Stereospecific Distribution of Methylphenidate Enantiomers in Rat Brain: Specific Binding to Dopamine Reuptake Sites

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To investigate the stereoselective distribution of methylphenidate (MPD) enantiomers in rats, the concentrations of each enantiomer were determined in plasma and brain regions (cerebellum, striatum, basal forebrain, brain stem, and cortex) after iv administration of racemic MPD and its individual enantiomers. The concentrations of MPD enantiomers in each brain region reached pseudo-steady state within 10 min after iv administration of racemic MPD (2 mg/kg dose). The influx clearances for MPD calculated from $K_{P_{app}}$ values in each brain region were not significantly different between MPD enantiomers and between the five brain regions. The mean $K_{P_{app}}$ values for (+)-MPD in the striatum at 120 and 240 min after administration of racemic MPD were 10.1 and 10.5, respectively, and these values at each time were significantly larger than the $K_{P_{app}}$ values (7.5 and 7.0, respectively) for the (−)-isomer ($P < 0.01$). The $K_{P_{app}}$ value for (+)-MPD in the striatum decreased by coadministration of mazindol as an inhibition of both dopamine and norepinephrine reuptake, but it was not changed by desipramine as a norepinephrine reuptake inhibitor. These results suggest that (+)-MPD was bound specifically to the dopamine reuptake site in the striatum.

KEY WORDS: methylphenidate enantiomers; intravenous administration; brain striatum; dopamine reuptake site; stereospecific binding; rat.

INTRODUCTION

Methylphenidate (MPD), a sympathomimetic agent with stimulant effects on the central nervous system, is used frequently in the treatment of narcolepsy and attention-deficit disorder. MPD interacts with the dopamine transport system and appears to exert its effect by blocking the reuptake of dopamine by the presynaptic neuron (1–4), while it was also reported that the pharmacological effects of MPD involve facilitation of noradrenergic transmission (5). On the other hand, although MPD is an enantiomeric drug (Fig. 1) and clinically used as a racemate, the pharmacological activity of (+)-MPD is reported to be more potent than that of the (−)-isomer (6).

Based on an *in vitro* binding study of [^{3}H]MPD to membrane preparations of the rat striatum, Janowsky *et al.* (7) showed that the specific binding sites of [^{3}H]-MPD in the striatum localized in the dopaminergic nerve terminals. A study using *in vitro* receptor autoradiographic techniques also showed that the highest specific binding was observed in the sections through the forebrain which corresponded to the caudate-putamen, olfactory tubercle, nucleus accumbens, bed nucleus of the stria terminals, and median eminence (8). However, the *in vivo* regional brain distribution of MPD enantiomers still remained unclear.

In this study, to elucidate the cause of the difference in pharmacological activity between (+)- and (−)- MPD, we investigated the *in vivo* regional distribution of MPD enantiomers in the brain after iv administration of the racemate and the individual enantiomer of MPD to rats. Further, we investigate the specific binding site of MPD in the *in vivo* rat brain using monoamine reuptake inhibitors.

MATERIALS AND METHODS

Materials

Racemic mixtures of *threo*-form MPD·HCl and desipramine · HCl were kindly supplied by Ciba-Geigy (Japan) Ltd. (Takarazuka), and mazindol was kindly provided by the Department of Pharmaceutics, Faculty of Pharmaceutical Science, University of Tokyo. The enantiomers of MPD·HCl were optically separated from racemic MPD according to the method of Patrick *et al.* (6). The optical rotation of (+)-MPD·HCl was [+88°], 0.8% in methanol (lit., [+89°]), and that of (−)-MPD · HCl was [−81°], 0.8% in methanol (lit., [+89°]). All other chemicals used were commercially available and of analytical grade.

Intravenous Administration Experiments

Male Wistar rats, weighing 270 to 300 g, were anesthetized lightly with ether, and polyethylene cannulas were implanted into the femoral artery for blood sampling and the femoral vein for drug administration. After recovery from anesthesia, a dose of 2 mg/kg of racemic MPD or of 1 mg/kg of each MPD enantiomer was administered intravenously over 40-sec periods. At 1, 3, 5, 10, 30, 120, and 240 min after administration, blood samples (0.5 mL) were collected and then rats were immediately killed by decapitation. The brain was removed quickly and dissected in five regions (cerebellum, striatum, midbrain, brain stem, and cortex) on a cold plate according to the method of Glowinski *et al.* (9). Plasma (0.2 mL) was separated by centrifugation at about 0°C. The plasma and brain samples were stored at −80°C until analysis.

