm, Knauer) at 1 ml/min flow rate. TH activity was determined incubating 60 µl of tissue homogenized in 0.25 M sucrose (3 ml for heart and 0.5 ml for adrenals) at 37° C for 15 min in a mixing bath after adding a solution containing 1 M acetate buffer pH 6.0 (60 µl), catalase (20 µl), 10 and 50 mM 6-methyl-tetrahydropteridine (20 µl) and 200 and 500 mM L-tyrosine (40 µl). Instead of L-Tyr, D-Tyr was added in control incubation (18). Incubation was stopped by adding 600 µl of 0.4 N $HClO_4$ and cooling in ice. After centrifugation at 10.000 rpm for 10 min, catecholamines were extracted as previously described and L-dopa was determined by HPLC/ED.

Pargyline (Research Biochemicals) was injected i.p. at 50 mg/kg doses in a volume of 0.2 ml/100 g.

Results and Discussion

A single injection of MPTP or MPP^+ drastically depleted heart NA to 25 % of control values in a linear way 12 h after administration (fig. 1). In this experiment, spleen NA decreased to the same extent, 25 % of control (813.8 ± 93.8 mg NA/g wet tissue in control animals and 204.0 ± 34.8 mg NA/g wet tissue in MPTP-treated animals), indicating a gen-

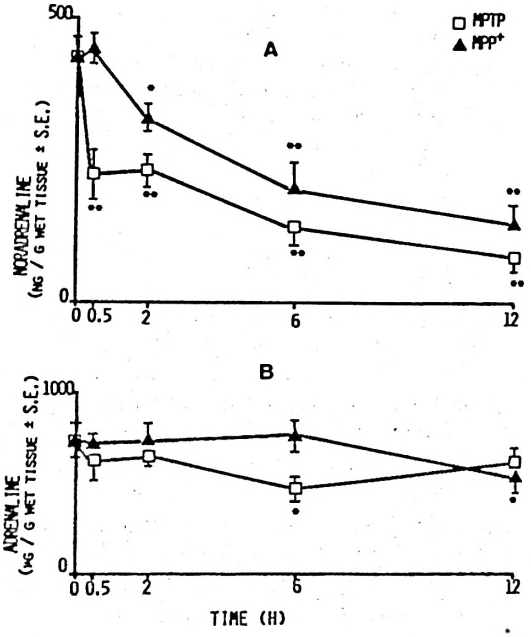

Fig. 1. *Time-course of noradrenaline in heart (a) and adrenaline in adrenal medullary glands (b) of rats (n = 5) after a single i.p. dose of 20 mg/kg MPTP and MPP^+.*

* $p < 0.05$, ** $p < 0.01$; Duncan's test vs. controls.

Table I. *TH activity (pmols L-dopa/min/mg tissue ± S.E.) in heart and adrenal medulla of rats (n = 5) treated i.p. with a single dose of MPTP (20 mg/kg) or saline solution and killed 12 h later.* * $p < 0.05$, ** $p < 0.01$; Student's t-test vs. controls.

	Control	MPTP-Treated
Heart	0.386 ± 0.18	0.269 ± 0.35*
Adrenal medulla	66.5 ± 14.8	22.0 ± 4.8**

eralized effect on the peripheral nervous system.

Contrarily, TH activity in the heart (table I) was only slightly reduced (70 % of control). Since the effects on the CA heart content produced by MPTP and MPP^+ were very similar, TH activity was tested only in animals treated with MPTP. The lack of correlation between the variation of CA and TH activity indicates that a single injection of MPTP does not produce any destructive effect on the nerve terminals, but it does produce an important depletion of the NA sympathetic vessels.

In contrast to the data obtained with heart, in the adrenal glands, treatments with MPTP or MPP^+ cause only a slight decrease in its CA content and a markedly reduced TH activity; a single dose of MPTP (20 mg/kg i.p.) causes a small transitory adrenaline depletion (73 % of control) 6 h after its administration which is totally recovered after 12 h. The same adrenaline depletion was found 12 h after the MPP^+ (20 mg/kg i.p.) administration (fig. 1). No changes were detected in adrenals noradrenaline content. As it has been previously reported in patients with Parkinson's disease (19), a markedly reduced TH activity was also found in the adrenal medulla of MPTP-treated rats (30 % of controls) (table I).

From these data, it is concluded that MPTP and MPP^+ have different effects on the peripheral catecholaminergic system and adrenal glands: on the heart a strong depleting effect of the noradrenergic vesicles accompanied by a slight reduction in TH activity was observed. The effects on the adrenal glands are opposite: an only limited decrease of adrenaline is accompanied by a more important loss of TH activity. It seems that the treatment does not cause any destructive effect on the nerve terminals but only a marked depleting effect on its NA vesicles. The reduction in TH activity may be partially due to a direct inhibitory effect on TH (16) and/or on the dihydropteridine reductase activity (13). In the adrenal glands, this lower TH activity is still sufficient to maintain a pool of NA that, via a normal phenylethanolamine N-methyltransferase activity, can restore the normal levels of adrenaline.

