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Neuronal Antioxidant System and MPTP-Induced Oxidative Stress in the Striatum and Brain Stem of the Rat

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DESOLE, M. S., M. MIELE, G. ESPOSITO, L. G. FRESU, R. MIGHELI, D. ZANGANI, S. SIRCANA, G. GRELLA AND E. MIELE. *Neuronal antioxidant system and MPTP-induced oxidative stress in the striatum and brain stem of the rat.* PHARMACOL BIOCHEM BEHAV **51**(4) 581-592, 1995.—Levels of ascorbic acid (AA), dehydroascorbic acid (DHAA), glutathione (GSH), uric acid, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), noradrenaline (NA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and 1-methyl-4-phenylpyridinium ion (MPP^+) were determined in the striatum, striatal synaptosomes, and/or brain stem of 3- and 6-month-old male Wistar rats given MPTP 35-52 mg/kg IP. In older rats, MPTP 35 mg/kg caused a 38% death rate within 15 min-12 h. Levels of MPTP and MPP^+ in the striatum, synaptosomes, and brain stem were directly correlated with the absolute MPTP dose/rat. MPTP decreased striatal DA metabolites and NA levels in the striatum and brain stem, and increased uric acid levels in all regions in all rats. All these changes were significantly correlated with MPP^+ levels. GSH levels were increased in younger rats and decreased in older rats. AA oxidation was increased mainly in older rats. We conclude that acute lethality and regional brain MPTP and MPP^+ levels depend upon the absolute dose of MPTP/rat rather than the relative dose/kg. In younger rats, the neuronal antioxidant GSH system is more efficient than in older rats, in which the response to MPP^+-induced oxidative stress also involves AA oxidation. The increase in uric acid levels provides further evidence for a mechanism of MPTP neurotoxicity involving oxidative stress mediated by xanthine oxidase.

Endogenous antioxidant system MPTP Striatum Brain stem Rat

ONE OF THE most significant neurochemical events following systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration is impairment of dopamine (DA) metabolism in the nigrostriatal system. The severity of damage varies among animal species, and within the same species differences arise between strains (16). Rodents are less sensitive than humans, primates, and nonhuman primates to MPTP neurotoxicity. Among rodents, the rat is resistant to MPTP toxicity, owing to its unusual MAO-B activity in brain microvessels (20); however, in rats systemic MPTP does induce DA striatal loss in those strains that have MAO-B activity in brain microvessels lower than resistant strains (27). When the MPTP four-electron oxidation product 1-methyl-4-phenylpyridinium ion (MPP^+) is injected directly in the rat substantia nigra, it does induce severe neuronal loss (38,39,41).

Oxidative stress has been hinted as a critical feature in MPTP neurotoxicity (1,19). Indeed, MPP^+ induces hydroxyl free radical formation when injected in the rat striatum or substantia nigra (41); moreover, intracerebroventricular MPP^+ administration increases striatal lipid peroxidation in mice (30).

The neuronal antioxidant system [glutathione (GSH), ascorbic acid (AA), vitamin E] may play an important role in limiting neuronal damage by MPP^+. Cleeter et al. (9) demonstrated that the irreversible MPP^+-induced inhibition of mitochondrial complex I (25) is prevented by free radical scaven-

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gers GSH and AA. MPTP depletes GSH in the brain stem of the rat (40) and in the substantia nigra of the mouse (14). The brain stem depletion is prevented by pretreatment with the antioxidant α-tocopherol (42). MPTP shows a greater neurotoxicity in vitamin E-deficient mice (2); in addition, MPP^+ shows greater neurotoxic effect in rat with vitamin E deficiency or with impaired GSH metabolism (40).

A constant finding in Parkinson's disease (PD) is the depletion of GSH in the substantia nigra (26,36,37). Riederer et al. (29) showed that in PD the brain regional depletion of GSH correlated well with the disease severity; in addition, a consistent overall decrease in regional AA levels was found.

Ageing is a factor that is claimed to increase nigrostriatal MPTP toxicity both in mice (3,32) and rats (18). An age-related increase in the susceptibility of dopaminergic cells to oxidative damage is heralded by Sershen et al. (32). Attention has been focused on the rate of MPTP biotransformation to MPP^+. It has been reported that in MPTP-treated mice striatal MPP^+ concentrations increase directly with age (21); however, according to Ricaurte et al. (28), the greater neurodegenerative effects of MPTP in older animals is not due to higher MPP^+ level or to enhanced uptake, but rather is related to a true increase in neuronal sensitivity. We have previously shown (11) that in aged rats the neuronal antioxidant system (AA and GSH) is less efficient than in young rats and we suggested that such impairment may play an enabling role in MPTP neurotoxic effects on the striatum and brain stem.

The present study was undertaken to assess the role of the endogenous striatal antioxidant system (AA oxidation, GSH and uric acid levels) during the MPTP-induced neuronal oxidative stress in the striatum, striatal synaptosomes (taken as a model on neuronal terminals), and brain stem of the young vs. adult rat.

METHOD

Six-month-old (b.wt. 410–600 g), and 3-month-old (b.wt. 260–330 g) male Wistar rats (Morini), maintained under standard animal care conditions on a 12L : 12D cycle and given food and water ad lib, were used in all experiments. MPTP (HCl, Sigma) was dissolved in distilled water and injected IP at the dose of 35 mg/kg to 6-month-old and 35–52 mg/kg to 3-month-old rats. Controls were given 2 ml/kg of saline IP. MPP^+, MPTP-3-ol, and 1-methyl-4-(2′-ethylphenyl)-1,2,3,6-tetrahydropyridine (2′Et-MPTP) were synthesised by one of us (G. Grella).

AA, dehydroascorbic acid (DHAA), uric acid, DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), NA, MPP^+, and MPTP determinations were performed by HPLC according to the method previously described (11,12), whereas reduced glutathione and glutathione disulfide (GSSG) were determined according to the enzymatic recycling method of Anderson (4). Because the GSSG recovered was no more than 1% of the total, the data will be given as "total glutathione" (GSH), which means the sum of GSH and GSSG in GSH equivalents.

Liquid chromatography with electrochemical detection (LCED) was done with a high-pressure pump Varian 9001 with a Rheodyne injector, column 15 cm × 46 mm i.d. TSK-OD S-80 TM, electrochemical detector BAS LC-4B, and integrator Spectra-Physics SP 4290. The mobile phase was citric acid 2%, K_2HPO_4 2%, EDTA 1 mM, MeOH 1.2%, and sodium octylsulphate 70 mg/l (pH 3.0); the flow rate was 1.2 ml/min and 10 μl of the sample was injected.