Coadministration Experiments with Mazindol or Desipramine

A dose of 8 mg/kg of mazindol or desipramine was administered intravenously to rats at 90 or 210 min after dosing 2 mg/kg of racemic MPD. The dose of these inhibitors was the same as that used by Takahashi *et al.* (10) and Riva *et al.* (11). At 30 min after the administration of mazindol or desipramine, corresponding to the time at 120 or 240 min after the administration of MPD, rats were killed by decapitation. The brain was removed and dissected as described above. The plasma and brain samples were stored at −80°C until analysis.

Analysis of MPD Enantiomers

The concentration of MPD enantiomers in plasma and brain tissues was determined according to the gas chromato-

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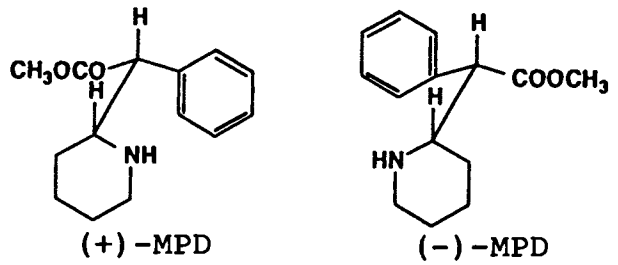

Fig. 1. Structural formulas of *threo*-methylphenidate (MPD) enantiomers.

graphic-chemical ionization-mass spectrometric method as reported previously (12).

Pharmacokinetic Analysis

For calculation of the pharmacokinetic parameters, the plasma concentrations after iv administration of MPD racemate or an individual enantiomer were fitted to the following equation (1):

$$C_p(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where $C_p(t)$ is the drug concentration at time t, A and B are the ordinate axis intercepts, and α and β are the corresponding first-order disposition rate constants. The terminal half-life ($t_{1/2}$) was calculated by dividing 0.693 by β. The area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal method and extrapolated to infinity. The steady-state distribution volume ($V_{d_{ss}}$) and the total-body clearance (CL_{tot}) were determined in accordance with the formulas given by Gibaldi and Perrier (13).

The apparent ratios of regional brain-to-plasma concentrations of MPD enantiomers ($K_{P_{app}}$) were calculated by dividing the concentration of MPD enantiomers in each brain region at time t [$C_b(t)$] by the plasma concentration [$C_p(t)$]. The influx clearance (K: mL/min/g brain) of MPD enantiomers across the blood–brain barrier in each brain region was obtained by fitting $C_p(t)$ and the apparent distribution volume, $C_b(t)/C_p(t)$ [$= K_{P_{app}}(t)$] at time t (1, 3, 5, and 10 min after administration) to the following equation, by nonlinear least-squares methods (14,15):

$$C_b(t)/C_p(t) = K \cdot \int_0^T \exp[-k_b \cdot (T - t)] \cdot C_p(t)dt/C_p(T) + (f \cdot V_e + V_b) \quad (2)$$

where k_b (min^{-1}) is the elimination rate constant from the tissue to blood, $f \cdot V_e + V_b$ (mL/g) is the distribution volume of the reversible binding site, and f, V_e (mL/g), and V_b (mL/g) are the ratio of the drug to transfer to blood, the distribution volume at steady state, and the volume in the capillary blood duct in the reversible compartment, respectively.

