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Acute effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on dopamine metabolism in mouse and rat striatum

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Monoamines and metabolites in mouse striatum were measured at intervals (0–6 h) after injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 50 mg kg^{-1} subcutaneously). In addition, the accumulation of 3,4-dihydroxyphenylalanine (dopa), induced by inhibition of the aromatic amino acid decarboxylase by 3-hydroxybenzylhydrazine (NSD 1015), was assessed during every 15 min (0–135 min) after MPTP administration. The alterations induced by MPTP during the first hour after injection were a transient acceleration followed by a marked retardation of dopa synthesis, a decrease in 3,4-dihydroxyphenylacetic acid (DOPAC; −55%) and an increase in 3-methoxytyramine (3-MT; +400%). Between 60 and 75 min after administration, some dramatic changes took place: a 40% reduction of dopamine (DA), a marked additional increase in 3-MT (to 1300% of control) and an increase in homovanillic acid (HVA; +50%). The period after 75 min was characterized by a further depletion of DA, a decrease in 3-MT and a transient increase in HVA (max. 240% of control). Six hours after the administration, all concentrations of DA and its metabolites were subnormal, i.e. DA (30% of control), 3-MT (10%), DOPAC (10%) and HVA (65%). The MPTP-induced retardation of dopa synthesis was not antagonized by haloperidol or by reserpine pretreatment. MPTP (25 or 50 mg kg^{-1} s.c.) produced similar acute changes in the levels of DA and its metabolites in rat as in mouse striatum, though much less pronounced. Fourteen days after injection of MPTP (2 × 50 mg kg^{-1}; 16 h interval) in mice, significant reductions in the striatal levels of NA, DA, 3-MT, DOPAC, HVA and 5-HT were observed. In rat striatum, no changes in the levels of monoamines and metabolites were noted 14 days after injection of MPTP (2 × 25 mg kg^{-1}; 16 h interval). The possible mechanisms underlying the rather dramatic acute biochemical changes induced by MPTP as well as their relationship to the neurotoxic effect are discussed.

Abuse of a pethidine (meperidine) congener contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered to cause parkinsonism in young addicts (Davis et al 1979; Langston et al 1983). Autopsy of one patient, who died of a drug overdose, showed histological and biochemical changes indistinguishable from those seen in idiopathic Parkinson's disease, i.e. loss of dopamine (DA) neurons and neuromelanin in substantia nigra and marked reductions in the concentrations of DA and its major metabolite homovanillic acid (HVA) in the caudate nucleus and the putamen (Davis et al 1979). Studies in monkeys (Burns et al 1983; Langston et al 1984a) and in mice (Hallman et al 1984; Heikkila et al 1984a) have verified that MPTP is able to destroy nigrostriatal DA neurons. In contrast, the effect of MPTP on the DA system in rat striatum has been reported to be poor (Chiueh et al 1984).

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We have earlier reported that administration of MPTP in mice initially leads to a release of striatal DA from the nerve terminals as indicated by an enormous increase in 3-methoxytyramine (3-MT) together with a reduction in DA and an acute increase in locomotor activity of habituated mice (Pileblad et al 1984). The possible relation between these acute changes in DA metabolism and the neurotoxicity of MPTP to DA neurons needs to be established.

The present investigation was undertaken to gain further information about the acute effects of MPTP on monoamine metabolism in mouse striatum; for comparative purposes, rats were also included. In addition, long-term effects of the substance in the striatum of both species will be presented.

MATERIALS AND METHODS

Male albino mice of the NMRI strain (20–30 g) and male Sprague-Dawley rats (200–300 g) were used.

The following drugs were used: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP; Research Biochemical Inc. Wayland, MA,

USA), haloperidol (Leo, Helsingborg, Sweden), reserpine (Ciba-Geigy, Basle, Switzerland) and 3-hydroxybenzylhydrazine (NSD 1015; synthezised by the Organic Chemistry Unit of this Department). MPTP and NSD 1015 were dissolved in 0·9% NaCl. Reserpine and haloperidol were dissolved in a few drops of glacial acetic acid and made up to volume with 5·5% glucose. Information on dosages and injection schedules are given in the figures and table texts.

