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Differences in dopamine efflux induced by MPP^+ and β-carbolinium in the striatum of conscious rats

Kazuo Matsubara [a,*], Tomoko Idzu [a], Yuta Kobayashi [b], Tatsuo Gonda [c], Hideki Okunishi [b], Kojiro Kimura [a]

[a] *Department of Legal Medicine, Shimane Medical University, Izumo 693, Japan*
[b] *Department of Pharmacology, Shimane Medical University, Izumo 693, Japan*
[c] *Institute of Experimental Animals, Shimane Medical University, Izumo 693, Japan*

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Abstract

The effects of *N*-methyl-4-phenylpyridinium cation (MPP^+) and of an endogenously formed analog, 2,9-di-methyl-norharmanium cation (2,9-Me_2NH^+), on extracellular dopamine were studied in the striatum of freely moving rats. Perfusion of either 2,9-Me_2NH^+ or MPP^+ through a microdialysis probe evoked a marked and dose-dependent increase of dopamine levels. Tetrodotoxin and Ca^{2+}-free medium prevented the increase in dopamine levels induced by 2,9-Me_2NH^+, but not that induced by MPP^+. Cocaine, 3 μM, intensified the 2,9-Me_2NH^+-induced increase in extracellular dopamine and slightly attenuated the MPP^+-induced efflux. *S*(−)-3-(3-Hydroxyphenyl)-*N*-propylpiperidine, that acts as an antagonist of dopamine autoreceptors in the presence of a dopamine reuptake inhibitor, markedly enhanced the increase in extracellular dopamine elicited by 2,9-Me_2NH^+, but not that by MPP^+. These results suggested that 2,9-Me_2NH^+ was a potent dopamine reuptake blocker, whereas MPP^+ acts as an amphetamine-like dopamine releaser rather than a reuptake inhibitor on the membrane transporter.

Keywords: Dopamine; β-Carboline; MPP^+ (*N*-methyl-4-phenylpyridinium); Uptake; Striatum; Microdialysis

1. Introduction

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces Parkinsonian symptoms in humans as a result of the degeneration of nigrostriatal neurons (Langston et al., 1983). After transport through the blood-brain barrier, MPTP is oxidized intracerebrally in glia or non-dopaminergic neurons by monoamine oxidase type B to an intermediate, *N*-methyl-4-phenyl-dihydropyridinium cation before undergoing disproportionate or spontaneous oxidation to form a neurotoxic metabolite, *N*-methyl-4-phenylpyridinium cation (MPP^+) (Chiba et al., 1985; Markey et al., 1984). Several classes of heterocyclic molecules structurally related to MPTP have been advanced as possible neurotoxin precursors underlying the nigrostriatal degeneration in Parkinsonism. Indoleamine-related β-carbolines, a class of heterocyclics that are basically plant alkaloids, are present in food (Gross et al., 1993) and mammalian brains (see Rommelspacher et al., 1991 and references cited therein). We have previously reported that an enzyme(s) in mammalian brain particulate fractions methylates β-carbolines, sequentially forming 2-mono-[*N*]-methylated (2-Me) and 2,9-di-[*N*,*N'*]-methylated (2,9-Me_2) β-carbolinium cations (Collins et al., 1992; Matsubara et al., 1992). These *N*-methylated β-carbolinium cations, structural analogs of MPP^+ with a nitrogen bridge, were found with higher concentrations localized in the substantia nigra than in the cortex in non-Parkinsonian human brains (Matsubara et al., 1993). Furthermore, these β-carbolinium cation levels, especially that of 2,9-di-methyl-norharmanium cation (2,9-Me_2NH^+), in cerebrospinal fluid are higher in Parkinsonian than non-Parkinsonian patients (Matsubara et al., 1995).

MPP^+ accumulates in nigrostriatal neurons via the dopamine transporter before being accumulated into the mitochondria by passive cationic gradient to shut down

* Corresponding author. Tel.: (81-853) 23-2111; Fax: (81-853) 25-0463.