Rats were sacrificed by decapitation at various time (1, 3, 8, and 24 h) after drug injection. Heads were cooled by rapid immersion in liquid nitrogen; thereafter, striata of both sides and the brain stem (including the substantia nigra) were rapidly removed. The striata of the left side were immediately processed for synaptosomes preparations and those on the right side and the brain stem were frozen at −40°C; thereafter, striata of the right side and the brain stem were weighed and homogenised in EDTA 1 mM containing meta-H_3PO_4 1%. After centrifugation (17,500 × *g* for 10 min at 4°C), the supernatant was divided into three aliquots. The first was filtered and immediately injected into the HPLC system for DOPAC, HVA, 3-MT, DA, NA, uric acid, and AA determinations. The second aliquot was adjusted to pH 7.0 with K_2PO_4 45% and DL-homocysteine 1% was added to reduce DHAA to AA. The sample was incubated for 30 min at 25°C, then adjusted to pH 3.0 with meta-H_3PO_4 30%, filtered, and injected (20 μl) for total AA determination. DHAA concentration was calculated from the difference in AA content between the first and second aliquots.

On the third aliquot MPTP and MPP^+ determinations were performed directly by injecting 50 μl of the filtered supernatant. Liquid chromatography was performed using a high-pressure pump (Varian 9001 with a Rheodyne injector), column (15 cm × 46 mm i.d. TSK-ODS-80 TM) and similar precolumn, UV detector (Star 9050 Varian) (MPTP 295 nm, MPP^+ 245 nm) and integrator (Spectra-Physics SP 4290). The mobile phase was composed of H_2S0_4 0.1 M, triethylamine 0.075 M, and acetonitrile 10% at pH 2.30; the flow was 1.5 ml/min. MPTP-3-ol (260 nm) was the internal standard. Analysis of samples spiked with free MPTP or MPP^+ yielded recovery ≥99%.

Crude synaptosomes of striata on the left side were prepared according to a modification of the Gray and Whittaker (17) method. Striata were homogenised in 30 vol. of ice-cold 0.32 M sucrose buffered at pH 7.4 with phosphate, using a Teflon-glass system. The homogenate was centrifuged at 4°C for 10 min at 1500 × *g* to remove nuclei and debris, and crude synaptosomes were isolated from the supernatant by centrifugation at 4°C at 22,000 × *g* for 20 min. To lyse synaptosomes, the pellet was resuspended by sonication in 0.9 ml ice-cold metaphosphoric acid and an aliquot of 50 μl was taken for protein analysis. After centrifugation (17,200 rpm for 7 min), 20 μl of the supernatant was divided into three aliquots for the above determinations.

PC12 cells were grown routinely in 35 cm^2 plastic tissue culture dishes under an atmosphere of 5% CO_2/95% air in Dulbecco-MEM containing 10% horse serum. DA determination was performed by HPLC as above, and GSH and GSSG analysis according to the enzymatic recycling method of Anderson (4).

All values were expressed in nmol or pmol/mg protein and given as mean ± SD. Biochemical data were analysed with ANOVA, and then with Student's two-tailed *t*-test or Bonferroni *t*-test. Pearson's correlation coefficient was also calculated in some instances.

All studies were carried out in accordance with the Decreto No. 116/1992 of the Italian Ministry of the Public Health (Directive 86/609/EEC).

RESULTS

Acute Lethality Rate and Behaviour

In 3-month-old rats (b.wt. 260–330 g, range absolute MPTP dose 9.8–14.56 mg/rat) MPTP treatment (35–52 mg/kg) induced neither lethality nor aberrant behaviour.

Of the 50 6-month-old rats, 19 (38%) died within 15 min-12 h after MPTP 35 mg/kg. Immobilisation and respiratory distress were observed in the rats that died within 1 h; Straub tail and convulsions were observed in those that died within 12 h. The mean body weight of the dead animals turned out to be 550 ± 35 g, the mean MPTP dose/rat was 19.17 ± 1.22 mg. Rats that survived and were randomly sacrificed at 1, 3, 8, and 24 h after MPTP had a mean body weight of 483.2 ± 43.8 g ($p < 0.05$ vs. b.wt. of dead rats); the mean dose of MPTP/rat was 16.88 ± 1.64 mg ($p < 0.05$).

In additional experiments in 3-month-old rats, MPTP 67 mg/kg induced a 100% mortality rate within 10–45 min. Immobilisation and respiratory distress were observed. The mean MPTP dose/rat turned out to be 18.83 ± 0.6 mg ($n = 5$). The lethal MPTP dose was therefore ≥ 18.5 mg/rat.

Levels of MPTP and MPP⁺ and Individual Correlations

The levels of MPTP and MPP^+ detected in the whole striatum, striatal synaptosomes, and brain stem of the rats sacrificed 1, 3, 8, and 24 h after MPTP are given in Table 1.

Regional levels of MPP^+ in younger rats given MPTP 35 mg/kg were considerably lower than levels in adult rats given the same MPTP dose/kg. Regional MPP^+ levels in younger rats given MPTP 52 mg/kg and adult rats given MPTP 35 mg/kg were fairly comparable in terms of peak level, which in the whole striatum and in the brain stem was reached 1 h after the drug. The regional MPP^+ clearance in younger rats was higher than older rats, in which the synaptosomal peak was reached 3 h after MPTP.

MPTP was detected neither in synaptosomes of all rats nor in striata of younger rats given the lower MPTP dose (35 mg/kg).

Individual absolute MPTP doses/rat were significantly correlated with MPTP and MPP^+ levels detected in the striatum, striatal synaptosomes, and brain stem 1 and 3 h after MPTP administration (Table 2).

Neurochemical Changes and Correlation With Individual MPP^+ Levels

GSH levels. Results are given in Table 3.

The two groups of younger saline-treated rats showed levels of GSH higher than older saline-treated rats in the striatum (+18% and +13%, respectively), striatal synaptosomes (+35%

TABLE 1

LEVELS OF MPTP AND MPP^+ (PMOL/MG PROTEIN) IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