The rates of tyrosine and tryptophan hydroxylation were measured as the amount of 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP), respectively, accumulated in-vivo during 15 min following inhibition of L-aromatic amino acid decarboxylase with NSD 1015 (Carlsson et al 1972; Carlsson & Lindqvist 1973).

The animals were decapitated and the corpus striatum, including the caudate nucleus and the putamen, was dissected on an ice-chilled glass plate. Noradrenaline (NA), dopa, DA, 3-MT, 3,4-dihydroxyphenylacetic acid (DOPAC), HVA, 5-HTP, 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were assayed by reversed phase liquid chromatography with electrochemical detection according to standard methods.

RESULTS

Levels of monoamines and their metabolites in mouse striatum during the first 6 h of MPTP treatment

As shown in Fig. 1, systemic injection of MPTP (50 mg kg^{-1} s.c.) caused a depletion of DA occurring in two phases, i.e. one rapid decrease of 40% between 60 and 75 min after administration and a slower reduction from 60% of control values at 75 min to 30% at 180 min ($P < 0·001$). The former change was accompanied by a thirteen-fold increase in the level of 3-MT, which then slowly decreased to one tenth of control values 6 h after the injection. The level of HVA started to increase 1 h after MPTP administration, reached a peak (240% of control) at 180 min and was also significantly reduced after 6 h, as was the concentration of DOPAC. The latter, however, showed a more complex pattern of alterations during the first hours, i.e. the level was reduced, except for two peak values at 75 and 120 min ($P < 0·001$, if compared to the groups immediately before and after) when the DOPAC