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ATP synthesis by inhibiting NADH dehydrogenase at Complex I of the respiratory chain (Javitch et al., 1985; Nicklas et al., 1985; Ramsay et al., 1986). The β-carbolinium cations also can enter dopaminergic neurons via the membrane transporter (Drucker et al., 1990); these cations also function as NADH-linked respiratory inhibitors in isolated mitochondria (Albores et al., 1990; Arora et al., 1990; Collins et al., 1992). MPP^+ and β-carbolinium cations increase extracellular dopamine (Johnson et al., 1989; Rollema et al., 1988a) and act as dopamine reuptake inhibitors in vitro (Drucker et al., 1990; Johnson et al., 1989). However, the mechanism of action of MPP^+, as well as of 2,9-Me_2NH^+, on the dopamine reuptake system in vivo is not well-established. The effects of these neurotoxins on the dopamine transporter are important if one is to understand their selective cytotoxicities. Furthermore, previous studies on the effects of MPP^+ on in vivo monoamine release have examined only a regimen ($> 10^{-3}$ M) strong enough to destroy striatal neurons (Booth et al., 1989; Johnson et al., 1989; Rollema et al., 1988b; Santiago et al., 1991). Thus, we studied the mechanism of the dopamine efflux induced by 2,9-Me_2NH^+ in comparison with that of MPP^+ in the striatum of freely moving rats.

2. Materials and methods

2.1. Chemicals

Dopamine and cocaine were purchased from Sigma (St. Louis, MO, USA) and Takeda (Osaka, Japan), respectively. MPP^+ and *S*(−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine ((−)-3-PPP) were obtained from RBI (Natick, MA, USA). 1-Octanesulfonic acid was purchased from Nacalai Tesque (Kyoto, Japan). Cation 2,9-Me_2NH^+ was prepared in our laboratory as previously reported (Matsubara et al., 1992). Other chemicals (Wako, Osaka, Japan) were of analytical or high performance liquid chromatographic (HPLC) grade.

2.2. Surgery

Anesthetized (50 mg/kg i.p., sodium pentobarbital) male Wistar rats (250–300 g, SLC, Japan) were stereotaxically implanted with 22-gauge cannulae in the left striata at AP +1.0 mm, L +2.8–2.9 mm from the bregma and −3.5 mm from the skull, according to the stereotaxic atlas of Paxinos and Watson (1986). Dummy probes were then placed inside the cannulae. The rats were housed in plastic cages (35 × 35 × 40 cm) with free access to food and water, and a 20-h recovery period was allowed. The animal experiments were done in accordance with the guidelines for care and use of laboratory animals from the Committee of Shimane Medical University.

2.3. Brain dialysis

The microdialysis probes with dialysis area of 3 mm length were of the I-shaped type prepared according to the method of Nakahara et al. (1989). The in vitro efficiency of the probe ($n = 4$, mean ± S.E.M.), perfused at 2 μl/min (37°C) in Ringer's solution (145 mM Na^+, 2.3 mM Ca^{2+}, 4 mM K^+ and 156 mM Cl^-), was 24.3 ± 0.3% for dopamine. After its insertion through the guide cannula, the probe was connected to a microinfusion pump and perfused with Ringer's solution at a flow rate of 2 μl/min for 180 min until stable conditions were achieved. Three consecutive samples were then collected at 20-min intervals to determine the basal level of dopamine. Immediately after the third sample was collected, 2,9-Me_2NH^+ (1, 10, 50 or 100 × 10^{-5} M) or MPP^+ (1, 10 or 100 × 10^{-5} M) in Ringer's solution was perfused for 60 min before the perfusion medium was replaced with the Ringer's solution for 180 min. The dialysate was collected at 20-min intervals during the perfusion. The Ca^{2+}-free Ringer solution consisted of 145 mM Na^+, 2.3 mM Mg^{2+}, 4 mM K^+ and 156 mM Cl^-.

The concentrations of 2,9-Me_2NH^+ and MPP^+ in the striatum were measured after 60-min perfusions with 10^{-5} M solutions of both cations followed by a 180-min washout with Ringer's solution. The animal was decapitated, and ipsi- and contra-lateral striatums were removed immediately. The tissue was sonicated in 1 ml of 0.2 M perchloric acid followed by the addition of 0.1 ml of 2 M KOH, and then centrifuged at 12 000 × *g* for 15 min. 2,9-Me_2NH^+ and MPP^+ in the supernatant were determined directly by the HPLC/fluorescence detection (ex. 400 nm and em. 460 nm for 2,9-Me_2NH^+; ex. 325 nm and em. 375 nm for MPP^+).

