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Further Investigation of Allopurinol Effects on MPTP-Induced Oxidative Stress in the Striatum and Brain Stem of the Rat

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DESOLE, M. S., G. ESPOSITO, L. FRESU, R. MIGHELI, S. SIRCANA, R. DELOGU, M. MIELE AND E. MIELE. *Further investigation of allopurinol effects on MPTP-induced oxidative stress in the striatum and brain stem of the rat.* PHARMACOL BIOCHEM BEHAV 54(2) 377-383, 1996.—Levels of uric acid, xanthine, hypoxanthine, ascorbic acid (AA), dehydroascorbic acid (DHAA), glutathione (GSH), noradrenaline (NA), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium ion (MPP^+) were determined in the striatum and/or in the brain stem of 3-month-old male Wistar rats given allopurinol (300 mg/kg day by gavage) for 3 days before a single MPTP 35 mg/kg dose IP. Allopurinol alone decreased uric acid and increased xanthine levels both in the striatum and in the brain stem; moreover, allopurinol decreased striatal DOPAC + HVA/DA ratio and increased 5-HIAA/5HT ratio in the brainstem. Allopurinol affected neither regional MPTP nor MPP^+ disposition. Allopurinol potentiated the MPTP-induced decrease in the DOPAC + HVA/DA ratio and increase in striatal AA oxidation; in addition, allopurinol antagonised the MPTP-induced: (i) increase in uric acid levels; (ii) decrease in NA levels in both regions, in DA levels, and in the 5-HIAA/5-HT ratio in the brain stem: (iii) increase in AA oxidation in the brain stem. In conclusion, the MPP^+-induced oxidative stress mediated by xanthine oxidase seems to be involved in DA depletion in the brainstem and in NA depletion in both regions; moreover, striatal uric acid may have an active role in the neuronal antioxidant pool.

Allopurinol MPTP Oxidative stress Striatum Brainstem Rat

OXIDATIVE stress has been hinted as a critical feature in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced damage of the nigro-striatal dopaminergic system (1,17). Indeed, the inhibition of mitochondrial function by 1-methyl-4-phenylpyridinium ion (MPP^+), the MPTP four-electron oxidation product, has a number of consequences, one of which is increased release of reactive oxygen species (ROS) from mitochondria, another is depletion of ATP (26). It has been shown that MPP^+ causes loss of ATP in the mouse brain synaptosomes (27); moreover, Chan et al. (7), have demonstrated that the regional brain ATP loss in the mouse is rapid, dose dependent, and selective for the striatum and the ventral mesencephalon. According to Bates et al. (3), the MPP^+-induced ATP depletion and the consequent energy depletion initiates the neuronal damage, which is further increased by ROS formation. The MPP^+-induced ROS formation following its injection in the rat striatum or substantia nigra has been demonstrated in vivo by Wu et al. (34).

The catabolism of ATP leads to inosine and then to hypoxanthine and xanthine, both of which are substrates for xanthine oxidase (4). The products of xanthine oxidase include uric acid and ROS (1,4,25).

MPTP impairs also the functioning of the noradrenergic (14,15,33) and 5-hydroxytryptaminergic (8,16) systems.

In previous papers (10,11,13) we have shown that MPTP increased uric acid and decreased noradrenaline (NA) levels in the striatum and brainstem of the rat. Individual levels of both uric acid and NA well correlated with individual MPP^+ levels, both in the striatum and in the brainstem; moreover, uric acid levels were indirectly correlated with NA levels. Because

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allopurinol, a well-known inhibitor of xanthine oxidase, antagonised both the MPTP-induced increase in uric acid levels and NA depletion (23), we concluded that an oxidative stress mediated by xanthine oxidase was involved at least in the MPTP-induced impairment of the functioning of the noradrenergic system.

The present study was, thus, undertaken to further assess the effects of allopurinol on MPTP-induced increase in purine catabolism and the role of uric acid as active component of the neuronal antioxidant pool in the striatum and in the brainstem of the rat.

