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The Effects of 1-Methyl-4-phenylpyridinium Ion (MPP^+) on the Efflux and Metabolism of Endogenous Dopamine in Rat Striatal Slices

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Abstract—1-Methyl-4-phenylpyridinium ion (MPP^+) was shown to accumulate concentration-dependently in slices from rat striatum. At 10 μM, MPP^+, the tissue concentration was found to be 118 ± 9 μM following 75 min of incubation. The accumulation of MPP^+ was reduced in the presence of 10 μM of the selective dopamine uptake inhibitor GBR 12909 (−50%) or by destruction of the dopaminergic terminals by complete hemisection of the forebrain 4 days before the experiments (−75%). Accumulation of MPP^+ in the catecholamine-poor occipital cortex and cerebellum was only 25% of that obtained in striatum. Reserpine pretreatment of the rats in-vivo did not modify the accumulation of MPP^+ in the striatal slices. MPP^+ (1–10 μM) increased the net efflux of dopamine and reduced the efflux of the dopamine metabolite DOPAC from the striatal slices. The effect on dopamine was readily diminished if MPP^+, after a 15 min incubation, was then omitted from the medium. In contrast, the DOPAC efflux was reduced for 75 min even though MPP^+ was present in the incubation medium only for the first 15 min. In the presence of the monoamine oxidase inhibitor, pargyline (350 μM), MPP^+ also produced an increase in dopamine efflux. In normal medium, the presence of the dopamine uptake inhibitor GBR 12909 (10 μM), or the absence of calcium, failed to modify the MPP^+-induced increase in dopamine efflux. MPP^+ also increased dopamine efflux from slices from reserpinized rats. In normal medium, MPP^+ (10 μM) was much more effective than GBR 12909 (10 μM) in increasing dopamine efflux, whereas in the presence of pargyline both drugs were equally effective. It is suggested that, in rat striatal slices, MPP^+ is selectively accumulated within the dopaminergic nerve terminals by means of a carrier mediated transport, sensitive to GBR 12909. It is concluded that MPP^+ increases dopamine efflux largely by inhibiting monoamine oxidase and by inhibiting dopamine uptake. These data are discussed in relation to the previously observed action of MPTP on dopamine metabolism in mouse brain in-vivo.

Although the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is well documented, both in man and in experimental animals, the mechanisms underlying the neurotoxic effects are not yet understood (reviewed by Irwin 1986; Snyder & D'Amato 1986). In humans intravenous self-administration of MPTP produced apparently irreversible symptoms of parkinsonism (Davis et al 1979; Langston et al 1983), accompanied by histological and biochemical changes in the nigrostriatal system which were indistinguishable from those seen in idiopathic Parkinson's disease (Davis et al 1979). A similar destruction of nigrostriatal dopamine neurons has been reported in animals including monkey (Burns et al 1983) and mouse (Hallman et al 1984; Heikkila et al 1984a). There is controversy surrounding the toxicity of MPTP in the rat and several authors have failed to show destruction of the striatal dopamine neurons in-vivo (e.g. Chiueh et al 1984). However, Steranka et al (1983), using higher doses of the drug administered by osmotic minipumps over 24 h, obtained a pronounced depletion of striatal dopamine indicating destruction of dopaminergic cells. Similar results have been obtained by Jarvis & Wagner (1985). Thus, the discrepancy between the rat and the mouse is probably quantitative rather than qualitative. In agreement with this, Mytelineou & Cohen (1984) observed MPTP-produced damage of dopamine neurons in explants of rat embryo mesencephalon.

Oxidative metabolism of MPTP to 1-methyl-4-phenyl pyridinium ion (MPP^+), by the action of monoamine oxidase appears to be of critical importance for the neurotoxic effects (Chiba et al 1984; Heikkila et al 1984b). MPP^+ accumulates selectively in dopamine-rich brain regions (Irwin & Langston 1985) and this accumulation is most likely due to specific transport of MPP^+ by the dopamine uptake carrier (Javitch & Snyder 1985). Pretreatment with inhibitors of dopamine uptake can prevent the cytotoxic effect of MPTP in mouse striatum (Pileblad et al 1985; Sundström & Jonsson 1985), suggesting that these effects depend upon the active accumulation of MPP^+ into the neuron.

The most striking acute biochemical change following MPTP administration is a profound rapid release of dopamine in mouse brain occurring 1 h after injection (Pileblad et al 1984, 1985). An acute increase in dopamine release has also been shown to occur in-vivo in rat striatum (Rollema et al 1986). Furthermore, MPTP- and MPP^+-produced dopamine release has been observed in-vitro in rat striatal preparations (Schmidt et al 1984; Markstein & Lahaye 1985; Chang & Ramirez 1986). In an attempt to gain further insight into the acute actions of the neurotoxin on dopamine metabolism, the effects of MPP^+—the toxic metabolite of MPTP—on the release and metabolism of dopamine were studied in rat striatal slices in-vitro.