RESULTS

Plasma and Regional Brain Concentration After iv Administration of Racemic MPD

As shown in Fig. 2, the plasma concentrations of both MPD enantiomers after iv administration of racemic MPD declined biexponentially. The $t_{1/2}$ of the (+)- and (−)-enantiomers was 0.7 and 0.6 hr, respectively (Fig. 2A). On the other hand, the concentrations of both MPD enantiomers in the striatum and the cerebellum increased rapidly after dosing and reached a maximum within 5 min in both regions (Fig. 2B). The mean maximum concentrations of (+)- and (−)-MPD were 2750 and 2897 ng/g in the striatum and 2409 and 2459 ng/g in the cerebellum, respectively. Thereafter the concentration of MPD enantiomer in both regions declined nearly in parallel with the plasma concentrations of each MPD enantiomer. The concentration of both enantiomers in the other brain regions (basal forebrain, brain stem, and cortex) was also similar to that in the striatum (data not shown).

The mean values of K for (+)-MPD in the brain regions were within the range of 0.401–0.570 mL/min/g, and that in the (−)-isomer was 0.396–0.506 mL/min/g. The K for the (+)- or (−)-isomer was not significantly different among the brain regions (Table I). The time courses of $K_{P_{app}}$ values for (+)- and (−)-MPD after administration of racemic MPD are shown in Fig. 3. The $K_{P_{app}}$ values of both enantiomers in the early phase increased markedly in all regions and reached pseudo-steady state at 10–30 min after administration. The mean $K_{P_{app}}$ values for (+)-MPD in the striatum at 120 and 240 min after administration were 10.1 and 10.5, respectively, and these values at each time were significantly larger than those for the (+)-enantiomer in the other four brain regions ($P < 0.01$, Student's t test) (Fig. 3A). The mean $K_{P_{app}}$ values for (−)-MPD in the striatum at 120 and 240 min after administration were 7.5 and 7.0, respectively (Fig. 3B). Compared with the $K_{P_{app}}$ values of MPD isomers at 120 and 240 min, the $K_{P_{app}}$ values for the (+)-isomer in the striatum were significantly larger than those for the (−)-isomer at each time ($P < 0.05$).

Plasma and Regional Brain Concentrations After iv Administration of MPD Enantiomers

The values of $V_{d_{ss}}$, CL_{tot}, and AUC for (+)-MPD were nearly the same between administrations of the racemate and the (+)-isomer (Table II). Similarly, these values for (−)-MPD were also the same between administration of the

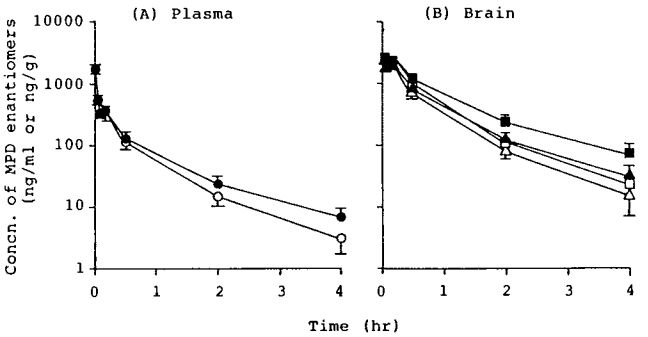

Fig. 2. Concentration–time courses of methylphenidate (MPD) enantiomers in plasma [A: ●, (+)-MPD; ○, (−)-MPD], striatum [B: ■, (+)-MPD; □, (−)-MPD], and cerebellum [B: ▲, (+)-MPD; △, (−)-MPD] after intravenous administration of racemic MPD (2 mg/kg dose) to rats. Each point and vertical bar represents the mean ± SD of three to six experiments.

Table I. Influx Clearance (K) of MPD Enantiomers in Various Brain Regions After Intravenous Administration of MPD Racemate (2 mg/kg) and Isomers (1 mg/kg) to Rats

Brain region	Isomer	Influx clearance (mL/min/g)	
		Racemate[a]	Isomers[b]
Striatum	(+)	0.570 ± 0.062[c]	0.794 ± 0.222[c]
	(−)	0.506 ± 0.171	0.463 ± 0.040
Cerebellum	(+)	0.401 ± 0.090	0.309 ± 0.082
	(−)	0.379 ± 0.190	0.421 ± 0.083
Midbrain	(+)	0.446 ± 0.111	0.559 ± 0.217
	(−)	0.471 ± 0.230	0.529 ± 0.120
Brain stem	(+)	0.414 ± 0.055	0.413 ± 0.235
	(−)	0.396 ± 0.183	0.583 ± 0.214
Cortex	(+)	0.481 ± 0.125	0.501 ± 0.182
	(−)	0.430 ± 0.222	0.526 ± 0.061