2.4. HPLC conditions

Dopamine was separated on a reverse phase C_{18} column (Eicompack MA-5ODS, 150 × 4.6 mm, Eicom, Japan), and detected electrochemically with a glassy carbon working electrode, set at +750 mV vs. Ag/AgCl (Eicom, Japan). The mobile phase, 0.1 M citric acid/sodium acetate buffer (pH 3.9) containing 0.75 mM 1-octanesulfonic acid, 13.4 μM EDTA and 15% methanol (v/v), was delivered at a flow rate of 0.8 ml/min by a Shimadzu LC-10A HPLC pump.

2,9-Me_2NH^+ and MPP^+ were analyzed according to the previously reported HPLC conditions (Matsubara et al., 1995).

2.5. Statistics

Statistical analysis was performed using a two-way analysis of variance (ANOVA) with repeated measures on

one factor or one factorial ANOVA, followed by the post-hoc Dunnett t-test.

3. Results

3.1. Dopamine efflux

The average basal concentrations of dopamine in the dialysate did not differ among the various experimental groups. The mean basal level of dopamine of all animals was 4.3 ± 0.3 fmol/min in 40 μl dialysate (mean ± S.E.M.). Perfusion with 2,9-Me_2NH^+ and MPP^+ evoked a marked, dose-dependent, effect on the dialysate levels of dopamine (for 2,9-Me_2NH^+, $F(52,247) = 12.54$, $P < 0.01$; for MPP^+, $F(39,234) = 43.94$, $P < 0.01$). A significant difference in dopamine levels was observed between the animals that received each dose of both drugs and controls ($P < 0.05$). The maximal dopamine efflux with 2,9-Me_2NH^+ was several fold less than that induced by the corresponding dose of MPP^+ (Table 1). The time-response curves for dopamine efflux with 2,9-Me_2NH^+ and MPP^+ were slightly different. Whereas the peak dopamine efflux induced by MPP^+ was rapidly established, within 20 min, that induced by 2,9-Me_2NH^+ was attained 60–80 min post-administration and the effect was relatively long-lasting (Figs. 1 and 2). The total dopamine overflow on perfusion with 2,9-Me_2NH^+ was 2–3-fold less than that induced by the corresponding dose of MPP^+ (Table 1).

After 60-min perfusions with 10^{-5} M solutions, the concentrations of 2,9-Me_2NH^+ and MPP^+ were 6.7 ± 4.5 and 23.1 ± 8.2 pmol/ipsilateral striatum (mean ± S.D.), respectively. Neither 2,9-Me_2NH^+ nor MPP^+ was detected in the contralateral region.