Materials and Methods

Male Sprague-Dawley rats (ALAB, Stockholm, Sweden), 200–300 g, were caged in groups of four, and maintained

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under standard conditions for at least 7 days before use (temperature 26°C, humidity 60–65%; free access to food and tap water; 14 h/10 h light/dark cycle, lights on at 0500 h). They were killed by cervical dislocation and decapitated between 0900 and 1300 h.

Hemisection

In some experiments, a complete hemisection of the rat forebrain was made 4 days before the in-vitro experiments, in order to damage the dopaminergic terminals in the striatum (Andén et al 1972). The cut was made through the caudal hypothalamus by a blunt-edged spatula under pentobarbitone anaesthesia (50 mg kg^{-1} i.p.) (Hassler & Bak 1969). The extent of the section was checked before the experiments.

The drugs used were: 1-methyl-4-phenylpyridinium (MPP^+) iodide (Research Biochemical Inc., Wayland, MA, USA), reserpine (Ciba-Geigy, Basle, Switzerland) and 1-(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)piperazine dihydrochloride (GBR 12909, Gist-Brocades, Harleem, The Netherlands). Reserpine was dissolved in a few drops of glacial acetic acid, and made up to volume with 5·5% glucose. Reserpine was administered by intraperitoneal injection (5 mg kg^{-1}, 18 h before death), with control animals receiving equivalent volumes of the vehicle.

The efflux rates for endogenous dopamine and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) from rat striatal slices were determined in an in-vitro incubation model, essentially as previously described (Snape & Holman 1985). The composition of the normal incubation medium was as follows (mM): NaCl 118·1, KCl 4·7, $CaCl_2$ 2·0, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25·0; D-glucose 10·0, pH 7·4, saturated with 95% O_2: 5% CO_2. L-ascorbic acid (0·1 mM) was included as an antioxidant (Shaskan & Snyder 1970), and L-tyrosine (0·05 mM) was included to protect endogenous stores of the catecholamine precursor (Delanoy et al 1982). Also, in some experiments pargyline (350 μM) was included. In some experiments, NaCl was substituted for $CaCl_2$. When MPP^+ iodide was included in test incubations, an equivalent concentration of KI was included in the control incubations.

Pooled slices of rat striatum (0·5 × 0·5 × 3·0 mm) were prepared using a manual tissue slicer of the type described by Bennett et al (1983). The slices (about 40 mg tissue per incubation tube) were subjected to 15 min of pre-incubation in 600 μL of normal medium, followed by a series of 15 min incubation periods in 600 μL of medium containing various drugs (see the individual Figure legends). At the end of each incubation, the medium was collected for analysis of dopamine and DOPAC. In some experiments, the post-incubation tissue slices were homogenized in chilled 0·2 M perchloric acid (Branson b-30 Cell Disruptor), and residual tissue dopamine and DOPAC were also quantified. In other experiments, the post-incubation tissue slices were quickly washed with 3·0 mL of chilled normal incubation medium, and then stored at −70°C for the subsequent analysis of tissue MPP^+ content.

Dopamine and DOPAC were isolated from the incubation media, or from the tissue extracts, by alumina extraction (Anton & Sayre 1962; Keller et al 1976), using α-methyl-DOPA as internal standard, and were quantified by high performance liquid chromatography with electrochemical detection. The chromatographic system consisted of a Waters M-45 high pressure pump, a Rheodyne 7010 injector, a Waters Guard-PAK precolumn (15·0 cm × 0·45 cm i.d.) and a stainless steel column packed with Nucleosil (5 μM) RP-18 (Macherey-Nagel). The composition of the mobile phase was as follows: citric acid (0·032 M), K_2HPO_4 $3H_2O$ (0·018 M), EDTA (20 mgL^{-1}), sodium octyl hydrogen-sulphate (75 mgL^{-1}), methanol (6–8%, v/v), pH 2·74, flow rate 1·5 mL min^{-1}. Amperometric detection was effected at a TL-5A thin layer cell, incorporating a glassy carbon working electrode and coupled to an LC-3 electrochemical controller (Bioanalytical Systems Inc). The detector potential was maintained at +0·75 V, relative to the Ag/AgCl reference electrode.

The concentrations of MPP^+ in the tissue slices were determined by gas chromatography/mass spectrometry (GC/MS) according to Irwin & Langston (1985). 1-Ethyl-4-phenylpyridinium ion (EPP^+) was used as internal standard. EPP^+ was prepared by reacting 4-phenylpyridine, dissolved in methylene chloride, with an excess of ethyl iodide at room temperature overnight. Purity of EPP^+ was verified by GC/MS. Calibration curves were obtained by adding known amounts of MPP^+ and EPP^+ to homogenates of brain tissue.