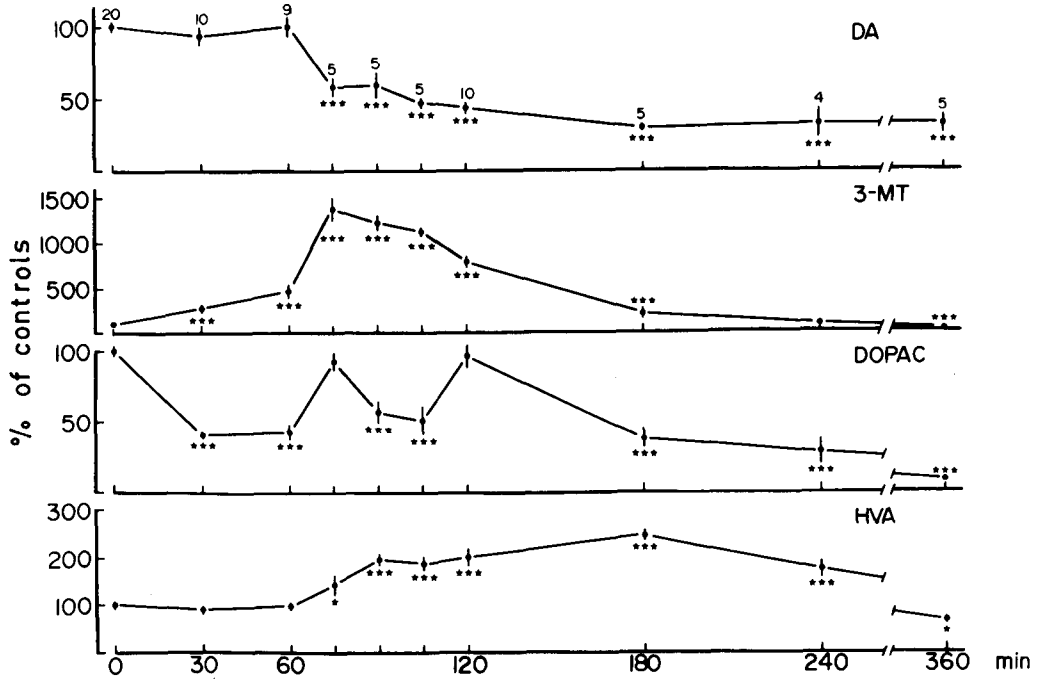

FIG. 1. Time course of the acute effects of MPTP (50 mg kg^{-1} s.c.) on dopamine and its metabolites in mouse striatum. Each point represents mean ± s.e.m. for the number of mice indicated in the upper curve. Percentages were calculated on the basis of the control values in each experiment. Pooled controls (ng g^{-1}; n = 20) were: DA = 10259 ± 526, 3-MT = 364 ± 27, DOPAC = 833 ± 42, HVA = 833 ± 37. Statistical evaluation was carried out by one-way analysis of variance followed by a *t*-test except in the case of 3-MT where only the *t*-test was applied because of large variations in the s.e.m. values; $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

concentration was back to control values. There were no changes in the striatal levels of NA, 5-HT and 5-HIAA at any time indicated in Fig. 1 (data not shown).

Levels of monoamines and their metabolites in rat striatum 2 h after MPTP administration

As illustrated in Table 1, MPTP 25 and 50 mg kg^{-1} caused an increase in striatal 3-MT (+78 and +147%, respectively) and decreased concentrations of DOPAC (−78 and −85%), HVA (−30 and −38%) and 5-HIAA (−15 and −23%). In addition, the larger dose significantly altered the levels of NA, DA and 5-HT (+38, −15 and −13%, respectively).

Table 1. Levels of monoamines and their metabolites in rat striatum 2 h after injection of MPTP (25 and 50 mg kg^{-1} s.c.). Data are means ± s.e.m. in ng g^{-1} for the number of animals indicated in parentheses. The statistical significances were calculated by a *t*-test after one-way analysis of variance.

	Striatal concns (ng g^{-1})		
		MPTP	
	Saline (10)	25 mg kg^{-1} (5)	50 mg kg^{-1} (5)
NA	111 ± 7	135 ± 4	153 ± 13**
DA	7521 ± 101	7810 ± 35	6367 ± 349**
3-MT	414 ± 21	739 ± 66***	1023 ± 50***
DOPAC	1017 ± 65	228 ± 20***	158 ± 14***
HVA	927 ± 52	648 ± 68**	575 ± 54***
5-HT	457 ± 11	467 ± 21	399 ± 19*
5-HIAA	738 ± 27	625 ± 18**	571 ± 12***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 2. Effects of haloperidol and reserpine on MPTP-induced reduction in dopa accumulation and effects of MPTP on 5-HTP accumulation in mouse striatum. MPTP (50 mg kg^{-1} s.c.), haloperidol (1·5 mg kg^{-1} i.p.) and NSD 1015 (100 mg kg^{-1} i.p.) were given 75, 25 and 15 min before death, respectively. Reserpine (10 mg kg^{-1} i.p.) was given 4 h before MPTP. 5-HTP was determined from two pooled brains. Data are means ± s.e.m. in ng g^{-1} for 5–6 observations. The statistical significances were calculated by a *t*-test.

Treatment	Dopa (ng g^{-1}) per 15 min	5-HTP (ng g^{-1}) per 15 min
Saline	630 ± 37	55 ± 3
Haloperidol	2060 ± 113*	—
MPTP	96 ± 7*	33 ± 2*
Haloperidol + MPTP	120 ± 9*[a]	—
Reserpine	713 ± 108	
Reserpine + MPTP	68 ± 7**	

* Differs from saline group $P < 0.001$.
[a] N.S. if compared to the MPTP group.
** Differs from reserpine group $P < 0.001$.