METHODS

Animals and Drug Treatment

All experiments were carried out on 3-month-old male Wistar rats (Morini), 270–330 g b.wt., maintained, under standard animal care conditions, on a 12-h day/night cycle and given food and water ad lib. Groups of 8 rats were given allopurinol (Sigma, Aldrich srl, Milan, Italy) (suspended in gum arabic 10%) by gavage (150 mg/10 ml x 2)/kg/per day for 3 days before MPTP administration. The last half of the allopurinol dose was given 1 h before MPTP administration in the 4th day. MPTP (HCl, Sigma) was dissolved in distilled water and injected IP at the dose of 35 mg/2 ml per kg (13). Controls and the MPTP groups (n = 8/groups) were also given gum arabic 10% by gavage.

All studies were carried out in accordance with the Decreto n° 116/1992 of the Italian Ministry of the Public Health (Directive 86/609/EEC).

Biochemical Aassays

Uric acid, xanthine, hypoxanthine, ascorbic acid (AA), dehydroascorbic acid (DHAA), NA, dopamine (DA), dihydroxy-phenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), MPTP and MPP^+ determinations were performed by HPLC according to the method previously described (13,23), and reduced glutathione (GSH) and glutathione disulphide (GSSG) were determined according to the enzymatic recycling method of Anderson (2). 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 (22).

Rats were killed by decapitation 1 h and 8 h after MPTP injection. Heads were cooled by rapid immersion in liquid nitrogen; thereafter, striata of both side and brainstem (including the substantia nigra) were rapidly removed and frozen at −40°C; thereafter, striata of the right side and brainstem were weighed and homogenised in 1% meta-H3PO4 containing EDTA 1 mM. After centrifugation (17,500 g for 10 min at 4°C), the supernatant was divided into four aliquots.

The first was filtered and immediately injected into the HPLC system for DOPAC, HVA, DA, NA, 5-HT, 5-HIAA, uric acid, and AA determinations. Liquid chromatography with electrochemical detection was done with a high-pressure pump Varian 9001 with a Rheodyne injector, column 15 cm × 46 mm i.d. TSK-ODS-80 TM, electrochemical detector BAS LC-4B and integrator Spectra-Physics SP 4290. The mobile phase was citric acid 0.1M, K2HPO4 0.1M, EDTA 1 mM, MeOH 5% and sodium octylsulphate 70 mg/l (pH = 3.0); the flow rate was 1.2 ml/min and 10 μl of sample was injected.

The second aliquot was adjusted to pH 7.0 with K2HPO4 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 metaH3PO4 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, SpA, Segrate, Italy 9001 with a Rheodyne injector), column (15 cm × 46 mm i.d. TSK-ODS-80 TM) and similar precolumn, UV detector (Star 9050 Varian, SpA, Segrate, Italy) (MPTP 245 nm, MPP^+ 295 nm) and integrator (Spectra-Physics, Spectra-Physics SpA, Milan, Italy SP 4290). The mobile phase was composed of H2S04 0.1M, triethylamine 0.075M, and acetonitrile 10% at pH 2.30; the flow was 1.5ml/min. MPTP-3-ol (260 nm) was the internal standard.

On the fourth aliquot, xanthine and hypoxanthine determinations were performed directly by injecting 50 μl of the filtered supernatant using the above high-pressure pump, column ODS-80 TM and similar precolumn, UV detector (254 nm), and integrator. The mobile phase was composed of two eluants: K2HP04-Me-OH (97 : 3) K2HP04-Me-OH (70 : 30). A 38-min linear gradient was employed; the flow rate was 1.2 ml/min.

Statistical Analysis

All values were expressed in nmol or pmol/mg protein and given as mean ± standard deviation (SD).

Biochemical data were analysed with the Kruskal–Wallis analysis by rank; following significant H values, post hoc comparisons were made using the Student–Newman–Keuls test.

RESULTS

Effects of Allopurinol

Allopurinol decreased uric acid levels in the striatum (−42%) and in the brain stem (−40%) compared to vehicle-treated rats (controls). Xanthine levels were increased in the striatum (+143%) and in the brain stem (+91%). Hypoxanthine levels were unchanged in both regions (Table 1).

Allopurinol slightly decreased striatal GSH and AA levels, but did not increase DHAA levels (Table 2 and 3). Allopurinol affected DA levels neither in the striatum nor in the brain stem, but lowered striatal DOPAC levels with a consequent decrease (−26%) in the DOPAC + HVA/DA ratio (Tables 4 and 5).

Striatal NA levels were increased by 30%, while NA levels in the brain stem were in the range of the control values (Table 5).

Allopurinol affected neither striatal 5-HT nor 5-HIAA levels, but increased 5-HIAA levels in the brain stem with a consequent increase (+19%) in the 5-HIAA/5-HT ratio (Table 6).