	Sacrifice After MPTP			
	1h	3h	8h	24h
		Striatum		
MPTP (3M-35)	ND	ND	ND	ND
(3M-52)	286.5 ± 75	74.4 ± 12	ND	ND
(6M-35)	185.4 ± 75[b]	25.7 ± 48	ND	ND
MPP^+ (3M-35)	889.4 ± 149	367.2 ± 64	ND	ND
(3M-52)	1522.1 ± 219[a]	633.5 ± 140[a]	109.0 ± 26	31.1 ± 13
(6M-35)	1302.2 ± 185[ab]	791.4 ± 245[a]	327.9 ± 158[b]	ND
ANOVA *F*	23.7	13.2		
p	<0.00001	<0.0002		
		Striatal synaptosomes		
MPP^+ (3M-35)	186.8 ± 34	36.7 ± 8	8.2 ± 3	ND
(3M-52)	345.7 ± 48[a]	186.0 ± 36[a]	20.8 ± 23	ND
(6M-35)	380.0 ± 65[a]	502.0 ± 113[ab]	162.5 ± 12[ab]	5.8 ± 12
ANOVA *F*	33.3	96.4	46.4	
p	<0.00001	<0.00001	<0.00001	
		Brain stem		
MPTP (3M-35)	67.6 ± 30	ND	ND	ND
(3M-52)	171.2 ± 35[a]	30.3 ± 5	ND	ND
(6M-35)	170.9 ± 72[a]	19.4 ± 28	ND	ND
ANOVA *F*	11.7			
p	<0.0005			
MPP^+ (3M-35)	354.6 ± 132	128.8 ± 39	49.1 ± 22	ND
(3M-52)	807.4 ± 169[a]	268.6 ± 35[a]	52.6 ± 10	ND
(6M-35)	756.5 ± 262[a]	498.7 ± 143[ab]	150.0 ± 61[ab]	ND
ANOVA *F*	12.7	36.2	21.1	
p	<0.0005	<0.00001	<0.00001	

ND not detectable.

[a] $p < 0.05$ vs. 3M-35; [b] $p < 0.05$ vs. 3M-52.

TABLE 2

CORRELATION BETWEEN INDIVIDUAL MPTP DOSE/RAT AND INDIVIDUAL LEVELS OF MPTP AND MPP^+ IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD AND 6-MONTH-OLD RATS 1H AND 3H AFTER MPTP 35-52 mg/kg IP

	3-Month-Old	3-Month-Old	6-Month-Old
MPTP mg/kg	35	52	35
MPTP mg/rat:			
a) 1 h groups	10.24 ± 0.38	13.94 ± 0.39	17.59 ± 1.15
b) 3 h groups	10.43 ± 0.43	13.99 ± 0.26	16.49 ± 1.37

N = 8/group.

Pearson's correlation coefficient (df = 22): a) 1 h groups: individual MPTP mg/rat vs: striatal MPTP, $r = +0.602$, $p < 0.02$; striatal MPP^+, $r = +0.548$, $p < 0.01$; synaptosomal MPP^+, $r = +0.826$, $p < 0.00001$; brain stem MPTP, $r = +0.618$, $p > 0.002$; brain stem MPP^+, $r = +0.615$, $p < 0.002$; b) 3 h groups: individual MPTP mg/rat vs: striatal MPTP, $r = +0.358$, $p > 0.08$; striatal MPP^+, $r = +0.796$, $p < 0.00001$; synaptosomal MPP^+, $r = +0.791$, $p < 0.00001$; brain stem MPTP, $r = +0.593$, $p < 0.005$; brain stem MPP^+, $r = +0.932$, $p < 0.00001$.

TABLE 3

LEVELS OF GSH (nmol/mg PROTEIN) IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

		Sacrifice After MPTP				ANOVA	
Groups	Controls	1h	3h	8h	24h	F	p
			Striatum				
3M-35	17.19 ± 1.53	19.04 ± 1.29*	18.34 ± 2.56	16.34 ± 1.37	17.66 ± 1.77	2.8	<0.05
3M-52	16.40 ± 1.49	19.91 ± 2.18*	18.65 ± 1.75*	17.02 ± 2.18	18.54 ± 2.17*	3.9	<0.02
6M-35	14.51 ± 1.41[ab]	12.58 ± 2.01*	14.74 ± 1.40	13.18 ± 1.24	13.73 ± 1.44	2.8	<0.05
ANOVA F	9.0						
p	<0.002						
			Synaptosomes				
3M-35	9.09 ± 0.95	9.18 ± 1.30	8.54 ± 0.98	8.98 ± 0.88	9.74 ± 1.30	1.2	>0.3
3M-52	8.13 ± 1.10	5.79 ± 0.94*	5.76 ± 1.06*	7.04 ± 0.85	7.45 ± 0.75	10.2	<0.0002
6M-35	6.75 ± 0.84[ab]	4.68 ± 1.75*	4.65 ± 1.08*	5.62 ± 1.17*	6.37 ± 0.55	6.1	<0.001
ANOVA F	14.8						
p	<0.0001						
			Brain stem				
3M-35	9.13 ± 0.82	9.64 ± 0.63	9.96 ± 1.12	8.87 ± 1.34	8.04 ± 1.33	1.4	>0.2
3M-52	9.80 ± 1.25	11.38 ± 1.39*	11.86 ± 1.92*	9.78 ± 1.27	10.03 ± 1.42	3.5	<0.02
6M-35	8.30 ± 0.51[ab]	6.37 ± 1.20*	7.58 ± 0.52*	8.24 ± 0.68	7.71 ± 0.76	8.9	<0.00005
ANOVA F	7.8						
p	<0.05						

*$p < 0.05$ vs. controls: [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 n = 8/group; 6M-35 control group n = 10, 1h, 3h and 8h n = 8, 24h n = 7.

Pearson's correlation coefficient (df, 3M groups n = 30, 6M groups n = 29): individual MPP^+ level vs. individual GSH levels in: Striatum: 3M-35 groups, $r = +0.417$, $p < 0.02$; 3M-52 groups, $r = +0.428$, $p < 0.02$; 6M-35 groups, $r = -0.126$, $p > 0.4$.

Synaptosomes: 3M-35 groups, $r = -0.144$, $p > 0.4$; 3M-52 groups, $r = -0.217$, $p > 0.2$; 6M-35 groups, $r = -0.523$, $p < 0.005$.

Brain stem: 3M-35 groups, $r = +0.204$, $p > 0.2$; 3M-52 groups, $r = +0.407$, $p < 0.05$; 6M-35 groups, $r = -0.556$, $p < 0.002$.

and +20%, respectively), and brain stem (+10% and +21%, respectively).

MPTP treatment led to an increase in GSH levels in the striatum and brain stem of younger rats, mainly in those given the higher dose (52 mg/kg), whereas in older rats a decrease was observed both in striatum and brain stem. MPTP induced a decrement in GSH content in the striatal synaptosomes of both young rats given the higher dose and adult rats. Recovery was almost complete 8 h (younger) to 24 h (older) after treatment.

Consistently, individual GSH values were directly correlated with MPP^+ levels in the striatum of both groups of younger rats, whereas in older rats the inverse correlation ($r = -0.126$) did not reach statistical significance. Conversely, the inverse correlation between individual GSH and MPP^+ levels reached statistical significance both in synaptosomes and brain stem of older rats (Table 3).