Effects of MPTP on dopa and 5-HTP accumulation in mouse striatum

Fig. 2 shows that MPTP (50 mg kg^{-1} s.c.) retarded the dopa accumulation, induced by inhibition of the aromatic amino acid decarboxylase, at all times assessed apart from the first 15 min when an increase was observed. The degree of reduction varied between 70 and 25% of control values (observations

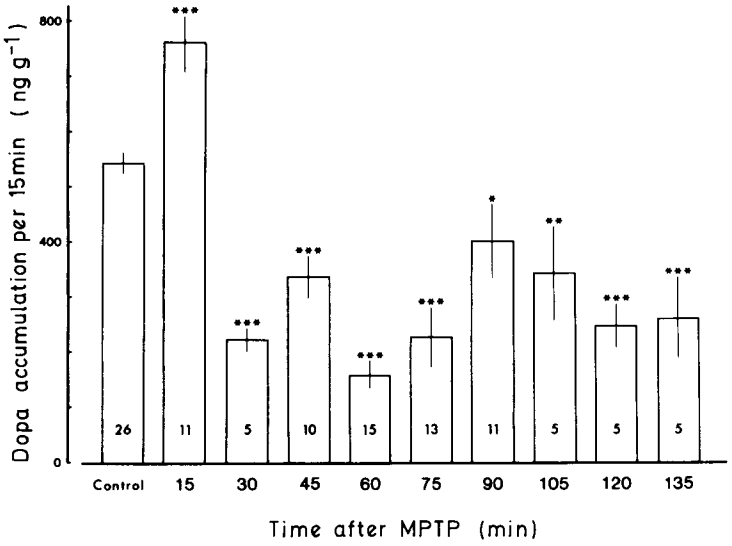

FIG. 2. Effects of MPTP on dopa accumulation in mouse striatum. The mice received NSD 1015 15 min before death; minutes on time scale indicate time between MPTP and death. Data are means ± s.e.m. in ng g^{-1} for the number of animals indicated in the bars. Statistical significances were calculated by a *t*-test after one-way analysis of variance; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3. Striatal levels of monoamines and metabolites 14 days after injections of MPTP in mice (2 × 50 mg kg^{-1} s.c.; 16 h interval) and rats (2 × 25 mg kg^{-1} s.c.; 16 h interval). Data are means ± s.e.m. in ng g^{-1} for 5–6 observations; striata from two mice were pooled. Statistical significances were calculated by *t*-test.

	Striatal concentrations (ng g^{-1})			
	Mice		Rats	
	Saline	MPTP	Saline	MPTP
NA	190 ± 12	114 ± 30*	138 ± 12	124 ± 22
DA	3784 ± 152	1269 ± 53***	7815 ± 534	7872 ± 393
3-MT	360 ± 34	223 ± 20**	298 ± 11	335 ± 20
DOPAC	1247 ± 59	654 ± 42***	966 ± 26	968 ± 30
HVA	909 ± 22	575 ± 32***	762 ± 51	677 ± 40
5-HT	445 ± 12	396 ± 13*	569 ± 23	529 ± 40
5-HIAA	348 ± 7	383 ± 18	692 ± 31	611 ± 58

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

at 60 and 90 min after MPTP, respectively; $P < 0.001$). Haloperidol (1·5 mg kg^{-1} i.p.; 10 min before NSD 1015) or reserpine (10 mg kg^{-1}; 4 h before MPTP) did not antagonize the MPTP-induced reduction in dopa formation (60–75 min after MPTP; Table 2). The accumulation of 5-HTP 60–75 min after MPTP was 60% of control values (Table 2).

Long-term effects of MPTP on the striatal levels of monoamines and their metabolites in mice and rats

Table 3 illustrates the effects of MPTP (2 × 50 mg kg^{-1}; 16 h interval) in mouse striatum 14 days after the injections. Significant decreases were observed for all amines and metabolites assayed apart from 5-HIAA, i.e. NA: −40%, DA: −66%, 3-MT: −38%, DOPAC: −48%, HVA: −37% and 5-HT: −11%.

In the long-term experiment with rats we were limited to injecting 25 mg kg^{-1} of MPTP each time because of the large mortality observed the first 16 h after administration of higher doses. Fourteen days after injection of MPTP (2 × 25 mg kg^{-1}; 16 h interval) no biochemical changes in rat striatum were observed (Table 3).