No alterations were found in adrenal medulla weights (33.3 ± 3.0 mg for controls and 29.5 ± 1.5 mg for MPTP-treat-

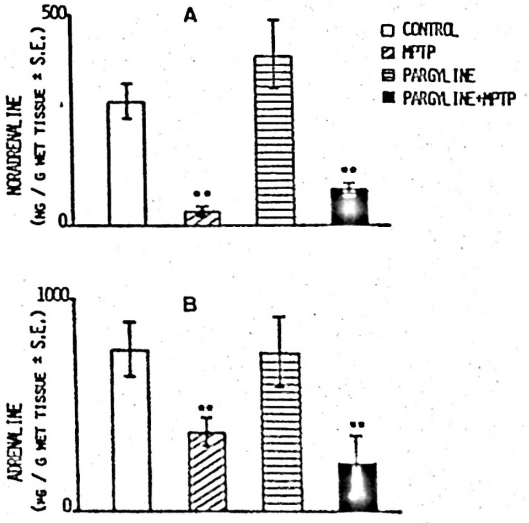

Fig. 2. *CA levels in heart (a) and adrenal medulla (b) in rats treated once i.p. with saline solution, MPTP (20 mg/kg), pargyline (50 mg/kg) 1 h before MPTP (20 mg/kg) (n = 5 for each group).* Animals were killed 6 h after MPTP treatment. * $p < 0.05$, ** $p < 0.01$; Duncan's test vs. controls.

ed animals), in contrast to data published for marmosets treated with four doses of MPTP (8).

When pargyline was administered (50 mg/kg i.p.) 1 h before receiving MPTP, CA contents of heart and adrenal medulla were depleted to the same extent as with MPTP treatment alone (fig. 2). Therefore, pargyline treatment does not prevent the peripheral neurotoxic actions of MPTP; indeed, a more extensive neurotoxicity for MPTP was revealed when the rats were pretreated with the monoamine oxidase (MAO) inhibitor: in one experiment, when pargyline was administered twice at 6 h and at 30 min before receiving MPTP, four of the five animals treated died within the next 12 h, but none of the animals treated only with pargyline, MPTP or saline did. The data agree with previous reports (4) that showed a lack of protective action of deprenyl, a MAO-B inhibitor, from depletion of catecholamines induced by MPTP from cultured chromaffin cells. These cells are known to differ from others containing MAO-B, including neurons (14), cultured dopaminergic neurons (9) and human platelets (7). Other reports (3) indicate that deprenyl fails to antagonize the neurotoxic actions of MPP^+ on dopaminergic neurons and, indeed, intesifies or extends its neurotoxic actions to noradrenergic neurons. These data agree with a previous report (12) that showed a lack of protective action of deprenyl on peripheral effects of MPTP suggesting that MPTP does not have to be converted to MPP^+ in order to deplete heart NA, a conversion required for depletion of striatal dopamine. In platelets, MPTP enters by diffusion; if this is also the case for heart and adrenal glands, pargyline would not be expected to prevent MPTP entry or the consequent damage. It seems likely that MPTP easily enters the cells and immediately initiates the release of stored catecholamines, probably as a consequence of its conversion to MPP^+.

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Resumen

Se estudia el efecto de las neurotoxinas parkinsonizantes, MPTP y MPP^+ (20 mg/kg i.p.), sobre el contenido de catecolaminas y la actividad tirosina hidroxilasa (TH) en corazón y glándula adrenal de ratas machos Sprague Dawley. En el corazón se produce una drástica depleción de noradrenalina, que llega a tan sólo un 20 % del normal al cabo de 12 h de tratamiento. La actividad TH se ve sólo ligeramente disminuida, indicando que, con toda probabilidad, no se produce destrucción tisular sino simplemente vaciamiento vesicular. La adrenalina de las glándulas suprarrenales, aunque no la noradrenalina, resulta ligeramente disminuida al cabo de 6 h de tratamiento con MPTP, recuperándose posteriormente los valores normales. La actividad TH de la médula adrenal resulta reducida a una tercera parte de su valor normal tras 12 h de la administración de MPTP. El pretratamiento con pargilina (50 mg/kg i.p.), inhibidor de la monoamino oxidasa (MAO), no protege contra los efectos periféricos del MPTP. La acción del MPTP sobre las aminas periféricas no parece estar mediada por la MAO.

Palabras clave: MPTP, MPP^+, Noradrenalina cardíaca, Catecolaminas adrenales, Pargilina, Tirosina hidroxilasa.

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