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The relative abilities of MPTP and MPP^+ to compete with [^{3}H]dopamine for the rat and marmoset striatal dopamine uptake site

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The principal neurotoxic effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and non-human primates is a profound destruction of the nigrostriatal dopamine system, causing severe motor impairments similar to those observed in Parkinson's disease [1–4].

Pretreatment of monkeys with monoamine oxidase B inhibitors fully or partially protects against the biochemical and behavioural impairments [4, 5], indicating that oxidation of MPTP to 1-methyl-4-phenylpyridinium (MPP^+) may be required to cause neurotoxicity [6, 7]. This may occur in glial or neuronal cells containing monoamine oxidase B, with the subsequent uptake of MPP^+ via the dopamine uptake process into the dopamine neurone [8, 9]. The partial protection afforded by dopamine uptake inhibitors against MPTP induced toxicity in the primate would support this hypothesis [10].

In contrast to the pronounced effects observed in the primate, rodents appear considerably less sensitive to MPTP induced toxicity, although the effects that do occur either *in vivo* or in cell cultures are also attenuated by monoamine oxidase B inhibitors [11–13]. In the rodent, as in the primate, the effects of MPTP may again be mediated by MPP^+, which has potent neurotoxic effects in cell cultures [14], although the reason for differences in *in vivo* sensitivity to MPTP between the rodent and primate species remains uncertain.

It was hypothesized that such species differences may relate to the second stage of the neurotoxic process involving MPTP and/or MPP^+ uptake into the dopamine neurones. Here we investigate this possibility and compare the abilities of MPTP, MPP^+ and uptake inhibitors to inhibit [^{3}H]dopamine uptake in striatal synaptosomal preparations from the rat and marmoset.

Methods and materials

Female Hooded Lister rats (Bradford bred) weighing 250–300g and male or female common marmosets (*Callithrix jacchus*) weighing 280–320g were killed by cervical dislocation and decapitation, respectively. The brains were immediately removed. The striatum was dissected out and homogenized in a glass–Teflon homogenizer (clearance 0.11–0.15 mm) by six movements up and down in 10 mL ice-cold 0.32 M sucrose followed by centrifugation at 1000 *g* (4°) for 10 min (Beckman L8-70 ultracentrifuge). The supernatant was retained and centrifuged at 48,000 *g* for 15 min (4°). The resulting pellet was gently resuspended in ice-cold 0.27 M sucrose at a concentration of 0.5–0.8 and 0.4–0.5 mg protein/mL for the rat and marmoset, respectively.

Krebs buffer (900 μL) was gassed using 95% O_2/5% CO_2 containing 115.0 mM NaCl, 4.97 mM KCl, 1.0 mM $CaCl_2$, 1.22 mM $MgSO_4$, 1.20 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 μM nialamide, 0.8 mM ascorbic acid and 0.1 μM [^{3}H]dopamine (50 Ci/mmol) in the absence (total uptake) or presence of uptake-competing compounds (2.0×10^{-10}–2.0×10^{-5} M, six concentrations) which were added to reaction tubes in triplicate and preincubated at 37° for 3 min. [^{3}H]Dopamine uptake was initiated by the addition of 100 μL of the crude synaptosomal preparation (or 100 μL 0.27 M sucrose for filter blank). The reaction was allowed to proceed at 37° for 6 min before termination by rapid filtration through pre-wet Whatmann GF/B filter paper, followed immediately by washing with 9.0 mL ice-cold Krebs buffer for 6 sec. The filtration and washing procedure was performed using a semi-automatic membrane harvester (Brandel). The filter discs were placed in 10.0 mL 'Insta-Gel' scintillant (Packard) and counted for tritium by liquid scintillation spectroscopy (Tri-Carb 1900 CA, Canberra Packard) at approximately 47% efficiency. Protein estimation was performed by the method of Bradford [15] using bovine serum albumin as the standard.