[a] Racemate was administered.
[b] Each isomer was administered.
[c] Mean ± SD; n = 3–6.

racemate and that of (−)-isomer. The values of K after administration of (+)- and (−)-isomers were similar to those after administration of racemic MPD (Table I). The $K_{P_{app}}$ values for MPD isomers at 120 and 240 min after administration of the individual MPD enantiomer were compared with that after racemic MPD (Fig. 4). No difference in $K_{P_{app}}$ for MPD isomers between the racemate and the enantiomers was observed in any brain region. The mean $K_{P_{app}}$ values for (+)-MPD in the striatum at 120 and 240 min (11.2 and 11.9, respectively) after administration of (+)-isomer were significantly larger than those for the (−)-isomer (9.2 and 7.0) after administration of (−)-isomer ($P < 0.01$). In addition, the $K_{P_{app}}$ values for each isomer in five brain regions after administration of the individual isomer were in good agreement with those after administration of racemic MPD.

Change in the $K_{P_{app}}$ Values of MPD Enantiomers After Coadministration with Monoamine Reuptake Inhibitors

Figure 5 shows the effects of mazindol (dopamine and norepinephrine reuptake inhibitor) and desipramine (norepinephrine reuptake inhibitor) on the $K_{P_{app}}$ for MPD enantiomers in the brain regions. The $K_{P_{app}}$ values for the (+)-isomer in the striatum at 120 and 240 min after administration of racemic MPD decreased significantly in the presence of mazindol, compared with those in the absence of the inhibitors (Fig. 5A). However, the $K_{P_{app}}$ value for the (+)-isomer in the cerebellum was not influenced significantly in the presence of both inhibitors (Fig. 5B). In contrast, the $K_{P_{app}}$ value for the (−)-isomer in the striatum and cerebellum was not influenced in the presence of either inhibitor (Figs. 5C and D).

DISCUSSION

It was shown that both (+)- and (−)-MPD were distributed extensively to the brain, and the K for (+)-MPD from circulation to the brain tissue was similar to that of the (−)-isomer. Further, it was estimated that the value of K was approximately half compared with the value of the blood