Allopurinol did not significantly modify MPTP and MPP^+ levels detected 1 h and 8 h after MPTP injection both in the striatum and in the brain stem (Table 7).

Effects of MPTP

MPTP did not affect hypoxanthine levels in the striatum and in the brain stem 1 h after its administration, but decreased them 8 h after (−27% and −40%, respectively), compared to controls. Levels of xanthine were increased 1 h after MPTP by 48% in the striatum and by 18% in the brain stem and, 8 h later, the levels were in the range of the control values in both regions. MPTP increased uric acid levels in the

TABLE 1

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN LEVELS OF XANTHINE, HYPOXANTHINE AND URIC ACID (pmol/mg PROTEIN) IN THE STRIATUM (STR) AND IN THE BRAINSTEM (BST) OF THE RAT

	Hypoxanthine		Xanthine		Uric acid	
Treatment	STR	BST	STR	BST	STR	BST
Vehicle	729.0 ± 122.4	1620.2 ± 166.9	281.2 ± 21.3	141.3 ± 29.6	28.01 ± 2.96	26.19 ± 3.56
Allopurinol	771.1 ± 76.2	1629.0 ± 381.4	682.5 ± 68.4*	269.7 ± 94.0*	15.05 ± 4.43*	14.44 ± 3.29*
MPTP 1 h	728.1 ± 216.2	1502.3 ± 204.3	416.5 ± 107.9*	167.1 ± 23.3	67.28 ± 10.51*	49.08 ± 13.34*
MPTP 1 h + allopurinol	720.8 ± 78.9	1558.8 ± 127.8	448.4 ± 59.3*	161.0 ± 17.9	18.38 ± 1.42*†	20.09 ± 6.69†
MPTP 8 h	534.5 ± 78.9*	970.2 ± 157.3*	267.5 ± 71.4	106.2 ± 12.5	35.45 ± 9.27*	33.91 ± 11.23*
MPTP 8 h + allopurinol	525.0 ± 50.3*	988.0 ± 54.0*	453.0 ± 42.7*‡	168.7 ± 16.7	16.32 ± 3.62*‡	18.96 ± 3.87*‡
Kruskal-Wallis (H)	24.6	32.0	37.9	32.8	39.9	33.1
(P)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. $n = 8$/group.

Student Newman-Keuls test, $p < 0.05$; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

TABLE 2

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN AA, DHAA AND GSH LEVELS (nmol/mg PROTEIN) AND IN THE DHAA/AA RATIO IN THE STRIATUM OF THE RAT

Treatment	AA	DHAA	DHAA/AA	GSH
Vehicle	12.96 ± 0.95	1.32 ± 0.17	0.103 ± 0.019	15.77 ± 0.54
Allopurinol	11.86 ± 0.53	1.31 ± 0.26	0.111 ± 0.021	14.30 ± 0.81*
MPTP 1 h	13.04 ± 1.15	1.92 ± 0.34*	0.141 ± 0.036	14.79 ± 1.69
MPTP 1 h + allopurinol	12.00 ± 1.65	1.71 ± 0.36	0.142 ± 0.024*	12.81 ± 1.32*†
MPTP 8 h	12.42 ± 1.10	1.95 ± 0.50*	0.163 ± 0.048*	13.21 ± 0.83*
MPTP 8 h + allopurinol	11.05 ± 0.36*	2.08 ± 0.42*	0.199 ± 0.030*	12.48 ± 1.07*
Kruskal-Wallis (H)	13.7	22.8	26.4	26.2
(P)	<0.02	<0.0001	<0.0001	<0.0001

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. $n = 8$/group.