DISCUSSION

As indicated by the present study, MPTP causes a puzzling sequence of rather dramatic changes in striatal DA synthesis and metabolism during the first few hours after a subcutaneous injection of a single dose to mice. The earliest change observed in this study was an increase in dopa synthesis occurring during the first 15 min after the injection. However, this effect was rapidly followed by a marked inhibition of dopa formation. A possible explanation of this biphasic response is afforded by the fact that MPTP can be metabolized to the quaternary 1-methyl-4-phenylpyridinium ion (MPP^+) through the action of monoamine oxidase; this metabolite appears to be responsible for the neurotoxic action (Chiba et al 1984; Heikkila et al 1984b; Langston et al 1984b; Markey et al 1984). The initial stimulation of DA synthesis may thus be caused by the parent compound, whereas the subsequent inhibition may be an action of the metabolite. Possibly MPTP is capable of blocking DA receptors to some extent, thus causing a feedback stimulation of DA synthesis. In fact, unpublished data from this laboratory show that the *N*-propyl analogue of MPTP, which seems to be devoid of neurotoxicity (Heikkila, personal communication), perhaps because it cannot be converted to a quaternary metabolite, causes an increase in the levels of DOPAC and HVA as well as sedation.

Apart from the inhibition of dopa formation (see later) the most striking change observed during the first hour after the injection is a decrease in the DOPAC level. Since there is no concomitant change in the level of DA, it seems logical to suggest that partial monoamine oxidase inhibition is responsible for this decrease. Against this hypothesis, there is no change in 5-HT and 5-HIAA levels. However, MPTP and/or its metabolite may be taken up selectively and concentrated in dopaminergic (and noradrenergic) neurons. Autoradiographic findings support such an assumption showing accumulation of radioactivity specifically in dopaminergic regions of the brain following administration of radioactive MPTP (Hartvig et al 1984; Larsson, personal communication; cf. Irwin & Langston 1985) as well as in-vitro data demonstrating that MPP^+ can be concentrated by the DA neuronal uptake system (Javitch & Snyder 1985). Moreover, in-vitro data indicate that MPTP, and possibly MPP^+, are capable of inhibiting monoamine oxidase (Parsons & Rainbow 1984).

Between 60 and 75 min after the injection, some dramatic changes took place: these were a decrease in DA and an increase in 3-MT. Presumably a rapid release of DA into the extraneuronal space occurred during this period. At the same time there was a rise in DOPAC and HVA levels, which, like the rise in 3-MT, may have been secondary to the release of DA.

The question arises if the inhibition of dopa formation can be explained by the DA-releasing action. Admittedly this inhibition was found to precede the rapid release occurring during the 60–75 min period. However, a several-fold increase in 3-MT occurred before this period. Receptor-mediated feedback inhibition of tyrosine hydroxylase can be excluded, since haloperidol did not antagonize the inhibition of dopa synthesis. Another

possibility would be that the release of DA into the synaptic cleft is preceded, and perhaps even caused, by release from the storage granules into the cytoplasm, thus causing a direct feedback inhibition of tyrosine hydroxylase. Such an action would be facilitated by the partial inhibition of intraneuronal monoamine oxidase discussed above. However, the lack of antagonism of reserpine pretreatment is against this assumption. If, on the other hand, a direct inhibition of the tyrosine hydroxylase by MPP^+ is suggested, it remains to be explained why MPTP does not retard dopa synthesis in noradrenergic neurons (unpublished data from this laboratory). The differences in accumulation of MPP^+ that exist between different brain regions (see above) may perhaps acount for this discrepancy. Further work is needed to support or reject these possibilities.

The period starting 75 min after the injection was characterized by a slow further depletion of DA, accompanied by a decrease in 3-MT and sustained high levels of HVA for several hours. These changes may be interpreted as consequences of a relatively slow release process, ensuing upon the more rapid release. The DOPAC level showed some irregularities which cannot be explained. Finally, after 6 h all levels of DA and its metabolites reached subnormal values, leading to a picture similar to that seen 2 weeks after MPTP ($2 \times 50\ mg\ kg^{-1}$). These data suggest that the changes seen during the latter part of the acute experiment are actually caused by the neurotoxic process, leading to long-standing cell damage. However, the relation between the early changes and the neurotoxic effect, as well as the mechanism underlying this action, remain to be clarified.