Analyses were performed on a HP 5700A GC interface with a HP 5970 Mass Selective Detector and equipped with a fused silica column (12 m, 0·22 mm i.d.) coated with crosslinked SE-54 (film thickness 0·3 μM, He carrier gas 1·1 mL min^{-1}). Oven temperature was set at 160°C and linearly programmed at 8°C per min up to 190°C. Injections were made in the split mode with split ratio 10:1.

Statistical evaluations were performed by Kruskal-Wallis one-way analysis of variance followed by Mann-Whitney U test.

Results

The concentrations of MPP^+ in rat striatal slices following 75 min of incubation with MPP^+ (5 successive 15 min periods) are shown in Table 1. In the range tested, the accumulation of MPP^+ in striatal tissue slices was clearly dependent upon the concentration of MPP^+ in the medium. Incubation with 10 μm MPP^+ resulted in a concentration of 118 μM (20·0 μg g^{-1}) MPP^+ in the striatal slices, whereas incubation with 1 and 5 μM of MPP^+ gave 17 and 62% of that value, respectively. Hemisection of the forebrain four days before the experiments, which is known to cause degeneration of the striatal dopaminergic terminals (Andén et al 1972), reduced the accumulation of MPP^+ by 75%. Furthermore, the dopamine uptake inhibitor GBR 12909 (10 μM; van der Zee et al 1980; Heikkila & Manzino 1984) reduced the MPP^+ accumulation by approximately 50%. GBR 12909 did not, however, further reduce the accumulation of MPP^+ in hemisected animals. Pretreatment of the rats with reserpine (5 mg kg^{-1} i.p.; 18 h) failed to modify the accumulation of MPP^+.

MPP^+ evoked a large increase in the net efflux of dopamine from rat striatal slices (Fig. 1a), together with a profound decrease in DOPAC efflux (Fig. 1b). Both effects were sustained throughout the 4 h incubation. The maximal MPP^+-induced increase in dopamine net efflux occurred between 15 and 30 min; i.e. 2·145 ± 0·566 μg $g^{-1} \times h$ vs 0·067 ± 0·016 in the controls (Fig. 1a). In the latter 2 h of

Table 1. Accumulation of MPP^+ in rat brain tissue: Effects of GBR 12909, reserpine and hemisection. Slices of striatum, cerebellum and occipital cortex were incubated with MPP^+ (1, 5 or 10 μM) for 75 min. The incubation medium was renewed every 15 min. Reserpine (5 mg kg^{-1}, i.p.) was administered in-vivo 18 h before the experiments, whereas GBR 12909 was added to the incubation medium. The hemisections (see Materials and Methods) were performed 4 days before the experiments. Data are means ± s.e.m. in per cent of the MPP^+ concentrations in the "Striatum; MPP^+ (10 μM) group"; mean of the pooled MPP^+ values in this group was: 20·0 ± 1·5 μg g^{-1} wet tissue or 118 ± 9·0 μM.

	Concn (μM)	Concn MPP^+ in tissue slices (%)	n
Striatum	MPP^+ 1	17 ± 3**	4
	MPP^+ 5	62 ± 3**	4
	MPP^+ 10	100 ± 5	22
	MPP^+ 10 + GBR 12909 10	54 ± 5**	8
Striatum (hemisection)	MPP^+ 10	28 ± 2**[a]	8
	MPP^+ 10 + GBR 12909 10	25 ± 3**[b]	4
Striatum (reserpine)	MPP^+ 10	107 ± 7	8
Cerebellum	MPP^+ 10	22 ± 3**	3
Occipital cortex	MPP^+ 10	30 ± 6**	3

**$P < 0.002$ vs controls; [a]$P = 0.014$ vs non-hemisected MPP^+ + GBR 12909; [b]$P = 0.048$ vs non-hemisected MPP^+ + GBR 12909.