Student Newman-Keuls test, $p < 0.05$; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

TABLE 3

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN AA, DHAA AND GSH LEVELS (nmol/mg PROTEIN) IN THE DHAA/AA RATIO IN THE BRAIN STEM OF THE RAT

Treatment	AA	DHAA	DHAA/AA	GSH
Vehicle	10.10 ± 0.39	1.48 ± 0.23	0.141 ± 0.024	10.77 ± 1.21
Allopurinol	10.11 ± 0.50	1.54 ± 0.34	0.153 ± 0.041	10.32 ± 1.07
MPTP 1 h	10.15 ± 0.68	2.04 ± 0.34*	0.202 ± 0.059*	8.90 ± 0.62*
MPTP 1 h + allopurinol	10.90 ± 0.95	2.03 ± 0.51*	0.191 ± 0.060*	9.28 ± 0.55*
MPTP 8 h	10.43 ± 0.73	1.94 ± 0.49*	0.187 ± 0.048*	9.33 ± 0.79*
MPTP 8 h + allopurinol	10.94 ± 0.37*	1.21 ± 0.26‡	0.110 ± 0.024‡	10.20 ± 1.49
Kruskal-Wallis (H)	13.0	21.1	19.4	16.5
(P)	<0.05	<0.0001	<0.005	<0.01

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. $n = 8$/group.

Student Newman-Keuls test, $p < 0.05$; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

TABLE 4

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN LEVELS OF DA, DOPAC AND HVA (pmol/mg PROTEIN) AND IN THE DOPAC + HVA/DA RATIO IN THE STRIATUM OF THE RAT

Treatment	DA	DOPAC	HVA	DOPAC + HVA/DA ratio
Vehicle	465.4 ± 49.6	87.91 ± 10.88	39.09 ± 6.48	0.274 ± 0.029
Allopurinol	503.6 ± 36.4	64.29 ± 12.76*	36.11 ± 8.03	0.204 ± 0.037*
MPTP 1 h	520.0 ± 77.6	16.65 ± 4.40*	44.93 ± 9.03	0.120 ± 0.013*
MPTP 1 h + allopurinol	531.5 ± 42.1	18.22 ± 6.52*	25.70 ± 5.52*†	0.080 ± 0.015*†
MPTP 8 h	460.1 ± 71.0	43.83 ± 12.39*	24.00 ± 6.22*	0.147 ± 0.030*
MPTP 8 h + allopurinol	430.9 ± 48.0	23.82 ± 6.06*‡	20.51 ± 3.38*	0.103 ± 0.017*‡
Kruskal-Wallis (H)	16.2	40.1	34.2	41.3
(P)	<0.01	<0.0001	<0.0001	<0.0001

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. n = 8/group.

Student Newman-Keuls test, $p <$ <0.05; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

striatum (+140% after 1 h and +27% after 8 h) and in the brain stem (+87% and +29%, respectively) (Table 1).

In the striatum (Table 2), MPTP did not affect AA levels, but increased DHAA levels (+45% after 1 h and +48% after 8 h), compared to controls; the increase in the DHAA/AA ratio reached statistical significance only 8 h after MPTP; also, the decrease in GSH levels (−19%) reached statistical significance 8 h after MPTP.

In the brainstem (Table 3), MPTP did not modify AA levels, compared to controls; the increase in DHAA levels and in the DHAA/AA ratio reached statistical significance only 1 h after MPTP. GSH levels were significantly reduced both 1 h (−17%) and 8 h (−13%) after MPTP.

MPTP did not affect striatal DA levels (Table 4), but DA levels in the brain stem were decreased by 35% 1 h and 74% 8 h after MPTP, compared to controls (Table 5). Striatal DOPAC levels were greatly decreased 1 h (−81%) and 8 h (−50%) after MPTP administration. HVA levels showed a late decrease (−39%). Consequently, the striatal DOPAC + HVA/DA ratio was greatly decreased at both time points (Table 4).

MPTP decreased NA levels in both regions at both time points (decrease range 32–48%) (Table 5).

MPTP affected neither striatal 5-HT nor 5-HIAA levels. In the brain stem, 5-HT levels were increased by 24% and 5-HIAA levels lowered by 19%, with a consequent decrease in the 5-HIAA/5-HT ratio (−35%), 1 h after MPTP. The changes in 5-HT and 5-HIAA levels were at the borderline of statistical significance 8 h after MPTP, but the 5-HIAA/5-HT ratio was still significantly reduced (−18%) (Table 6).