The rat data presented indicate that MPTP is capable of producing similar acute changes in the rat as in the mouse, though much less pronounced. The decrease in 5-HT and 5-HIAA may indicate a lower degree of selectivity in the rat. On the whole, however, the difference between rat and mouse may be quantitative rather than qualitative. In support of this, Steranka et al (1983) observed a 40% depletion of DA in rat striatum one week after MPTP administration, in contrast to our findings and those of Chiueh et al (1984). The probable reason why only Steranka et al could observe a sustained neurotoxic effect was that they used a higher dosage, administered over 24 h by means of osmotic minipumps. Moreover, Mytilineou & Cohen (1984) observed a damage of DA neurons in explants of rat embryo mesencephalon.

The more pronounced decrease in DA than in its metabolites, as observed in the long term mouse experiment, is in agreement with Hallman et al (1984); it may be due to a compensatory increase in DA turnover, as proposed by these authors.

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REFERENCES

Burns, R. S., Chiueh, C. C., Markey, S. P., Ebert, M. H., Jacobowitz, D. M., Kopin, I. J. (1983) Proc. Natl. Acad. Sci. (USA) 80: 4546–4550

Carlsson, A., Davis, J. N., Kehr, W., Lindqvist, M., Atack, C. V. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 275: 153–168

Carlsson, A., Lindqvist, M. (1973) J. Neural Transmission 34: 79–91

Chiba, K., Trevor, A., Castagnoli, Jr., N. (1984) Biochem. Biophys. Res. Com. 120: 574–578

Chiueh, C. C., Markey, S. P., Burns, R. S., Johannessen, J. N., Pert, A., Kopin, I. J. (1984) Eur. J. Pharmacol. 100: 189–194

Davis, G. C., Williams, A. C., Markey, S. P., Ebert, M. H., Caine, E. D., Reichert, C. M., Kopin, I. J. (1979) Psychiat. Res. 1: 249–254

Hallman, H., Olson, L., Jonsson, G. (1984) Eur. J. Pharmacol. 97: 133–136

Hartvig, P., Aquilonius, S. M., Bergström, K., Bondesson, U., d'Argy, R., Eckernäs, S. Å., Gullberg, P., Larsson, B., Lydén, A., Lindberg, B., Lindquist, N. G., Lundqvist, H., Långström, B., Malmborg, P., Någren, K. (1984) Proceedings from the Annual Swedish Pharmaceutic Congress; Abstr. p. 58

Heikkila, R. E., Hess, A., Duvoisin, R. C. (1984a) Science 224: 1451–1453

Heikkila, R. E., Manzino, L., Cabbat, F. S., Duvoisin, R. C. (1984b) Nature 311: 467–469

Irwin, I., Langston, J. W. (1985) Life Sci. 36: 207–212

Javitch, J. A., Snyder, S. H. (1985) Eur. J. Pharmacol. 106: 455–456

Langston, J. W., Ballard, P., Tetrud, J. W., Irwin, I. (1983) Science 219: 979–980

Langston, J. W., Forno, L. S., Rebert, C. S., Irwin, I. (1984a) Brain Res. 292: 390–394

Langston, J. W., Irwin, I., Langston, E. B., Forno, L. S. (1984b) Neurosci. Lett. 48: 87–92

Markey, S. P., Johannessen, J. N., Chiueh, C. C., Burns, R. S., Herkenham, M. A. (1984) Nature 311: 464–467

Mytilineou, C., Cohen, G. (1984) Science 225: 529–531

Parsons, P., Rainbow, T. C. (1984) Eur. J. Pharmacol. 102: 375–377

Pileblad, E., Nissbrandt, H., Carlsson, A. (1984) J. Neural Transmission 60: 199–203

Steranka, L. R., Polite, L. N., Perry, K. W., Fuller, R. W. (1983) Res. Comm. S. 4: 315–323