[^{3}H]Dopamine (50 Ci/mmol, Amersham International, Amersham, U.K.), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (Research Biochemical Incorporated, RBI), 1-methyl-4-phenylpyridinium (RBI), GBR12909 1-[2-[bis-(4-fluorophenyl) methoxyl]-ethyl-4-[3-phenyl-propyl]-piperazine dihydrochloride (RBI), desipramine hydrochloride (Sigma Chemical Co., Poole, U.K.), hemicholinium-3 (Sigma), mazindol (Sandoz, Middlesex, U.K.), fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN), benztropine mesylate (Merck Sharp & Dohme, Herts, U.K.) and cocaine hydrochloride (May & Baker, Dagenham, U.K.).

All drugs were dissolved in distilled water except mazindol and GBR 12909 which were dissolved in a minimum quantity of dilute hydrochloric acid and made to volume with distilled water. All drugs were used as received.

Results and discussion

Preliminary experiments established that [^{3}H]dopamine uptake into the synaptosomes was linear with respect to time of incubation (up to 15 min). Furthermore, the uptake was comparable (1.8 ± 0.5 and 2.4 ± 0.2 pmol/min/mg protein, mean ± SE of 4–7 determinations) in rat and marmoset striatal preparations respectively.

The uptake of [^{3}H]dopamine into rat and marmoset striatal synaptosomes was inhibited by nanomolar concentrations of the selective dopamine uptake inhibitor

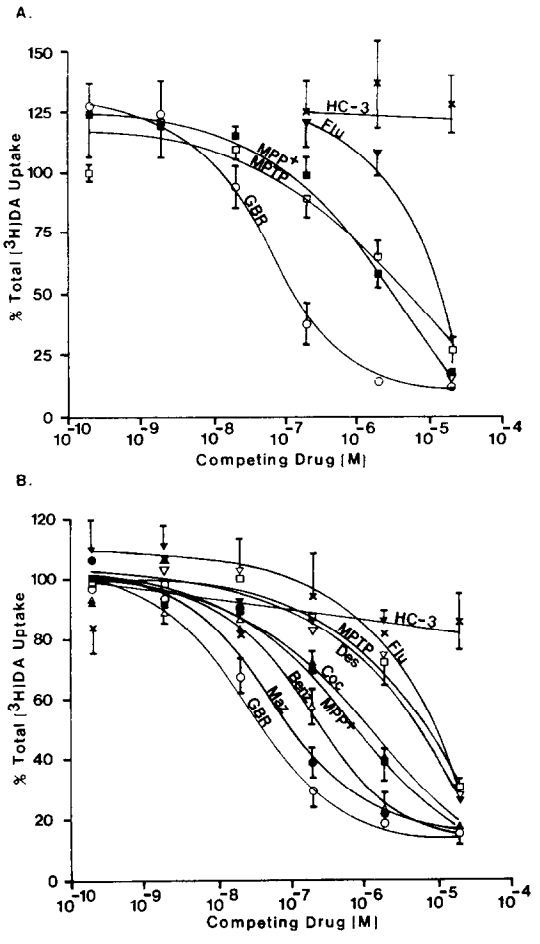

Fig. 1. Inhibition of [^{3}H]DA uptake into (A) marmoset and (B) rat striatal synaptosomes by MPTP (□), MPP$^+$ (■) and various uptake inhibitors: GBR12909 (GBR, ○), mazindol (Maz, ●), benztropine (Benz, △), cocaine (Coc, ▲), desipramine (Des, ▽), fluoxetine (Flu, ▼) and hemicholinium-3 (HC-3, X). Data represent the mean ± SE obtained from 3–7 individual experiments.

GBR12909 [16]. The other dopamine inhibitors mazindol, benztropine and cocaine were effective at micromolar concentrations and similar to those reported elsewhere [16, 17]. The ability of such compounds to inhibit 85–90% of the [^{3}H]dopamine uptake, and the much reduced potency of the noradrenaline and 5-hydroxytryptamine uptake inhibitors desipramine and fluoxetine and failure of the choline uptake inhibitor hemicholinium-3 to inhibit [^{3}H]dopamine uptake indicate the selectivity of action at the dopamine uptake site.