Effects of MPTP and 2'Et-MPTP on DA and GSH Metabolism in PC12 Cells

The opposite effects of MPTP on GSH levels in young adult and adult rats prompted us to study the effects of MPTP and 2'Et-MPTP on GSH metabolism in PC12 cells. This catecholamine-containing cell line also contains GSH and thus is a useful model system in which to investigate features of the MPTP action on DA and GSH metabolism. Because PC12 cells contain relatively high levels of MAO-A and little or no MAO-B (24,43), we also used the MPTP analogue 2'Et-MPTP, which is metabolised by MAO-A to 2'Et-MPP^+ (5). Cells were exposed to 1 mM MPTP or 1 mM 2'Et-MPTP for 1–3 and 24 h. The results are given in Table 4. Exposure to both neurotoxins resulted in a time-dependent decrease in DA levels. In this regard, 2'Et-MPTP was more active than MPTP. The neurotoxins increased both GSH and GSSG levels; however, the MPTP effect occurred earlier (3 h) than 2'Et-MPTP (24 h).

AA levels. Results are given in Table 5. Both groups of saline-treated 3-month-old rats showed levels of AA higher than saline-treated older rats in striatal synaptosomes (+28% and +19%, respectively) and brain stem (+50% and +35%, respectively).

MPTP treatment led to a significant increase in AA levels in the striatum of younger rats given the higher dose (52 mg/kg); all other changes were of minor importance (Table 5).

DHAA levels. Results are given in Table 6. Older rats showed control levels of DHAA higher than younger rats in the brain stem (+41% and +24%,respectively).

MPTP increased DHAA levels in all regions of older rats; however, the increase was late in the striatum and early in synaptosomes and brain stem. DHAA levels remained unaffected in all regions of younger rats given the lower MPTP dose and in synaptosomes and brain stem of rats given the higher dose (52 mg/kg). In the striatum of these rats, DHAA levels underwent a biphasic change: decrease 1 h after and increase 3 h after MPTP.

Individual DHAA levels were inversely correlated with MPP^+ levels in the striatum of younger rats given the higher MPTP dose and older rats ($r = -0.495$ and -0.439, respectively). However, the correlation was direct in synaptosomes and brain stem of older rats ($r = +0.378$ and $+0.345$, respectively) (Table 6).

Uric acid levels. Results are given in Table 7. Baseline uric acid levels in the striatum and brain stem of adult rats were slightly lower than in younger rats.

An increase in uric acid levels was found 1, 3, and 8 h after MPTP in the striatum, striatal synaptosomes, and brain stem of all treated rats. The peak increase (two to three times the control values) occurred 1 h after MPTP administration in all regions.

Individual levels of uric acid were directly correlated with MPP^+ levels in all regions of all groups (Table 7). Moreover, individual uric acid levels were inversely correlated with NA levels in the striatum and brain stem of all groups (3M-35 groups, $r = -0.565$ and -0.715, respectively; 3M-52 groups, $r = -0.583$ and -0.864, respectively; 6M-35 groups, $r = -0.678$ and -0.658, respectively).

NA Levels

Results are given in Table 8. MPTP decreased NA levels in the striatum and brain stem of all groups; recovery was complete 24 h after treatment.

Individual levels of NA were indirectly correlated with MPP^+ levels in all groups (Table 8).

DA, DOPAC, HVA, and 3-MT levels. Results are given in Table 9. Baseline DA levels in adult rats were higher than young rats both in striatum (by about 3–11%) and synaptosomes (19–39%).

MPTP induced uneven changes that were not related to MPP^+ levels. A significant decrease was found in the striatum of all groups 3–8 h after MPTP. The recovery was complete 24 h later. Striatal DA levels in older rats were even higher than control values 24 h after MPTP.

Changes in synaptosomal DA levels were of minor importance in young rats; in older rats the levels were significantly increased even after 24 h. These findings, however, may be biased by the DA loss during synaptosomal preparation.

Baseline striatal DOPAC levels in adult rats were higher than younger rats (68–99%).

TABLE 4

LEVELS OF DA, GSH AND GSSG (nmol/mg PROTEIN) IN PC12 CELLS INCUBATED AT VARIOUS TIMES WITH MPTP OR 2'ET-MPTP

		MPTP 1mM Incubation Time (h)			2'Et-MPTP 1mM Incubation Time (h)			ANOVA	
(Controls)	——	1	3	24	1	3	24	*F*	*p*
DA	12.07 ± 1.40	6.48 ± 0.66[a]	4.89 ± 1.39[a]	2.97 ± 0.66[a]	3.78 ± 1.82[ab]	1.21 ± 0.24[ab]	0.97 ± 0.31[ab]	99.0	<0.00001
GSH	8.26 ± 1.14	8.94 ± 1.33	10.56 ± 2.15[a]	8.09 ± 2.25	6.26 ± 1.09	7.07 ± 1.01[b]	11.50 ± 3.06[ab]	9.1	<0.0001
GSSG	0.33 ± 0.10	0.32 ± 0.13	0.57 ± 0.07[a]	0.29 ± 0.09	0.43 ± 0.15	0.47 ± 0.11	0.55 ± 0.18[ab]	7.0	<0.0001

N of determinations = 8/group.
Bonferroni *t*-test: [a]$p < 0.05$ vs. controls; [b]$p < 0.05$ vs. MPTP at corresponding incubation time.

TABLE 5

LEVELS OF AA (nmol/mg PROTEIN) IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

Groups	Controls	Sacrifice After MPTP				ANOVA	
		1h	3h	8h	24h	*F*	*p*
			Striatum				
3M-35	13.35 ± 0.58	13.73 ± 1.02	13.76 ± 1.39	13.57 ± 0.99	13.73 ± 0.66	1.4	>0.2
3M-52	13.41 ± 1.49	14.77 ± 2.18*	14.05 ± 1.75*	13.34 ± 2.18	14.26 ± 2.18*	3.8	<0.02
6M-35	14.41 ± 1.39	15.46 ± 1.08	15.70 ± 1.43	15.54 ± 1.17	15.59 ± 0.69	1.7	>0.1
ANOVA *F*	3.1						
p	>0.06						
			Synaptosomes				
3M-35	7.85 ± 0.55	8.20 ± 0.75	8.60 ± 0.70	8.05 ± 0.89	7.66 ± 0.77	1.9	>0.1
3M-52	7.28 ± 1.00	7.36 ± 0.75	7.57 ± 1.08	7.08 ± 1.62	8.26 ± 1.61	1.6	>0.4
6M-35	6.14 ± 0.58[ab]	6.68 ± 0.99	7.25 ± 0.99	6.87 ± 1.00	7.17 ± 1.13	2.0	>0.1
ANOVA *F*	12.9						
p	<0.0002						
			Brain stem				
3M-35	11.78 ± 0.68	12.23 ± 0.83	12.17 ± 0.92	11.89 ± 0.88	11.87 ± 1.30	0.4	>0.8
3M-52	10.59 ± 0.72	10.71 ± 0.73	9.78 ± 0.89	9.65 ± 0.73*	10.73 ± 0.92	3.5	<0.02
6M-35	7.84 ± 0.57[ab]	7.25 ± 1.04	7.70 ± 0.98	7.47 ± 0.76	7.55 ± 0.79	0.7	>0.6
ANOVA *F*	87.3						
p	<0.00001						

*$p < 0.05$ vs. controls; [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 $n = 8$/group; 6M-35 control group $n = 10$, 1h, 3h and 8h $n = 8$, 24h $n = 7$.