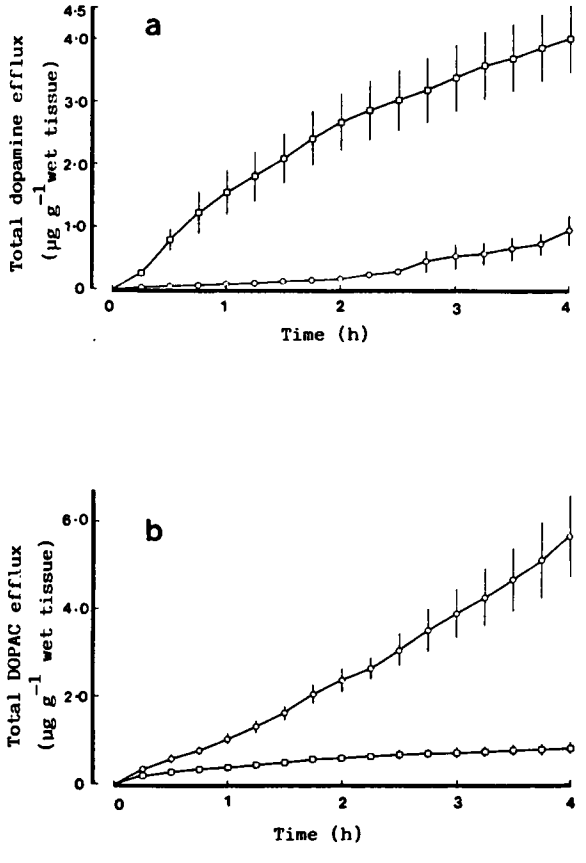

FIG. 1. The effects of MPP^+ on the net efflux of dopamine and DOPAC from rat striatal slices in-vitro. Dopamine (a) and DOPAC (b) efflux were determined in successive 15 min periods, as described in Materials and Methods, and the cumulative efflux at each time point was calculated. Values are means ± s.e.m. (in $\mu g\ g^{-1}$ wet tissue) for groups of 4 individual incubations, including either 10 μM MPP^+ iodide (□) or 10 μM KI (○), throughout incubation. Cumulative values of dopamine and DOPAC at 4 h in the MPP^+-incubated group were significantly different from controls; $P < 0.028$.

incubation, however, the efflux of dopamine from the control slices increased and became more variable, presumably reflecting a decline in tissue viability. At the end of the 4 h of incubation, the residual dopamine content was 7·1 ± 1·1 $\mu g\ g^{-1}$ in the controls and 1·2 ± 0·03 $\mu g\ g^{-1}$ in the MPP^+ group, while the tissue DOPAC contents were 0·25 ± 0·072 and 0·01 ± 0·001 $\mu g\ g^{-1}$, respectively (n = 4).

Fig. 2 shows the concentration-dependent increase in net efflux of dopamine following incubation of rat striatal slices with MPP^+ (1–10 μM) for 75 min. A slight but significant effect was seen at 1 μM MPP^+. Following incubation with 10 μM of MPP^+, the net efflux of dopamine was 9 times higher than in the control incubation. The efflux of DOPAC during 75 min of incubation was: Controls = 427 ± 57 ng g^{-1} wet tissue; 1 μM MPP^+ = 198 ± 16 ng g^{-1}; 5 μM = 290 ± 17 ng g^{-1}; 10 μM = 314 ± 29 ng g^{-1}. Thus, all concentrations of MPP^+ significantly ($P < 0.028$) reduced DOPAC efflux, the lowest concentration (1 μM) appeared to be most effective and DOPAC efflux at this concentration was significantly ($P < 0.028$) lower than after 5 and 10 μM of MPP^+.

In the presence of pargyline (350 μM), MPP^+ also concentration-dependently increased the net efflux of dopamine from rat striatal slices during 30 min of incubation (Fig. 3). At 1 μM, MPP^+ was ineffective, whereas at 10 and 20 μM, MPP^+ increased dopamine by 550 and 775%, respectively. The efflux of DOPAC during 30 min of incubation was: Controls = 55 ± 2 ng g^{-1} wet tissue; 1 μM MPP^+ = 40 ± 8 ng g^{-1} ($P = 0.028$); 10 μM = 67 ± 6 ng g^{-1}; 20 μM = 76 ± 4 ng g^{-1} ($P = 0.028$); i.e. 1 μM MPP^+ slightly decreased the DOPAC efflux, whereas 20 μM caused a small increase.

The MPP^+-induced increase in the net efflux of dopamine was not sustained throughout 75 min of incubation when striatal slices were exposed to the drug for only one 15 min incubation period (Fig. 4a). The decrease in DOPAC efflux induced by MPP^+ was, however, sustained for 75 min following an initial 15 min exposure to the drug (Fig. 4b).

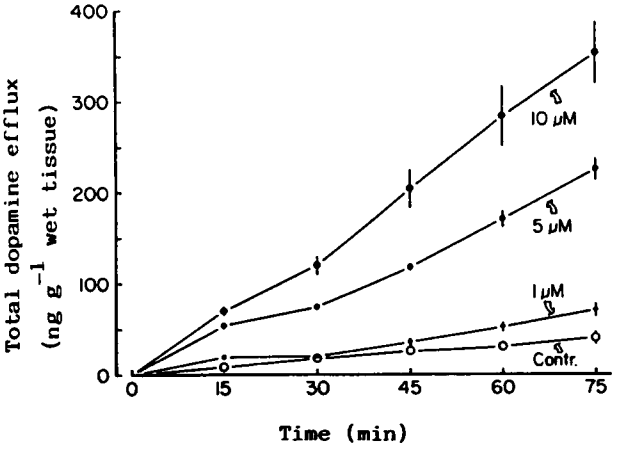

FIG. 2. Effects of various concentrations of MPP^+ on the net efflux of dopamine from rat striatal slices in-vitro. Dopamine release was determined in successive 15 min incubation periods, as described in Materials and Methods, and the cumulative value to each time point was calculated. Tissue slices were incubated continually in normal medium containing either 1, 5 or 10 μM MPP^+ iodide or 10 μM KI. Values are means ± s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations. Cumulative values of dopamine at 75 min in all MPP^+-incubated groups were significantly different from controls; $P < 0.028$.