MPTP inhibited [^{3}H]dopamine uptake in both the rat and marmoset striatal synaptosomes with a similar affinity. MPP$^+$ was approximately 10 times more potent than MPTP to inhibit [^{3}H]dopamine uptake into rat synaptosomes but retained a similar inhibitory potency to MPTP using the marmoset synaptosomal preparations. As a consequence of such effects, and in contrast to all other treatments, MPP$^+$ was approximately three to four times more potent to inhibit [^{3}H]dopamine uptake into synaptosomes from the rat than from the marmoset (Fig. 1, Table 1).

No evidence was obtained that MPTP has a greater affinity for the marmoset dopamine uptake sites, MPTP

Table 1. pIC_{50} values for drug induced inhibition of [^{3}H]dopamine uptake into striatal synaptosomal preparations from rat and marmoset

Drug	N	pIC_{50} Rat	pIC_{50} Marmoset
MPTP	4	5.38 ± 0.08	5.66 ± 0.22
MPP$^+$	4–5	6.31 ± 0.52	5.77 ± 0.09*
GBR12909	4–7	7.66 ± 0.13	7.22 ± 0.18
Mazindol	6	7.50 ± 0.15	NT
Benztropine	4	6.70 ± 0.13	NT
Cocaine	4	6.32 ± 0.08	NT
Desipramine	3	5.47 ± 0.07	NT
Fluoxetine	3	5.31 ± 0.05	5.17 ± 0.06
Hemicholinium-3	3	<5.0	<5.0

Each value represents the mean ± SE from N separate experiments where each assay was performed in triplicate. A significant difference in drug affinity between primate and rodent dopamine uptake sites is indicated.

* $P < 0.05$ (Dunnett's t-test).

NT, not tested.

preventing [^{3}H]dopamine uptake into both rat and marmoset synaptosomal preparations to the same extent and with low and similar potencies. It is therefore unlikely that the greater *in vivo* toxicity of MPTP in the marmoset reflects a greater affinity for the dopamine uptake process on the dopamine neurone. Similar comments would apply to MPP$^+$ which indeed appeared to be slightly less potent to inhibit [^{3}H]dopamine uptake in the marmoset than rat synaptosomes. These observations are also consistent with those of Willoughby *et al.* [18] who reported that MPP$^+$ displayed similar affinity for the dopamine uptake channels in rat and human striatal synaptosomes. However, there appear differences between MPTP and MPP$^+$ in the two species. Thus MPP$^+$ was an order of magnitude more potent than MPTP to inhibit [^{3}H]dopamine uptake in the rat, similar results being reported by Javitch *et al.* [8] and in mesencephalic rat cell cultures [14]. In contrast, MPTP and MPP$^+$ have equal affinity for the dopamine uptake site in the marmoset striatal synaptosomes. It remains uncertain as to whether this equal potency of MPTP and MPP$^+$ to block the dopamine uptake process contributes to the *in vivo* sensitivity of the marmoset to MPTP, particularly when MPP$^+$ is considerably more potent than MPTP as a neurotoxic agent in marmoset mesencephalic cell cultures [19].

There is no single hypothesis which can yet explain all aspects of the acute and chronic neurotoxicity caused by MPTP. The greater retention of MPTP/MPP$^+$ in glial, serotonergic or other cells in primate rather than rodent brain [20] may permit a continuing source of toxic material for release and uptake into the dopamine neurone. Certainly, the protection afforded against MPTP in the primate by dopamine uptake inhibitors, provided that they were administered for many weeks after MPTP treatment, would support this hypothesis [10, 21]. However, whilst the affinity of MPTP/MPP$^+$ for the dopamine uptake system may contribute importantly to their selectivity of neurotoxic action on the dopamine neurone, the present study indicates that this is unlikely to be a key factor in explaining the greater sensitivity of the primate to MPTP induced toxicity.

Postgraduate Studies in Pharmacology
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