Pearson's correlation coefficient (*df*, 3M groups $n = 30$, 6M groups $n = 29$): individual MPP^+ level vs. individual AA levels in: Striatum: 3M-35 groups, $r = +0.232$, $p > 0.2$; 3M-52 groups, $r = +0.341$, $p = 0.056$; 6M-35 groups, $r = +0.03$, $p > 0.8$.

Synaptosomes: 3M-35 groups, $r = +0,128$, $p > 0.4$; 3M-52 groups, $r = +0.365$, $p < 0.05$; 6M-35 groups, $r = +0.05$, $p > 0.7$.

Brain stem: 3M-35 groups, $r = +0.194$, $p > 0.2$; 3M-52 groups, $r = +0.02$, $p > 0.8$; 6M-35 groups, $r = -0.06$, $p > 0.7$.

MPTP greatly decreased DOPAC levels in all groups, both in the striatum and synaptosomes. Individual DOPAC levels were always inversely correlated with MPP^+ levels. Young rats given the lower MPTP dose showed an early trend to recovery.

MPTP also greatly decreased striatal HVA levels in all groups, mainly 3–8 h after MPTP. Young rats given the lower MPTP dose showed an early trend to recovery. Individual HVA levels did not correlate with MPP^+ levels, owing to the late decrease.

Striatal 3-MT levels showed an early and great increase (six- to eightfold the control values) in all groups. Individual 3-MT levels correlated well with MPP^+ levels (Table 9).

DOPAC + HVA/DA and DOPAC/DA ratios. Baseline DOPAC + HVA/DA ratio values in adult rats were higher than young rats (32–44%).

MPTP treatment led to a decrease in the DOPAC + HVA/DA ratio in the striatum of all groups. Younger rats given the lower MPTP dose showed an early trend to recovery; in the other groups, the ratio was still reduced 24 h after treatment.

MPTP treatment also led to a decrease in the DOPAC/DA ratio in the striatal synaptosomes of all rats. The recovery was complete 24 h after treatment.

Individual levels of MPP^+ were always inversely correlated with both DOPAC + HVA/DA and DOPAC/DA ratio values in all groups (Table 10).

DISCUSSION

The results of the present study shows that in 3- and 6-month-old rats given a single MPTP dose, acute lethality and regional brain disposition of both MPTP and MPP^+ depend upon the absolute dose of MPTP/rat, rather than upon the relative dose/kg.

Langston et al. (21) reported an age-related increase in striatal MPP^+ levels in mice (range 6–32 weeks) given single or repeated doses of MPTP/kg; in this regard, it must be recalled that the body weight of a 6-week-old mouse is considerably lower than the body weight of a 32-week-old one. Fuller et al. (15) suggested that MPP^+ formed in the brain was mainly responsible for the acute MPTP lethality in mice. The results of the present study suggest that the age-related disposition of MPP^+ in the brain needs a reassessment in terms of absolute MPTP dose/individual rather than in terms of relative dose/kg; however, following MPTP treatments, which initially led to equal striatal MPP^+ levels, the MPP^+ clearance in the striatum, striatal synaptosomes, and brain stem of adult rats was significantly lower than younger rats. This finding may be taken into account to explain the claimed greater neurodegenerative effects of MPTP in older animals (28).

TABLE 6

LEVELS OF DHAA (nmol/mg PROTEIN) IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

Groups	Controls	Sacrifice After MPTP				ANOVA	
		1h	3h	8h	24h	*F*	*p*
				Striatum			
3M-35	1.25 ± 0.41	1.54 ± 0.39	1.43 ± 0.34	1.62 ± 0.44	1.70 ± 0.28	1.7	>0.1
3M-52	1.40 ± 0.34	0.86 ± 0.20*	1.79 ± 0.30*	1.60 ± 0.48	1.50 ± 0.50	6.5	<0.001
6M-35	1.41 ± 1.23	1.49 ± 1.08	1.85 ± 1.43*	1.91 ± 1.17*	1.92 ± 0.69*	6.7	<0.001
ANOVA *F*	1.2						
p	>0.5						
				Synaptosomes			
3M-35	0.92 ± 0.22	0.94 ± 0.22	1.00 ± 0.29	1.02 ± 0.21	0.88 ± 0.22	0.4	>0.8
3M-52	1.09 ± 0.34	1.08 ± 0.27	1.17 ± 0.40	1.09 ± 0.24	1.30 ± 0.18	0.5	>0.7
6M-35	0.76 ± 0.16	1.33 ± 0.15*	1.20 ± 0.29*	1.00 ± 0.36	0.99 ± 0.11*	7.8	<0.0002
ANOVA *F*	2.5						
p	>0.1						
				Brain stem			
3M-35	1.30 ± 0.19	1.17 ± 0.45	1.43 ± 0.45	1.58 ± 0.51	1.54 ± 0.52	1.2	>0.3
3M-52	1.47 ± 0.72	1.27 ± 0.73	1.41 ± 0.89	1.69 ± 0.73	1.22 ± 0.92	1.7	>0.1
6M-35	1.83 ± 0.57[ab]	2.34 ± 1.04*	1.91 ± 0.98	1.86 ± 0.76	2.03 ± 0.79	4.2	<0.01
ANOVA *F*	11.5						
p	<0.0005						

*$p < 0.05$ vs. controls; [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 $n = 8$/group; 6M-35 control group $n = 10$, 1h and 3h $n = 8$, 24h $n = 7$.

Pearson's correlation coefficient (*df*, 3M groups $n = 30$, 6M groups $n = 29$): individual MPP^+ level vs. individual DHAA levels in: Striatum: 3M-35 groups, $r = -0.212$, $p > 0.2$; 3M-52 groups, $r = -0.495$, $p < 0.005$; 6M-35 groups, $r = -0.439$, $p < 0.02$.

Synaptosomes: 3M-35 groups, $r = +0.03$, $p > 0.8$; 3M-52 groups, $r = -0.220$, $p > 0.2$; 6M-35 groups, $r = +0.378$, $p < 0.05$.

Brain stem: 3M-35 groups, $r = -0.310$, $p > 0.08$; 3M-52 groups, $r = -0.222$, $p > 0.2$; 6M-35 groups, $r = +0.345$, $p < 0.05$.