Effects of Allopurinol on MPTP-induced Changes

Allopurinol-pretreated rats, 1 h after MPTP administration. Hypoxanthine levels were in the range of the control values both in the striatum and in the brain stem. Striatal xanthine levels were increased by 59% compared to controls, but did not differ from levels in the corresponding MPTP-treated group. Xanthine levels in the brain stem were in the range of controls. Striatal uric acid levels were lower than either controls (−34%) or the corresponding MPTP-treated

TABLE 5

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN NA AND DA LEVELS IN THE STRIATUM (STR) AND/OR IN THE BRAINSTEM (BST) OF THE RAT

	NA		DA
Treatment	STR	BST	BST
Vehicle	8.38 ± 1.09	29.17 ± 3.12	5.73 ± 1.70
Allopurinol	10.93 ± 2.44*	26.29 ± 2.65	5.84 ± 1.23
MPTP 1 h	4.39 ± 0.86	15.33 ± 2.12*	3.75 ± 0.77*
MPTP 1 h + allopurinol	6.06 ± 1.51†	22.72 ± 3.54†	5.75 ± 1.21†
MPTP 8 h	5.29 ± 1.27*	19.88 ± 5.10*	1.47 ± 0.52*
MPTP 8 h + allopurinol	8.75 ± 3.09‡	23.52 ± 5.00	3.96 ± 1.56‡
Kruskal-Wallis (H)	33.7	29.6	30.1
(P)	<0.0001	<0.0001	<0.0001

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. n = 8/group.

Student Newman-Keuls test, $p < 0.05$; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

TABLE 6

EFFECTS OF ALLOPURINOL ON MPTP-INDUCED CHANGES IN LEVELS OF 5-HT AND 5-HIAA (pmol/mg PROTEIN) AND IN THE 5-HIAA/5-HT RATIO IN THE STRIATUM (STR) AND IN THE BRAIN STEM (BST) OF THE RAT

	5-HT		5-HIAA		5-HIAA/5-HT ratio	
Treatment	STR	BST	STR	BST	STR	BST
Vehicle	25.90 ± 3.33	33.44 ± 3.29	20.39 ± 2.19	25.13 ± 2.32	0.796 ± 0.117	0.754 ± 0.085
Allopurinol	27.28 ± 5.55	35.98 ± 5.10	20.24 ± 2.94	31.62 ± 3.86*	0.757 ± 0.120	0.898 ± 0.060*
MPTP 1 h	24.94 ± 6.07	41.41 ± 8.23*	22.69 ± 4.07	20.25 ± 4.12*	0.952 ± 0.272	0.488 ± 0.129*
MPTP 1 h + allopurinol	24.98 ± 7.95	44.91 ± 7.37*	22.96 ± 4.11	21.52 ± 17.9	0.977 ± 0.271	0.483 ± 0.082*
MPTP 8 h	30.83 ± 8.18	38.72 ± 6.213	22.12 ± 4.91	23.37 ± 4.19	0.737 ± 0.139	0.618 ± 0.163*
MPTP 8 h + allopurinol	28.93 ± 4.89	46.30 ± 6.91*	22.77 ± 2.56	36.91 ± 2.05*‡	0.798 ± 0.103	0.797 ± 0.149‡
Kruskal-Wallis (H)	5.4	32.0	5.1	32.5	6.9	32.0
(P)	<0.4	<0.0001	>0.4	<0.0001	<0.05	<0.0001

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. Controls and the MPTP groups were given allopurinol vehicle by gavage for 3 days. n = 8/group.

Student Newman-Keuls test, $p < 0.05$; * vs. vehicle; † vs. MPTP 1 h group; ‡ vs. MPTP 8 h group.

group (−73%). In the brainstem, the level of decrease reached statistical significance only vs. the corresponding MPTP group (−59%) (Table 1).

Striatal AA levels were slightly decreased; also, the increase in DHAA levels (+29%) was at the borderline of statistical significance, compared to controls. However, the increase in the DHAA/AA ratio (+39%) reached statistical significance (Table 2). In the brainstem, AA levels were in the range of control values, and DHAA levels and the DHAA/AA ratio increased by +37% and +35%, respectively (Table 3).

Striatal GSH levels were reduced by 19% compared to controls and 13% compared to the corresponding MPTP-treated group (Table 2). In the brainstem, GSH levels were decreased by 14%, compared to controls (Table 3).

Striatal DA levels were slightly increased (+14%) at the borderline of statistical significance, and DOPAC levels were reduced by 79%, compared to controls. HVA levels were reduced compared either to controls (−34%) or the corresponding MPTP group (−43%). Consistently, the DOPAC + HVA/DA ratio was reduced compared either to controls (−71%) or the corresponding MPTP group (−33%) (Table 4). In the brainstem, DA levels were in the range of controls and higher (+53%) than the corresponding MPTP group (Table 5).