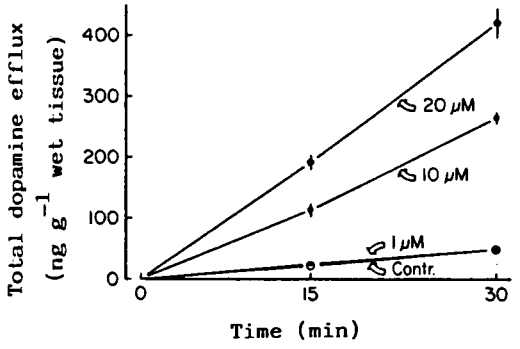

FIG. 3. Effects of various concentrations of MPP+ on the net efflux of dopamine from rat striatal slices in the presence of pargyline (350 μM). Dopamine efflux was determined in successive 15 min incubation periods, as described in Materials and Methods, and the cumulative value to each time point was calculated. Tissue slices were incubated continually in pargyline medium containing either 1, 10 or 20 μM MPP+ iodide or 20 μM KI. Values are means ± s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations. Cumulative values of dopamine after 30 min of incubation with MPP+, 10 or 20 μM, were significantly different relative to controls ($P = 0.028$).

Reserpine, administered in-vivo 18 h before the experiments, slightly reduced the spontaneous efflux of dopamine and increased the efflux of DOPAC (Table 2); these differences were, however, insignificant. In the presence of MPP+, dopamine efflux after reserpine pretreatment was 50% of that after vehicle pretreatment. However, in percentage of the baseline efflux in the two groups, MPP+-induced approximately the same increase in dopamine in vehicle-treated and reserpinized animals: 390 versus 340%, respectively. Reserpine did not affect the MPP+-induced reduction in DOPAC efflux (Table 2). Reserpine alone produced a 95% reduction in striatal dopamine at death (588 ± 86 ng g^{-1} vs 11 528 ± 354 μg g^{-1} in vehicle-injected controls).

Incubation of striatal slices in calcium-free medium failed to modify the net efflux of endogenous dopamine (Control = 168 ± 40 ng g^{-1} wet tissue/75 min, Ca-free = 97 ± 30) or DOPAC (Control = 1310 ± 126, Ca-free = 1280 ± 135). Neither did omission of calcium affect the changes induced by 10 μm MPP+ (Dopamine: MPP+ = 864 ± 86 ng g^{-1} wet tissue/75 min, MPP+ in Ca-free medium = 859 ± 114; DOPAC: MPP+ = 468 ± 48, MPP+ in Ca-free medium = 486 ± 62). Calcium-free incubation, under similar conditions in an independent experiment, abolished the increase in dopamine release evoked by 40 mM KCl during 90 sec of incubation, however (703 ± 26 ng g^{-1} reduced to 22 ± 6 ng g^{-1}).

FIG. 4. The time-dependent effects of a single 15 min exposure to MPP+ on dopamine and DOPAC efflux from rat striatal slices in vitro. Dopamine (a) and DOPAC (b) efflux were determined in successive 15 min incubation periods, as described in Materials and Methods, and the cumulative value to each time point was calculated. Tissue slices were incubated continually in normal medium containing 10 μM KI (○) or 10 μM MPP+ iodide (□), or for 15 min only in normal medium containing 10 μM MPP+ iodide, followed by continual incubation in normal medium containing 10 μM KI (△). Values are means ± s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations. Statistical evaluations were performed on cumulative values of dopamine and DOPAC at 75 min: All MPP+ groups were different from controls ($P = 0.028$); in addition the dopamine values in the "continuous MPP+ group" were significantly different from the "single MPP+ exposure group" ($P = 0.028$).

GBR 12909 (10 μM) alone produced a 270% increase in net dopamine efflux over the 75 min incubation, whereas MPP+ (10 μM) increased dopamine efflux by 900% (Fig. 5a). When the slices were incubated with MPP+ in combination with GBR 12909 the net efflux of dopamine was not significantly different from that obtained with MPP+ alone. GBR 12909 did not significantly influence the MPP+-induced reduction in DOPAC efflux (Fig. 5b).