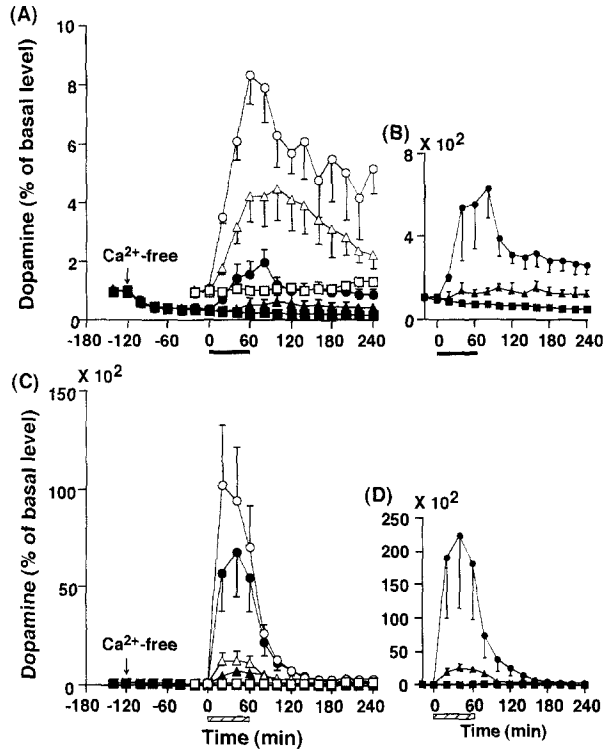

Fig. 1. Effects of Ca^{2+}-free Ringer's solution on dopamine efflux induced by (A) 2,9-Me_2NH^+ (black horizontal bar) and (C) MPP^+ (hatched horizontal bar). The addition of Ca^{2+}-free medium was started at the time indicated by an arrow. Data are mean ± S.E.M. values, expressed as percentages of basal values. When the dopamine level reduced by the Ca^{2+}-free treatment was used as basal value, the dopamine levels evoked by 2,9-Me_2NH^+ and MPP^+ are represented in Fig. 1B and D, respectively. □, control ($n = 5$); ○, 10^{-4} M 2,9-Me_2NH^+ ($n = 4$) or MPP^+ ($n = 6$); △, 10^{-5} M 2,9-Me_2NH^+ ($n = 7$) or MPP^+ ($n = 4$); ■, Ca^{2+}-free treatment ($n = 5$); ●, 10^{-4} M 2,9-Me_2NH^+ ($n = 5$) or MPP^+ ($n = 5$) with Ca^{2+}-free treatment and ▲, 10^{-5} M 2,9-Me_2NH^+ ($n = 4$) or MPP^+ ($n = 5$) with Ca^{2+}-free treatment.

3.2. Effects of low Ca^{2+} and tetrodotoxin

The addition of Ca^{2+}-free Ringer solution, or tetrodotoxin (1×10^{-6} M) to the perfusate effectively prevented the 2,9-Me_2NH^+-induced increase in extracellular dopamine (for Ca^{2+}-free treatment, $F(1,7) = 48.58$, $P < 0.01$ for the 10^{-4} M dose and $F(1,9) = 12.46$, $P < 0.01$ for the 10^{-5} M dose, Fig. 1A; for tetrodotoxin treatment, $F(1,7) = 70.23$, $P < 0.01$ for the 1×10^{-4} M dose and $F(1,10) = 17.78$, $P < 0.01$ for the 1×10^{-5} M dose, Fig. 2A). 2,9-Me_2NH^+ still slightly increased extracellular dopamine from the levels reduced by Ca^{2+}-free or tetrodotoxin treatment (for Ca^{2+}-free treatment, $F(26,143) = 4.28$, $P < 0.01$, Fig. 1B; for tetrodotoxin treatment,

Table 1
Effects of 60-min perfusions with 2,9-Me_2NH^+ and MPP^+ on the extracellular dopamine in the rat striatum

Dose in medium	Maximal levels (% of basal value)		Total efflux [a] (pmol)	
($\times 10^{-5}$ M)	2,9-Me_2NH^+	MPP^+	2,9-Me_2NH^+	MPP^+
1	496.3 ± 108.2 (7)	1 337.8 ± 439.1 (4)	1.16 ± 0.30	3.09 ± 0.79
10	878.1 ± 78.6 (4)	11 030.5 ± 3 265.4 (6)	8.86 ± 3.71	15.50 ± 2.64
50	2 102.1 ± 437.0 (4)	Not tested	11.84 ± 2.50	Not tested
100	8 227.4 ± 2 116.0 (4)	20 306.9 ± 2 730.1 (6)	23.33 ± 8.39	62.74 ± 13.56

The total dopamine efflux into extracellular fluid elicited by the cation was obtained by subtraction of basal output of dopamine (0.63 ± 0.17 pmol in control animals, $n = 5$). The values are expressed as means ± S.E.M. Number of animals used are shown in parentheses. All experimental doses of both drugs induced significant increases of dopamine efflux compared with controls ($P < 0.05$).

[a] Total dopamine amount induced by test compounds in the dialysate collected for 240 min during and after perfusion with the compounds.