It has been suggested that the rat is resistant to the neurotoxic effects of systemic MPTP, owing to the unusual MAO-B activity in its brain microvessels (20). However, systemic MPTP does induce DA striatal loss in those strains of rat that have MAO-B activity in brain microvessels lower than resistant (like the Sprague–Dawley) strains (27). In the present study, in which Wistar rats were used, the recovery of MPP^+ and not of MPTP in the striatal synaptosomes (which are a reliable model of neuronal terminals) demonstrates that following systemic MPTP its active metabolite, MPP^+, reaches the terminals of target neurons. The MPTP-induced changes in striatal and synaptosomal DA levels, in the present study, were biphasic and not related to the individual MPP^+ concentrations; moreover, the recovery was complete 24 h after MPTP. The MPTP-induced changes in DA metabolites (early decrease in DOPAC, later decrease in HVA, and early increase in 3-MT levels) indicate an inhibition of the intraneuronal oxidative metabolism of DA and an increase of the extracellular metabolism by COMT, well correlated with individual MPP^+ concentrations.

The fact that, in the rat, MPP^+ intranigrally administered does induce severe neuronal loss (38,39,41) and the MPP^+ derived from systemic MPTP and reaching dopaminergic terminals does not raises the question whether this event may be related to an efficient defence mechanism in dopaminergic terminals. In the latter, GSH may play a key role. GSH not only provides cells with their reducing milieu, thus protecting them against the toxic effects of reactive oxygen species (22), but also functions as an antioxidant by promoting the reduced forms of other antioxidants such as α-tocopherol (7) and AA (22). MPP^+ has greater neurotoxic effects in selenium-deficient rats, in which the GSH system is considerably impaired (40). Cleeter et al. (9) demonstrated that irreversible MPP^+-induced inhibition of mitochondrial complex I is prevented by free radical scavengers GSH and AA. In the present study, MPTP increased GSH levels in the striatum and brain stem and decreased them in synaptosomes of young rats given the higher dose. These findings are suggestive of an MPP^+-induced increase in intraneuronal synthesis, utilisation, and extracellular release of GSH. Such hypothesis is supported by the results of MPTP and 2'Et-MPTP effects on PC12 cells, in which both neurotoxins decreased DA and increased GSH and GSSG contents.

The increase in GSH activity in young rats probably spared AA from oxidation (21), because levels of DHAA did not increase. On the contrary, in older rats MPTP induced decrease in GSH levels in the striatum, in synaptosomes, and in brain stem; moreover, AA underwent oxidation, because

TABLE 7

LEVELS OF URIC ACID (pmol/mg PROTEIN) IN THE STRIATUM, STRIATAL SYNAPTOSOMES AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

		Sacrifice After MPTP				ANOVA	
Groups	Controls	1h	3h	8h	24h	*F*	*p*
			Striatum				
3M-35	30.92 ± 3.77	67.73 ± 7.31*	41.16 ± 6.15*	29.78 ± 5.33	26.97 ± 2.66	79.8	<0.00001
3M-52	32.78 ± 2.45	73.14 ± 14.53*	60.88 ± 17.49*	44.61 ± 9.09*	32.42 ± 6.90	19.6	<0.00001
6M-35	27.83 ± 4.74[b]	75.44 ± 16.01*	44.75 ± 9.04*	39.75 ± 8.46*	31.67 ± 6.15	32.0	<0.00001
ANOVA *F*	3.8						
p	<0.05						
			Synaptosomes				
3M-35	30.80 ± 2.97	69.27 ± 10.80*	35.44 ± 2.75	29.38 ± 2.81	24.42 ± 8.55	60.5	<0.00001
3M-52	27.24 ± 2.60	59.56 ± 8.90*	45.58 ± 7.61*	38.07 ± 7.65*	27.04 ± 3.17	34.2	<0.00001
6M-35	28.44 ± 5.12	74.12 ± 24.32*	57.75 ± 18.70*	41.77 ± 4.74*	36.23 ± 10.59	13.3	<0.00001
ANOVA *F*	1.8						
p	<0.1						
			Brain stem				
3M-35	33.94 ± 2.46	61.98 ± 17.18*	41.02 ± 3.25*	33.36 ± 3.28	30.01 ± 2.48	20.1	<0.00001
3M-52	31.97 ± 0.72	62.55 ± 0.73*	56.83 ± 0.89*	44.59 ± 0.73*	32.10 ± 0.92	26.5	<0.00001
6M-35	28.25 ± 6.14[a]	83.37 ± 23.14*	45.87 ± 13.31*	32.35 ± 6.49	30.84 ± 7.25	33.8	<0.00001
ANOVA *F*	4.7						
p	<0.02						

*$p < 0.05$ vs. controls; [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 $n = 8$/group; 6M-35 control group $n = 10$, 1h,3h and 8h $n = 8$, 24h $n = 7$.

Pearson's correlation coefficient (*df*, 3M groups $n = 30$, 6M groups $n = 29$): individual MPP^+ level vs. individual uric acid levels in: Striatum: 3M-35 groups, $r = +0.969$, $p > 0.00001$; 3M-52 groups, $r = +0.771$, $p < 0.00001$; 6M-35 groups, $r = +0.785$, $p < 0.00001$.

Synaptosomes: 3M-35 groups, $r = +0.922$, $p < 0.00001$; 3M-52 groups, $r = +0.509$, $p < 0.0005$; 6M-35 groups, $r = +0.641$, $p < 0.0002$.

Brain stem: 3M-35 groups, $r = +0.941$, $p < 0.00001$; 3M-52 groups, $r = +0.796$, $p < 0.00001$; 6M-35 groups, $r = +0.743$, $p < 0.00001$.

TABLE 8

LEVELS OF NA (pmol/mg PROTEIN) IN THE STRIATUM AND BRAIN STEM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

		Sacrifice After MPTP				ANOVA	
Groups	Controls	1h	3h	8h	24h	*F*	*p*
			Striatum				
3M-35	9.04 ± 0.78	6.66 ± 0.98*	8.35 ± 2.39	8.52 ± 4.06	8.92 ± 3.02	4.9	<0.005
3M-52	8.74 ± 3.4	5.73 ± 1.7*	6.88 ± 1.9*	9.47 ± 3.1	9.81 ± 2.6	5.0	<0.005
6M-35	7.78 ± 4.74	3.69 ± 16.01*	5.32 ± 9.04*	8.23 ± 8.46	8.63 ± 6.15	17.6	<0.00001
ANOVA *F*	1.8						
p	>0.1						
			Brain stem				
3M-35	41.16 ± 4.04	24.66 ± 3.98*	28.96 ± 2.39*	34.84 ± 4.06*	39.67 ± 3.02	31.0	<0.00001
3M-52	36.9 ± 3.4	17.0 ± 1.7*	20.0 ± 1.9*	27.5 ± 3.1*	33.1 ± 2.6	94.8	<0.00001
6M-35	37.72 ± 4.74	15.03 ± 16.01*	21.42 ± 9.04*	28.06 ± 8.46*	31.99 ± 6.15	30.0	<0.00001
ANOVA *F*	3.2						
p	>0.06						

*$p < 0.05$ vs. controls. 3M-35 and 3M-52 $n = 8$/group; 6M-35 control group $n = 10$, 1h,3h and 8h $n = 8$, 24h $n = 7$.