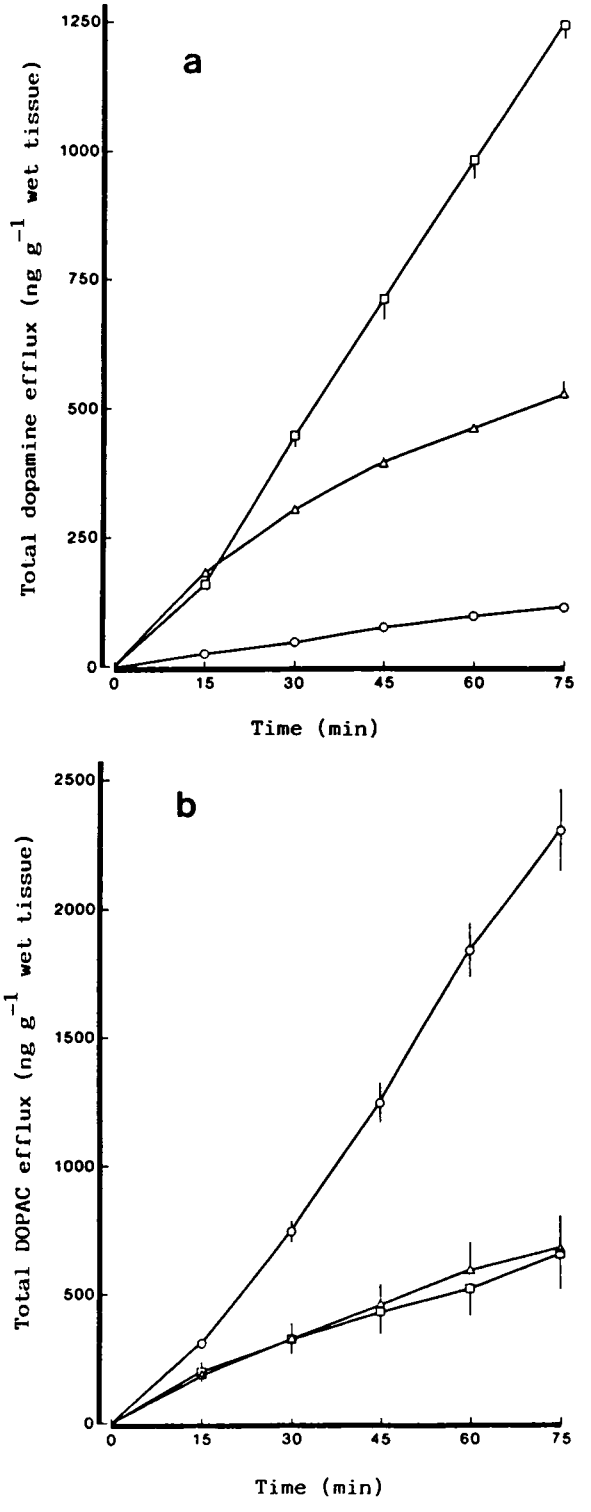

Table 2. The effects of reserpine pretreatment on the MPP^+-induced changes in endogenous dopamine and DOPAC efflux from rat striatal slices. Efflux was determined in two successive 15 min periods, as described in Materials and Methods, and the cumulative values after 30 min was calculated. Tissue slices from animals pretreated with vehicle or reserpine (5 mg kg^{-1} i.p., 18 h before death) were incubated in the continual presence of either 10 μM MPP^+ or 10 μM KI. Cumulative values are means $\pm$ s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations.

	Efflux during 30 min (ng g^{-1} wet tissue)	
	Dopamine	DOPAC
Controls	21 ± 3	254 ± 43
Reserpine	13 ± 5	378 ± 21
MPP^+	90 ± 16**	185 ± 29
Reserpine + MPP^+	45 ± 3**[a,b]	140 ± 7**[a]

**$P = 0.028$ vs controls; [a]$P = 0.028$ vs reserpine; [b]$P = 0.028$ vs MPP^+

Fig. 6 shows the results from a basically identical experiment to the latter, except that pargyline (350 μM) was present in the incubation medium. In this case, both MPP^+ (10 μM) and GBR 12909 (10 μM) produced 450% increases in the net efflux of dopamine. The two drugs in combination appeared to induce a larger net efflux than either drug alone (significant compared to GBR 12909). In this experiment, there were no differences in DOPAC efflux between the groups (mean value $= 70 \pm 8$ ng g^{-1} wet tissue/30 min; $n = 16$).

Discussion

Incubation of rat striatal slices with MPP^+ resulted in a large accumulation of the drug within the tissue. After 75 min, the striatal concentration was more than 10-fold that in the incubation medium. The striatal uptake was much reduced in the presence of the selective dopamine re-uptake inhibitor GBR 12909 and to a greater extent after destruction of the striatal dopaminergic terminals by complete brain hemisection before the in-vitro incubation. Furthermore, the accumulation of MPP^+ in the catecholamine-poor occipital cortex and cerebellum was much lower than in the striatum, the uptake in the former two regions being the same range as that in the striatum after hemisection. It thus seems likely that MPP^+ to a great extent accumulated within the dopaminergic nerve terminals.

Reserpine pretreatment did not affect the accumulation of MPP^+ in the striatal slices. Whether MPP^+ is taken up into the storage vesicles by a reserpine-insensitive mechanism or if MPP^+ is not, to any great extent, accumulated within the vesicles remains to be established.