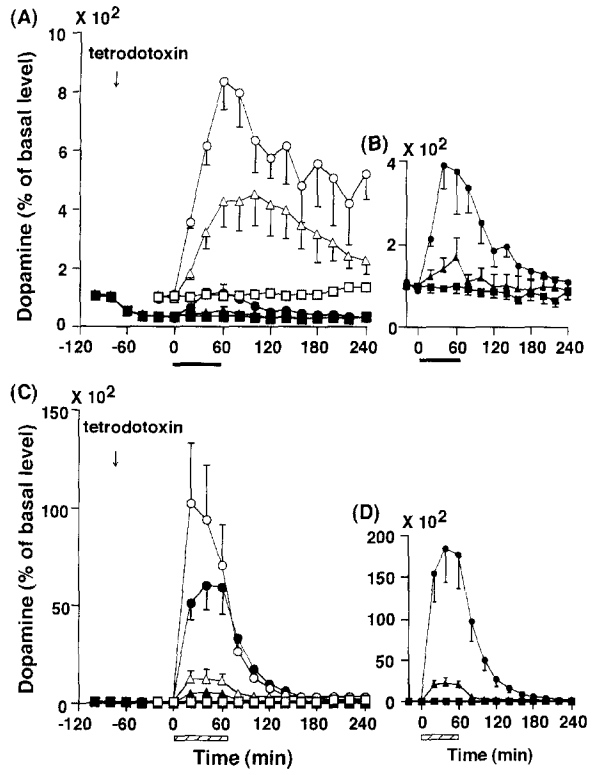

Fig. 2. Effect of tetrodotoxin on dopamine release caused by (A) 2,9-Me_2NH^+ (black horizontal bar) and (C) MPP^+ (hatched horizontal bar). Tetrodotoxin (10^{-6} M) was added to the perfusion medium (arrow). Data are mean ± S.E.M. values, expressed as percentages of basal values. When the dopamine level reduced by the tetrodotoxin treatment was used as a basal value, dopamine levels elicited by 2,9-Me_2NH^+ and MPP^+ are represented in Fig. 2B and D, respectively. □, control ($n = 5$); ○, 10^{-4} M 2,9-Me_2NH^+ ($n = 4$) or MPP^+ ($n = 6$); △, 10^{-5} M 2,9-Me_2NH^+ ($n = 7$) or MPP^+ ($n = 4$); ■, tetrodotoxin treatment ($n = 6$); ●, 10^{-4} M 2,9-Me_2NH^+ ($n = 5$) or MPP^+ ($n = 6$) with tetrodotoxin treatment and ▲, 10^{-5} M 2,9-Me_2NH^+ ($n = 5$) or MPP^+ ($n = 4$) with tetrodotoxin treatment.

$F(26,169) = 9.33$, $P < 0.01$, Fig. 2B). The basal levels of dopamine during Ca^{2+}-free and tetrodotoxin additions were 1.4 ± 0.2 and 1.9 ± 0.3 fmol/min in 40 μl dialysate (mean ± S.E.M.), respectively. On the other hand, the effects of both treatments were only slight on the MPP^+-inducing dopamine efflux (for Ca^{2+}-free treatment, $F(1,9) = 0.98$, $P > 0.3$ in the 1×10^{-4} M dose and $F(1,7) = 3.60$, $P > 0.09$ in the 1×10^{-5} M dose, Fig. 1C and D; for tetrodotoxin treatment, $F(1,10) = 0.57$, $P > 0.4$ in the 1×10^{-4} M dose and $F(1,6) = 5.41$, $P > 0.05$ in the 1×10^{-5} M dose, Fig. 2C and D).

3.3. Effect of cocaine

The effect of cocaine on both cations that induced dopamine efflux was tested. Cocaine (3×10^{-6} M) was perfused continuously from 20 min prior to cation administration to the end of the experiment. The continuous perfusion of cocaine itself increased the extracellular dopamine level. The dopamine level reached 200% of its basal value, and then declined to 110–130% of the basal level. The effect of cocaine on the cation-induced dopamine efflux is shown in Fig. 3. Cocaine intensified the 2,9-Me_2NH^+-induced increase in extracellular dopamine ($P < 0.05$) and attenuated the MPP^+-induced efflux ($P < 0.05$) only during cation perfusion.