Na levels were in the range of controls, both in the striatum and in the brainstem, and higher than the corresponding MPTP group (+38% and +44%, respectively) (Table 5).

Striatal 5-HT and 5-HIAA levels were in the range of controls. In the brainstem, allopurinol pretreatment did not affect MPTP-induced changes in 5-HT and 5-HIAA levels.

Allopurinol-pretreated rats, 8 h after MPTP administration. Hypoxanthine levels were lower than in controls both in the striatum (−28%) and in the brainstem (−39%), but did not differ from the levels in the corresponding MPTP-treated group. Striatal xanthine levels were higher than either controls (+61%) or the corresponding MPTP group (+70%). Xanthine levels in the brainstem were in the range of the control values; the increase vs. the corresponding MPTP group (+59%) was at the borderline of statistical significance. Uric acid levels were lower than either controls or the corresponding MPTP group (range decrease 28–54%) (Table 1).

Striatal AA levels were decreased by 15%, compared to controls; the decrease (−11%) vs. the corresponding MPTP-treated group was at the borderline of statistical significance.

TABLE 7

MPTP AND MPP^+ LEVELS (pmol/mg PROTEIN) IN THE STRIATUM AND IN THE BRAINSTEM OF RATS GIVEN MPTP ALONE OR ASSOCIATED WITH ALLOPURINOL

	1 h		8 h	
Treatment	MPTP	MPP^+	MPTP	MPP^+
MPTP				
Striatum	81.3 ± 29.2	637.4 ± 161.9	ND	48.0 ± 25.8
Brainstem	63.2 ± 34.8	407.0 ± 222.8	ND	57.4 ± 25.7
MPTP + allopurinol				
Striatum	115.8 ± 38.7	730.6 ± 112.7	ND	100.5 ± 52.7
Brainstem	68.2 ± 52.2	501.2 ± 143.6	ND	41.4 ± 17.4

MPTP single 35 mg/kg dose was given IP; rats were killed 1 h and 8 h later. Allopurinol (300 mg/kg/day) was given by gavage for 3 days before MPTP. The last allopurinol dose was given 1 h before MPTP injection. n = 8/group. ND, not detectable.

DHAA levels and the DHAA/AA ratio increased by 29% and 93%, respectively, compared to controls. In the brainstem, AA levels were increased by 8%, compared to controls; DHAA levels were slightly decreased (−18%) compared to controls, and significantly decreased (−38%) compared to the corresponding MPTP group.

Striatal GSH levels were reduced by 21%, compared to controls. In the brainstem, GSH levels were in the range of controls.

Striatal DA levels were in the range of controls, and DOPAC levels were reduced compared either to controls (−13%) or to the corresponding MPTP group (−46%). HVA levels were reduced compared to controls (−34%). Consistently, the DOPAC + HVA/DA ratio was reduced compared either to controls (−62%) or the corresponding MPTP group (−30%) (Table 4). In the brainstem, DA levels were in the range of controls and higher (+65%) than the corresponding MPTP group (Table 5).

Na levels were in the range of controls, both in the striatum and in the brainstem, and higher than the corresponding MPTP group (+65% and +18%, respectively) (Table 5).

Striatal 5-HT and 5-HIAA levels were in the range of controls. In the brainstem, 5-HT levels were higher than controls (+38%) and 5-HIAA levels were higher than both controls (+47%) and the MPTP group (+58%). Consequently, the 5-HIAA/5-HT ratio was higher (+29%) than the ratio in the MPTP group.

DISCUSSION

The results of the present study show that allopurinol inhibited xanthine oxidation to uric acid, with a consequent decrease in uric acid levels and increase in xanthine levels, both in the striatum and in the brainstem. Hypoxanthine levels were unchanged in both regions. The lack of increase in hypoxanthine levels may be explained by the the fact that allopurinol increases the conversion of hypoxanthine to inosinic acid (6,18) and inhibits also the rate of de novo purine biosynthesis (35). Such findings confirm that xanthine and hypoxanthine are metabolised by xanthine oxidase (1,23). Allopurinol decreased striatal DA oxidative metabolism. Such an effect seems to be specific for the striatal dopaminergic system, because allopurinol did not affect striatal 5-HT oxidative metabolism and even increased it in the brainstem. The allopurinol-induced decrease in DA oxidative metabolism has been suggested as one of the protective mechanisms against manganese-induced oxidative stress in the rat striatum (12).