MPP^+ induced a profound increase in the net efflux of endogenous dopamine from striatal tissue slices (cf. Chang & Ramirez 1986). It also reduced the efflux of DOPAC, probably by inhibiting MAO (Kinemuchi et al 1985; Pileblad et al 1985). The latter effect probably contributes to the increase in dopamine efflux, but is unlikely to be the only

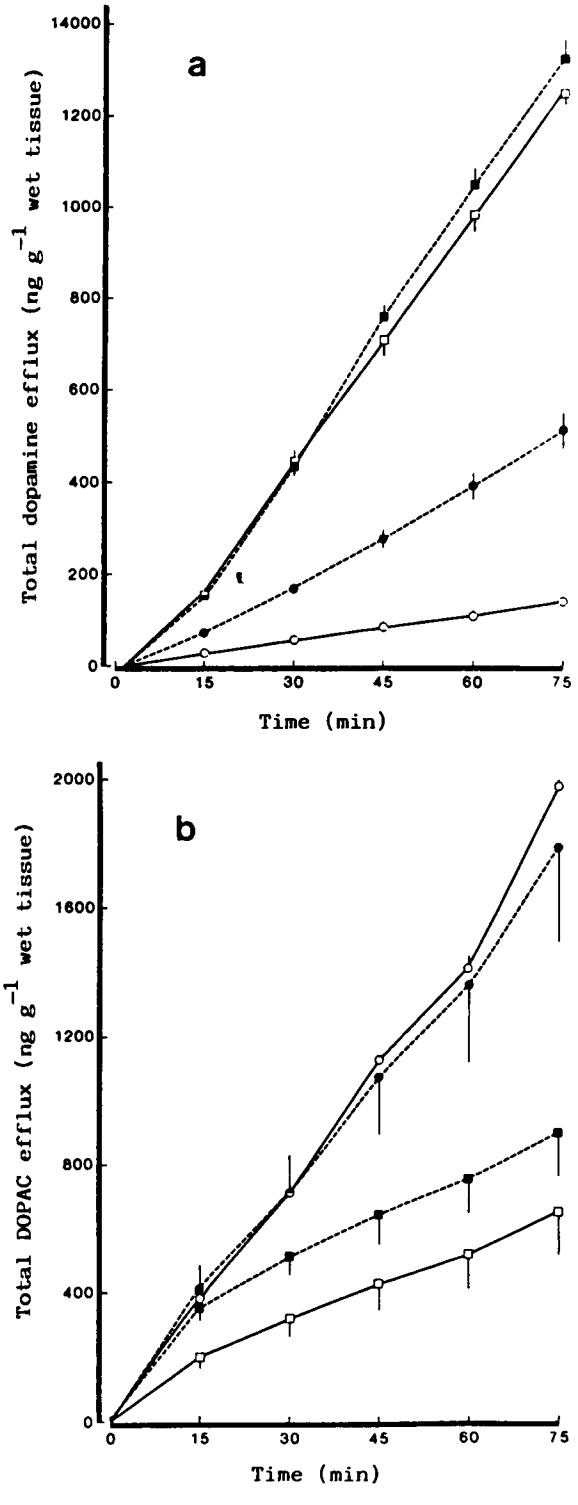

FIG. 5. The effects of GBR 12909, MPP^+, or both in combination, on the efflux of dopamine and DOPAC from rat striatal slices. Dopamine (a) and DOPAC (b) efflux were determined in successive 15 min incubation periods, as described in Materials and Methods, and the cumulative value to each time point was calculated. All 4 groups were subjected to 30 min of pre-incubation in normal medium, with GBR 12909 present where appropriate. Tissue slices were then incubated continually in normal medium containing 10 μM KI (○), 10 μM MPP^+ iodide (□), 10 μM KI plus 10 μM GBR 12909 (●), or 10 μM MPP^+ iodide plus 10 μM GBR 12909 (■). Values are means $\pm$ s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations. Statistics were performed on the cumulative values at 75 min: Dopamine: All "treated" groups are significantly different relative to controls ($P = 0.028$); "GBR group" vs "MPP^+ group" ($P = 0.028$); "GBR group" vs "GBR + MPP^+ group" ($P = 0.028$). DOPAC: All MPP^+ groups significantly different relative to controls ($P = 0.028$).