3.4. Effect of (−)-3-PPP

(−)-3-PPP (1×10^{-5} M) slightly increased the dopamine efflux in the striatum. Co-perfusion with (−)-3-PPP and 2,9-Me_2NH^+ induced a more than 5-fold increase in the extracellular dopamine levels elicited by 2,9-Me_2NH^+ alone ($F(1,9) = 40.49$, $P < 0.01$; Fig. 4A).

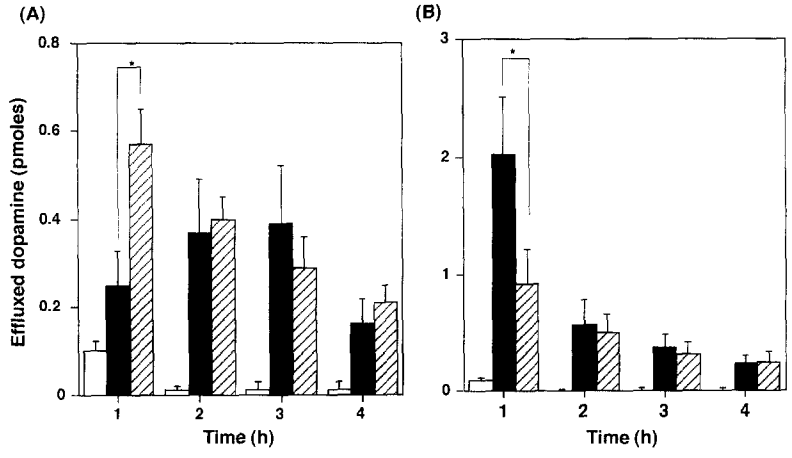

Fig. 3. Effect of cocaine (3×10^{-6} M) on (A) 2,9-Me_2NH^+ and (B) MPP^+ induced dopamine efflux. The cations (10^{-5} M) were administered through a microdialysis probe during the first hour, and cocaine was continuously perfused from 20 min prior to the cation administration. The drug-induced dopamine efflux was obtained by subtraction of the basal output. The data were expressed as mean ± S.E.M. (pmol). The dopamine efflux induced by both cations was significantly higher than the cocaine-induced efflux at each period. Open columns, cocaine alone; solid columns, the cation alone; and hatched columns, co-perfusion with the cation and cocaine. * $P < 0.05$.

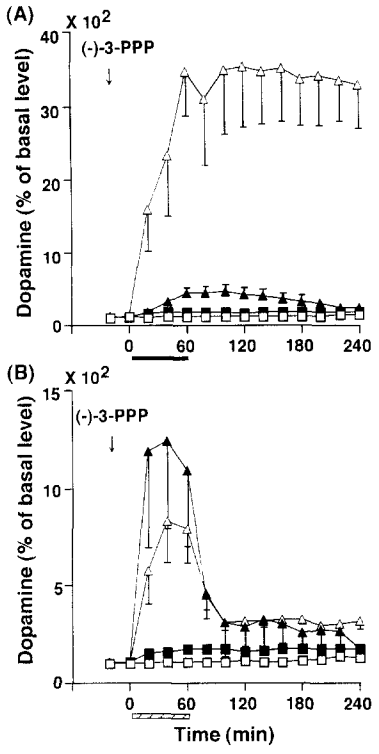

Fig. 4. Effect of (−)-3-PPP co-perfusion with (A) 2,9-Me_2NH^+ (black horizontal bar) and (B) MPP^+ (hatched horizontal bar) on extracellular dopamine levels. (−)-3-PPP (10^{-5} M) was added to the perfusion medium (arrow). Data are mean ± S.E.M. values, expressed as percentages of basal values. □, control ($n = 5$); ▲, 10^{-5} M 2,9-Me_2NH^+ ($n = 7$) or MPP^+ ($n = 4$); ■, (−)-3-PPP alone ($n = 4$) and △, 10^{-5} M 2,9-Me_2NH^+ ($n = 4$) or MPP^+ ($n = 5$) with (−)-3-PPP.

On the contrary, co-perfusion of (−)-3-PPP with MPP^+ did not affect dopamine levels induced by MPP^+ ($F(1,7) = 0.38$, $P > 0.50$; Fig. 4B).