Pearson's correlation coefficient (*df*, 3M groups $n = 30$, 6M groups $n = 29$): individual MPP^+ levels vs. individual NA levels: a) in the striatum, in: 3M-35 groups, $r = -0.565$, $p < 0.001$; 3M-52 groups, $r = -0.583$, $p < 0.0005$; 6M-35 groups, $r = -0.643$, $p < 0.00005$. b) in the brain stem, in: 3M-35 groups, $r = -0.789$, $p < 0.00001$; 3M-52 groups, $r = -0.796$, $p < 0.00001$; 6M-35 groups, $r = -0.744$, $p < 0.00001$.

TABLE 9

LEVELS OF DA, DOPAC, HVA AND 3-MT (pmol/mg PROTEIN) IN THE STRIATAL SYNAPTOSOMES AND/OR STRIATUM OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

Groups	Controls	Sacrifice After MPTP 1h	3h	8h	24h	ANOVA F	ANOVA p
			Striatal DA				
3M-35	555.5 ± 31.3	511.1 ± 34.3	500.8 ± 54.3*	549.1 ± 26.4	542.1 ± 51.9	2.8	<0.05
3M-52	595.9 ± 52.1	520.0 ± 101.5	404.3 ± 53.2*	539.8 ± 68.6*	565.4 ± 54.4	9.2	<0.00005
6M-35	614.8 ± 53.8[a]	671.3 ± 52.61*	603.6 ± 63.4	536.1 ± 62.6*	709.0 ± 57.3*	10.1	<0.00002
ANOVA F	3.5						
p	<0.05						
			Synaptosomal DA				
3M-35	86.5 ± 7.7	98.4 ± 5.1*	88.0 ± 17.0	79.1 ± 7.6	84.6 ± 12.3	3.4	<0.02
3M-52	73.9 ± 8.8[a]	70.8 ± 12.1	72.1 ± 7.1	69.3 ± 9.6	75.3 ± 18.1	0.3	>0.8
6M-35	102.7 ± 14.0[ab]	171.5 ± 36.2*	155.2 ± 50.0*	165.8 ± 51.4*	134.4 ± 11.7*	5.3	<0.002
ANOVA F	15.7						
p	<0.0001						
			Striatal DOPAC				
3M-35	76.4 ± 6.7	12.0 ± 2.7*	20.9 ± 3.1*	45.5 ± 8.6*	68.4 ± 9.4	143.6	<0.00001
3M-52	90.7 ± 0.72[a]	14.4 ± 0.73*	9.3 ± 0.89*	55.3 ± 0.73*	56.2 ± 0.92*	75.3	<0.00001
6M-35	152.1 ± 21.8[ab]	20.9 ± 7.3*	19.4 ± 9.31*	27.3 ± 7.2*	129.8 ± 19.1*	159.3	<0.00001
ANOVA F	56.7						
p	<0.00001						
			Synaptosomal DOPAC				
3M-35	75.0 ± 6.7	19.2 ± 2.7*	26.3 ± 3.1*	54.7 ± 8.6*	76.2 ± 9.4	100.5	<0.00001
3M-52	86.0 ± 3.8[a]	18.6 ± 4.4*	16.3 ± 3.8*	57.0 ± 12.3*	78.7 ± 9.1	150.9	<0.00001
6M-35	89.5 ± 7.6[a]	37.4 ± 7.8*	27.2 ± 13.8*	21.2 ± 6.1*	99.7 ± 21.3	74.4	<0.00001
ANOVA F	9.7						
p	<0.001						
			Striatal HVA				
3M-35	54.9 ± 7.4	45.4 ± 8.6*	21.4 ± 3.9*	46.1 ± 6.5*	53.6 ± 6.1	32.0	<0.00001
3M-52	61.5 ± 9.3	38.5 ± 8.7*	10.2 ± 5.1*	24.9 ± 11.6*	50.9 ± 6.3*	45.3	<0.00001
6M-35	55.61 ± 8.3	32.3 ± 14.0*	7.5 ± 4.7*	5.9 ± 3.8*	55.1 ± 7.0*	72.7	<0.00001
ANOVA F	1.7						
p	>0.1						
			Striatal 3-MT				
3M-35	4.07 ± 0.74	23.40 ± 2.7*	3.02 ± 3.1*	2.86 ± 8.6*	2.34 ± 9.4*	128.5	<0.00001
3M-52	5.51 ± 2.13	37.30 ± 10.25*	10.34 ± 5.33*	11.44 ± 5.26*	7.26 ± 2.87	38.4	<0.00001
6M-35	3.39 ± 2.72[b]	28.72 ± 7.45*	6.23 ± 2.67*	4.95 ± 1.43	9.94 ± 2.45*	58.4	<0.00001
ANOVA F	3.8						
p	<0.05						

*$p < 0.05$ vs. controls; [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 n = 8/group; 6M-35 control group n = 10, 1h,3h and 8h n = 8, 24h n = 7.

Pearson's correlation coefficient (degree of freedom, 3M groups n = 30, 6M groups n = 29): individual MPP^+ levels vs.: a) striatal DA: 3M-35 groups, $r = -0.303$, $p > 0.09$; 3M-52 groups, $r = -0.04$, $p > 0.7$; 6M-35 groups, $r = +0.06$, $p > 0.7$; b) synaptosomal DA: 3M-35 groups, $r = +0.510$, $p < 0.005$; 3M-52 groups, $r = +0.140$, $p > 0.4$; 6M-35 groups, $r = +0.298$, $p > 0.1$; c) striatal DOPAC: 3M-35 groups, $r = -0.816$, $p > 0.00001$; 3M-52 groups, $r = -0.716$, $p < 0.00001$; 6M-35 groups, $r = -0.695$, $p < 0.00001$; d) synaptosomal DOPAC: 3M-35 groups, $r = -0.716$, $p < 0.00001$; 3M-52 groups, $r = -0.815$, $p < 0.00001$; 6M-35 groups, $r = -0.562$, $p < 0.00001$; e) striatal HVA: 3M-35 groups, $r = -0.03$, $p > 0.8$; 3M-52 groups, $r = -0.02$, $p > 0.8$; 6M-35 groups, $r = -0.259$ $p < 0.1$; f) striatal 3-MT: 3M-35 groups, $r = +0.882$, $p < 0.00001$; 3M-52 groups, $r = +0.898$, $p < <0.00001$; 6M-35 groups, $r = +0.682$, $p < 0.00001$.