MPTP increased hypoxanthine and xanthine catabolism in the striatum and in the brainstem, with a consequent increase in uric acid levels in these brain regions. Because the products of the xanthine oxidase action on hypoxanthine and xanthine include ROS (4,25), a question arises about the relevance of the claimed (10,11,13) oxidative stress mediated by xanthine oxidase in the MPTP-induced neurochemical changes. In the present study, MPTP inhibited striatal DA oxidative metabolism, decreased NA levels both in the striatum and in the brainstem, and decreased DA levels and 5-HT turnover in the brainstem. The response of the neuronal antioxidant system (increase in AA oxidation, decrease in GSH levels) was consistent with an increase in ROS formation.

Allopurinol potentiated the MPTP-induced inhibition of striatal DA oxidative metabolism, but antagonised the MPTP-induced decrease in NA levels in both regions, and in DA levels and 5-HT turnover in the brainstem. Also, allopurinol attenuated the response of the neuronal antioxidant pool in the brainstem.

These findings, taken together with the allopurinol-induced antagonism of uric acid production, not only confirm the hypothesis (23) that the MPTP-induced oxidative stress mediated by xanthine oxidase is involved in the decrease in NA levels in the striatum and in the brainstem, but also demonstrate that such oxidative stress is involved in the DA decrease in the brainstem. Moreover, in this brain region, uric acid does not seem to play an active role as free radical scavenger (4). On the contrary, in the striatum uric acid does seem to play a role in the neuronal antioxidant pool.

According to Sevanian et al. (28), one of the uric acid scavenging activities is to maintain AA in its reduced form in biological fluids. Church and Ward (9) showed a postmortem decrease in uric acid levels in the substantia nigra and in the caudate of human parkinsonism patients, and AA levels did not differ from controls in either brain region. In addition, according to Martenson and Meister (22), an important in vivo function of GSH is to maintain tissue AA, which may have reductive functions that are not efficiently performed by GSH. Indeed, L-buthionine-(S,R)-sulfoximine, an inhibitor of GSH synthesis, greatly decreased AA and increased DHAA levels in the brain of the newborn rat (22). AA is the main ROS scavenger in the extracellular compartment (24). AA is not synthesized in the mammalian brain; it is supplied to the brain by active uptake at the choroid plexus site (30), by a carrier-mediated saturable process and by simple diffusion at the blood-brain barrier site (19). Brain AA concentrations are kept constant by an efficient homeostatic mechanism (29). AA is claimed to protect against kainate (20) and NMDA-receptor mediated neurotoxicity (21); the latter seems to play a key role in MPTP-induced neuronal damage. Indeed, it has been shown that NMDA-receptor antagonists protect against MPTP-induced neurotoxicity (31,32). Bates et al. (3) recently suggested that the MPP^+-induced ATP depletion and the consequent energy depletion initiates the neuronal damage, which is further increased by ROS formation. In turn, ROS are known to release excitatory aminoacids (25), whose excitotoxicity is exacerbated by collapse of the ion gradient consequent to ATP depletion (5). Therefore, the functioning of the neuronal antioxidant system seems to play a critical role in the mechanisms underlying MPTP neurotoxicity. In the present study, MPTP increased striatal levels of DHAA but did not significantly decrease AA levels. In allopurinol-pretreated rats, however, AA levels were significantly reduced 8 h after MPTP administration, with a consequent significant increase in the DHAA/AA ratio. Also, GSH levels were further decreased. These findings suggest that striatal uric acid may be not a mere end-product of ATP catabolism, but may have a physiological role as active component of the neuronal antioxidant pool (AA, GSH, vitamin E) because uric acid, like GSH (22), keeps AA in its reduced form (28). In addition, it has been shown that uric acid decreases the rate of caudate DA oxidation (9).

In conclusion, the allopurinol effects on MPTP-induced changes in uric acid production, NA levels. and levels of DA in the brainstem, suggest that the MPP^+-induced oxidative stress mediated by xanthine oxidase may be involved at least in the depletion of NA (in the striatum and in the brainstem) and DA (in the brainstem). The increase in striatal DHAA/AA ratio, following the block of uric acid production by allopurinol, suggests that uric acid is an active component of the neuronal antioxidant pool and its scavenging activity contributes to maintain striatal AA in its reduced form.

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