4. Discussion

The 60-min perfusion with the highest concentration of 2,9-Me_2NH^+ (10^{-3} M), as well as MPP^+ (Collins et al., 1992; Johnson et al., 1989; Rollema et al., 1988a,b), should cause nerve degeneration, resulting in massive dopamine release. In fact, the log-concentration-response curves from Table 1 shows that the maximal effects on dopamine release induced by $1–50 \times 10^{-5}$ M of 2,9-Me_2NH^+ increased almost linearly, but that on perfusion with 10^{-3} M, the cation induced a sudden and steep rise of dopamine release. A 15-min perfusion with 10^{-3} M 2,9-di-methyl-harmanium has been reported to induce irreversible effects on dopamine release (Collins et al., 1992). Thus the pharmacological experiments in this study were carried out with the lower (10^{-5} and 10^{-4} M) concentrations of the cations.

The cations, 2,9-Me_2NH^+ and MPP^+, strongly induced a dopamine efflux in the striatum of freely moving rats, even at the 1×10^{-5} M dose. Intrastriatal perfusions with 1×10^{-5} M 2,9-Me_2NH^+ and MPP^+ for 60 min caused increases of dopamine efflux to about 500 and 1300% of the basal values, respectively. The concentrations of the test compounds in the extracellular fluid around the dialysis probe during the perfusion with 1×10^{-5} M solution were estimated as $1.5–2 \times 10^{-6}$ M. This was based on the in vitro efficiency of the probe where the partition across the dialysis membrane was 15–20% of the concentrations of both compounds in the medium, although it is difficult to predict the amounts actually delivered in vivo. Delivery into the brain depends greatly on the rate of removal of infused compounds from around the dialysis membrane. After the 60-min perfusion with a 10^{-5} M concentration of the compound and 180-min washout with Ringer's solution, the concentrations of MPP^+ and 2,9-Me_2NH^+ in the ipsilateral striatum were 23.1 and 6.7 pmol, respectively.

MPP^- is a strong dopamine reuptake inhibitor, with an IC_{50} value less than 3×10^{-6} M (Johnson et al., 1989; Drucker et al., 1990). However, the present results suggested that this cation might not act as a potent blocker of dopamine reuptake in vivo. The increase in dopamine efflux elicited by MPP^+ was tetrodotoxin-insensitive and not Ca^{2+}-dependent. This increase was attenuated by co-perfusion with cocaine, indicating that MPP^+ uptake via the dopamine transporter was inhibited by cocaine. These results indicated that MPP^+ should be an amphetamine-like dopamine releaser to evoke a marked increase in extracellular dopamine levels. Tetrodotoxin independence of the effects of 30 min 10^{-4} M MPP^+ (Westerink et al., 1987b), as well as amphetamine-like effects of 1 min 10^{-2} M MPP^+ (Rollema et al., 1988b), have been reported previously. The mechanism of dopamine release by amphetamine and its derivatives is Ca^{2+}-independent and tetrodotoxin-insensitive (Carboni et al., 1989; Hurd and Ungerstedt, 1989). Molecular sites of action of these compounds are membrane and vesicular transporters (Schuldiner et al., 1993). The accumulation of MPP^+ has been shown to occur by an active process in striatal synaptosomal preparations, and the kinetic characteristics of MPP^+ uptake are similar to those of dopamine (Chiba et al., 1985; Javitch et al., 1985). Methamphetamine protects against MPTP neurotoxicity, possibly due to competition with MPP^+ on a membrane transporter site (Sziraki et al., 1994). Thus MPP^+ pumped into dopamine neurons would further accumulate in the vesicular storage, possibly through the amine transporter.

On the other hand, the effect of 2,9-Me_2NH^+ on extracellular dopamine levels was tetrodotoxin-sensitive and Ca^{2+}-dependent. Uptake blockers, such as cocaine and nomifensine, cannot evoke an increase in dopamine under Ca^{2+}-free conditions and during tetrodotoxin treatment in vivo (Carboni et al., 1989; Hurd and Ungerstedt, 1989; Tateyama et al., 1993). In other words, these uptake inhibitors inhibit the reuptake of dopamine released exocytotically. Perfusion with 10^{-4} M 2,9-Me_2NH^+ solution