TABLE 10

DOPAC + HVA/DA RATIO IN THE STRIATUM AND DOPAC/DA RATIO IN STRIATAL SYNAPTOSOMES OF 3-MONTH-OLD RATS GIVEN MPTP 35 mg/kg (3M-35) OR 52 mg/kg (3M-52) AND 6-MONTH-OLD RATS GIVEN MPTP 35 mg/kg IP (6M-35)

Groups	Controls	Sacrifice After MPTP				ANOVA	
		1h	3h	8h	24h	*F*	*p*
			Striatum				
3M-35	0.236 ± 0.020	0.113 ± 0.025*	0.085 ± 0.014*	0.158 ± 0.025*	0.228 ± 0.025	73.6	<0.00001
3M-52	0.256 ± 0.034	0.099 ± 0.010*	0.044 ± 0.013*	0.141 ± 0.038*	0.200 ± 0.032*	70.6	<0.00001
6M-35	0.339 ± 0.036[ab]	0.044 ± 0.018*	0.079 ± 0.025*	0.062 ± 0.020*	0.279 ± 0.021*	233.1	<0.00001
ANOVA *F*	27.6						
p	<0.00001						
			Synaptosomes				
3M-35	0.891 ± 0.139	0.194 ± 0.049*	0.295 ± 0.104*	0.701 ± 0.195*	0.907 ± 0.172	44.7	<0.00001
3M-52	1.180 ± 0.170	0.249 ± 0.046*	0.227 ± 0.050*	0.799 ± 0.157*	1.051 ± 0.156	95.9	<0.00001
6M-35	0.862 ± 2.004	0.227 ± 2.80*	0.179 ± 2.69*	0.129 ± 3.14*	0.744 ± 3.27	87.7	<0.00001
ANOVA *F*	11.2						
p	<0.0005						

*$p < 0.05$ vs. controls; [a]$p < 0.05$ vs. 3M-35 group; [b]$p < 0.05$ vs. 3M-52 group. 3M-35 and 3M-52 $n = 8$/group; 6M-35 control group $n = 10$, 1h, 3h and 8h $n = 8$, 24h $n = 7$.

Pearson's correlation coefficient (*df*, 3M groups $n = 30$, 6M groups $n = 29$): individual MPP^+ levels vs.: a) striatal DOPAC + HVA/DA ratio: 3M-35 groups, $r = -0.615$, $p < 0.0005$; 3M-52 groups, $r = -0.487$, $p < 0.005$; 6M-35 groups, $r = -0.633$, $p < 0.00002$; b) synaptosomal DOPAC/DA ratio: 3M-35 groups, $r = -0.700$, $p < 0.00001$; 3M-52 groups, $r = -0.830$, $p < 0.00001$; 6M-35 groups, $r = -0.602$, $p < 0.0005$.

DHAA levels increased in all these brain regions. These data may suggest an age-related impairment of the antioxidant GSH system, already shown in aged (20-month-old) rats (11). In a previous study [in young adult vs. aged rats (13)] on the response of the neuronal antioxidant system to manganese, a well-know neurotoxin that impairs DA metabolism in mammals (33), we obtained similar results: increase in striatal and decrease in synaptosomal GSH levels in young rats, decrease in striatal and synaptosomal GSH levels in aged rats, in which manganese showed a greater neurotoxicity. The fact that GSH may play a key role also in Mn neurotoxicity is supported by our results (unpublished observations) about manganese effects on monoamine and GSH metabolism in PC12 cells. In this neuronal cell model, Mn exposure decreased DA and NA and increased GSH and GSSG contents. The manganese-induced monoamine depletion was potentiated by L-buthionine-(*S,R*)-sulfoximine, an inhibitor (21) of GSH synthesis.

Brain AA concentrations are kept constant by an efficient homeostatic mechanism (35). Also, according to Martenson and Meister (22), an important in vivo function of GSH is to maintain tissue AA, which may have reducing functions that are not efficiently performed by GSH. In addition, one of the uric acid scavenging activities is to maintain AA in its reduced form in biological fluids (34). In the present study, MPTP administration did not reduce striatal levels of AA; in young rats given the higher MPTP dose, the AA levels were even increased. Endogenous ascorbic acid may protect from MPTP neurotoxicity with a mechanism independent from its scavenging activity (i.e., by limiting MPP^+ uptake by dopaminergic terminals). Indeed, Debler et al. (10) found that ascorbic acid inhibits MPP^+ uptake by striatal synaptosomes in vitro, the IC_{50} being 0.1 mM, considerably lower than the concentrations (0.2–0.4 mM) of striatal extracellular ascorbic acid found in vivo in the rat (6). The experimental design of the present study was not suitable for a direct experimental evidence supporting such additional mechanism (i.e., an inverse correlation between striatal levels of ascorbic acid and MPP^+ levels in striatal synaptosomes in homolateral striata).

The great MPTP-induced increase in uric acid levels in all brain regions deserves attention. Uric acid levels correlated well with MPP^+ concentrations in all experimental groups and in all brain regions. Also, individual uric acid levels were inversely correlated with NA levels in the striatum and brain stem of all rats. It has been shown that MPP^+ causes loss of ATP in the mouse brain synaptosomes (31) and striatum (8). It is well known that the catabolism of ATP leads to xanthine and hypoxanthine, both of which are metabolised by xanthine dehydrogenase or, as suggested by Adams and Odunze (1), also by xanthine oxidase. The products of xanthine oxidase include uric acid and superoxide radical anion. Thus, if xanthine and hypoxanthine are metabolised by xanthine oxidase, the superoxide radical anion could participate to the MPP^+-induced oxidative stress. Indeed, we demonstrated (23) that allopurinol, a well-known inhibitor of xanthine oxidase, decreased baseline levels of uric acid and hypoxanthine in the striatum and in the brain stem of 3-month-old rats; moreover, allopurinol fully antagonised the MPTP-induced increase in uric acid levels and decrease in xanthine, hypoxanthine, and NA levels in these brain regions. Such findings demonstrate that the claimed oxidative stress mediated by xanthine oxidase (11,12) is involved at least in the MPP^+-induced NA depletion in the striatum and brain stem.

In conclusion, in 3- and 6-month-old rats acute lethality and regional brain MPTP and MPP^+ levels depend upon the absolute dose of MPTP/rat; MPP^+ levels correlated well with the changes in levels of DA metabolites, NA, and uric acid. In young rats the neuronal antioxidant GSH system is more

efficient than in adult rats, in which the response to MPP^+-induced oxidative stress also involves AA oxidation. The increase in uric acid levels provides further evidence for a mechanism of MPTP neurotoxicity involving oxidative stress mediated by xanthine oxidase.

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