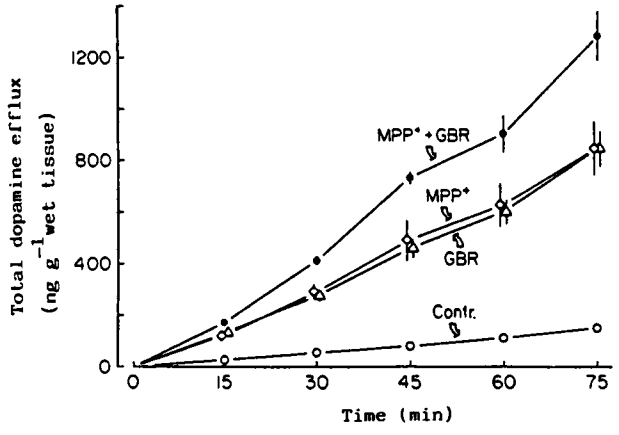

FIG. 6. The effects of GBR 12909, MPP+, or both in combination, on the net efflux of dopamine from rat striatal slices in the presence of pargyline (350 μM). Dopamine efflux was determined in successive 15 min incubation periods, as described in Materials and Methods, and the cumulative value to each time point was calculated. All 4 groups were subjected to 30 min of pre-incubation in normal medium, with GBR 12909 present where appropriate. Tissue slices were then incubated continually in normal medium containing 10 μM KI, 10 μM MPP+ iodide, 10 μM KI plus 10 μM GBR 12909, or 10 μM MPP+ iodide plus 10 μM GBR 12909. Values are means $\pm$ s.e.m. (in ng g^{-1} wet tissue) for groups of 4 individual incubations. Statistics were performed on the cumulative values at 30 min: All "treated" groups are significantly different relative to controls ($P=0.028$); "GBR group" vs "GBR+MPP+ group" ($P=0.028$).

mechanism involved since MPP+ also produced a concentration-dependent increase in dopamine efflux in the presence of the MAO inhibitor pargyline, without further reducing DOPAC efflux. Furthermore, the effect of MPP+ on dopamine efflux was immediately diminished when the drug was omitted from the incubation medium, whereas DOPAC efflux continued to be reduced for a considerable time after MPP+ had been omitted.

The MPP+-induced release of dopamine was clearly concentration-dependent in the range tested. However, although GBR 12909 reduced the MPP+ accumulation in the slices by ca 50%, it failed to modify the MPP+-induced increase in dopamine efflux in the non-pargyline preparation. Thus, it seems unlikely that MPP+ induced an increase in the net efflux of dopamine by an action within the dopaminergic terminals. Moreover, GBR 12909 alone produced an increase in the next efflux of dopamine but had no effect on the efflux of DOPAC. Compared with the observed increment in dopamine efflux following incubation with MPP+ the effect of GBR 12909 was small. However, when pargyline was present in the incubation medium the increase in dopamine efflux produced by GBR 12909 and MPP+ was in the same range. These data may suggest that the comparably larger effect of MPP+ on dopamine efflux observed after equal concentrations of the two drugs in the non-pargyline preparation could be due to the fact that MPP+ inhibits MAO whereas GBR 12909 does not. Furthermore, the data may also suggest that the inhibition of dopamine uptake could be one of the mechanisms by which MPP+ evokes an increase in the net efflux of dopamine. That MPP+ also increased dopamine efflux from slices from reserpine-pretreated rats is also in accord with such an assumption.

In summary, MPP+ appears to be selectively accumulated in dopamine nerve terminals in rat striatal tissue slices and to induce a net efflux of dopamine from them. The mechanisms behind the efflux are suggested to include MAO inhibition together with inhibition of dopamine uptake. These effects of MPP+ may well contribute to the increase in striatal dopamine release observed in-vivo following the administration of MPTP to mice (Pileblad et al 1985). However, it seems most unlikely that MAO inhibition and uptake inhibition are factors of major importance for the effect of MPTP on dopamine release in mouse brain. Thus, the magnitude of dopamine release is much greater than would be expected from the combination of MAO inhibition and of dopamine uptake inhibition alone. In fact, dopamine release in mouse brain following administration of the uptake inhibitor GBR 13098 (an analogue of GBR 12909) in combination with pargyline is definitely not in the same range as that after MPTP administration (Pileblad & Carlsson, unpublished observation). Furthermore, the time course of dopamine release in mouse brain in-vivo following MPTP administration (Pileblad et al 1985) does not resemble that of dopamine release in rat striatum in-vitro. In the present study, MPP+ induced an almost constant efflux of dopamine during the 4 h test period, whereas in mouse brain in-vivo a dramatic increase in dopamine release occurred for 15 min at 1 h after the administration of MPTP (Pileblad et al 1985). In addition, in contrast to the present results, GBR 13098, in a dose that prevented the neurotoxic events of MPTP in the mouse (Pileblad & Carlsson 1985), markedly reduced the MPTP-induced acute increase in mouse striatal dopamine release (Pileblad & Carlsson 1988). These data may suggest that, in contrast to the present observations in rat striatum in-vitro, the increase in dopamine release in mouse brain in-vivo is related to the uptake of MPP+ by the dopaminergic nerve terminals and may also suggest that this dopamine release is somehow related to the neurotoxic events underlying the destruction of the neurons. Thus, it appears that in mouse brain in-vivo, MPTP releases dopamine by a mechanism different from that by which MPP+ produces an increase in the net efflux of dopamine from rat striatal slices in-vitro.

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