still produced a dopamine increase both during treatment with tetrodotoxin and under Ca^{2+}-free conditions, although the net amount was small. These results could indicate that mechanisms other than uptake inhibition, such as amphetamine-like release and/or nerve damage, could be partially involved in the dopamine release elicited by the perfusion with 10^{-4} M 2,9-Me_2NH^+ solution. Thus only concentrations of 10^{-5} or lower of 2,9-Me_2NH^+ would produce pure dopamine reuptake blocking effects without contribution of other, amphetamine-like or toxic effects. It was also possible that the cation still inhibited dopamine reuptake under conditions of reduced neuronal firing. (−)-3-PPP strongly potentiated the effect of 2,9-Me_2NH^+ on extracellular dopamine, which was consistent with dopamine reuptake inhibition activity. It is well established that the combination of a dopamine reuptake inhibitor and a dopamine antagonist induces a synergistic increase in the extracellular dopamine level (See, 1994; Westerink et al., 1987a). (−)-3-PPP itself increased the extracellular dopamine concentration in vivo as reported by See (1994), probably due to its post- and presynaptic dopamine antagonistic properties (Arnt et al., 1983; Clark et al., 1991). Perfusion with (−)-3-PPP (10^{-4} M) and nomifensine (10^{-5} M) produces a dopamine overflow twice as high as that induced by nomifensine alone in the caudate-putamen (See, 1994), suggesting that 2,9-Me_2NH^+ might be a more potent inhibitor than nomifensine. Thus the results from the (−)-3-PPP study further supported the possibility that 2,9-Me_2NH^+ could be a potent dopamine reuptake blocker. The 2-Me β-carbolinium cations are accumulated into synaptosomes in a Na^+-dependent and nomifensine-sensitive manner (Drucker et al., 1990), but with far higher IC_{50} values for dopamine reuptake than with MPP^+ (Johnson et al., 1989; Drucker et al., 1990). In contrast to 2-Me β-carbolinium cations, based on the present results dopamine reuptake inhibition would be a more potent in vivo characteristic of 2,9-Me_2NH^+. Carrier-mediated influx of this cation into neurons could not be evaluated from the present results, although a significant amount of the compound was detected in the striatum after the 60-min perfusion with 10^{-5} M solution. This concentration of 2,9-Me_2NH^+ in the ipsilateral striatum was two-sevenths of that of MPP^+. These various efficiencies of delivery into the brain cell could explain the differences in pharmacological effects on the dopamine transporter between the two cations.

One might consider that the reduction in dopamine reuptake caused by both cations could be due to mitochondrial dysfunction, since dopamine uptake is ATP-dependent and mitochondria are major sites of ATP supply (Cao et al., 1990). This may be partly attributable to the increasing dopamine concentration, especially to sustained high dopamine concentrations even after the removal of both cations from the perfusion medium and/or the slight increase in dopamine levels elicited by 2,9-Me_2NH^+ under Ca^{2+}-free conditions and during tetrodotoxin treatment. The dopamine concentration evoked by typical dopamine reuptake inhibitors, such as cocaine and nomifensine, and amphetamine quickly returned to the basal level after removal of the inhibitors from the perfusion medium (Chen and Reith, 1994; Tateyama et al., 1993).

In these acute perfusion experiments in rats, 2,9-Me_2NH^+ increased extracellular dopamine levels differently from MPP^+. This difference may not prevent the conceptualization of a Parkinsonian toxicant, however. Although the carrier-mediated influx of this cation into neurons should be evaluated in future work, 2,9-Me_2NH^+ could accumulate over several decades within nigrostriatal neurons before the onset of Parkinson's disease, due to impaired liver metabolism of precursors, excess intranigral biosynthesis or impaired removal. If 2,9-Me_2 β-carbolinium cations, such as 2,9-Me_2NH^+, are appreciably formed within dopaminergic neurons in Parkinsonian individuals by the indole *N*-methylating enzymes, uptake-dependent specificity is circumvented. Indeed, the 2-Me β-carbolinium cation contents in the cerebrospinal fluid increase significantly with the progression of Parkinson's disease, but 2,9-Me_2NH^+ decreases as the disease is exacerbated (Matsubara et al., 1995).

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