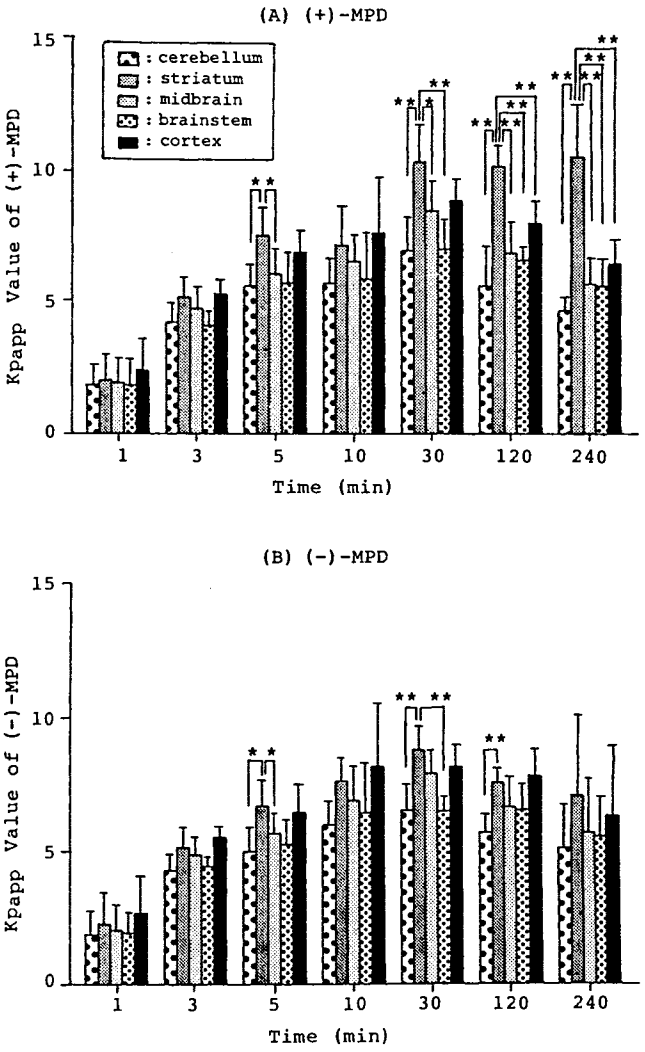

Fig. 3. $K_{P_{app}}$ values of (+)-MPD (A) and (−)-MPD (B) in regional brains after intravenous administration of racemic MPD (2 mg/kg dose) to rats. Each bar represents the mean ± S.D. of three to six experiments. (*) $P < 0.05$ and (**) $P < 0.01$ comparing the $K_{P_{app}}$ value in striatum with that in other brain regions (Student's t test).

flow rate in the rat brain as reported by Patlak *et al.* (14,15). This distribution characteristic of MPD may be closely related to its high lipophilicity (n-butanol/water partition coefficient of racemic MPD, 7.8) (16). We found that the $K_{P_{app}}$ value for (+)-MPD in the striatum at 120 and 240 min after iv administration of racemic MPD was larger than that for the (−)-isomer. The difference in this $K_{P_{app}}$ value would not be due to the interaction between the enantiomers such as a competition in binding, because similar results on the $K_{P_{app}}$ were also obtained after iv administration of the individual isomer. Therefore, these results suggested that (+)-MPD may be stereospecifically bound to the striatum. This finding is not incompatible with reports that MPD acts mainly on the striatum, in which the terminal of the dopaminergic and norepinephrinergic neuron was localized, and that (+)-MPD is more active pharmacologically than the (−)-isomer (6). On the other hand, stereospecific binding of MPD to the striatum was not observed in the early phase (within 10 min)

Table II. Pharmacokinetic Parameters of the Individual MPD Enantiomers After Intravenous Administration of Racemic (2 mg/kg Dose) and Enantiomeric MPD (1 mg/kg Dose)

Parameter	Isomer	Dose (+)-MPD (2 mg/kg)	Dose (+)-MPD (1 mg/kg)	Dose (−)-MPD (1 mg/kg)
β (hr^{-1})	(+)	0.973 ± 0.405[a]	0.901 ± 0.270[a]	
	(−)	1.187 ± 0.398		1.186 ± 0.092[a]
$V_{d_{ss}}$ (L/kg)	(+)	3.14 ± 0.84	3.36 ± 1.10	
	(−)	2.86 ± 0.56		2.58 ± 0.54
CL_{tot} (L/hr/kg)	(+)	3.06 ± 0.90	3.03 ± 0.76	
	(−)	3.40 ± 0.94		3.06 ± 0.54
AUC (ng · hr/mL)	(+)	326.8 ± 146.6	329.6 ± 110.1	
	(−)	293.9 ± 111.9		327.1 ± 69.6

[a] Mean ± SD; n = 3–6.

after iv administration, suggesting that no big difference in blood–brain barrier transport rates was observed between (+)- and (−)-isomers.

It has been reported that both dopamine and norepinephrine reuptake sites are localized mainly in the striatum, and norepinephrine reuptake sites in the cerebellum (17). In the experiments on coadministration of MPD and the monoamine reuptake inhibitors (mazindol and desipramine), the $K_{P_{app}}$ values for (+)-MPD in the striatum were decreased by supplementation of mazindol (dopamine and norepinephrine reuptake inhibitor), while desipramine (norepinephrine reuptake inhibitor) did not induce a change. However, the $K_{P_{app}}$ values in the cerebellum were not influenced by the supplementation of both inhibitors. These findings strongly suggest that (+)-MPD binds stereospecifically to dopamine reuptake sites in the striatum.

In conclusion, (+)-MPD was distributed stereoselectively in the rat brain, especially in the striatum. Further, it was shown that the (+)-isomer binds specifically to dopamine uptake sites in the striatum. The difference in this stereospecific binding might result in the difference in pharmacological activity.

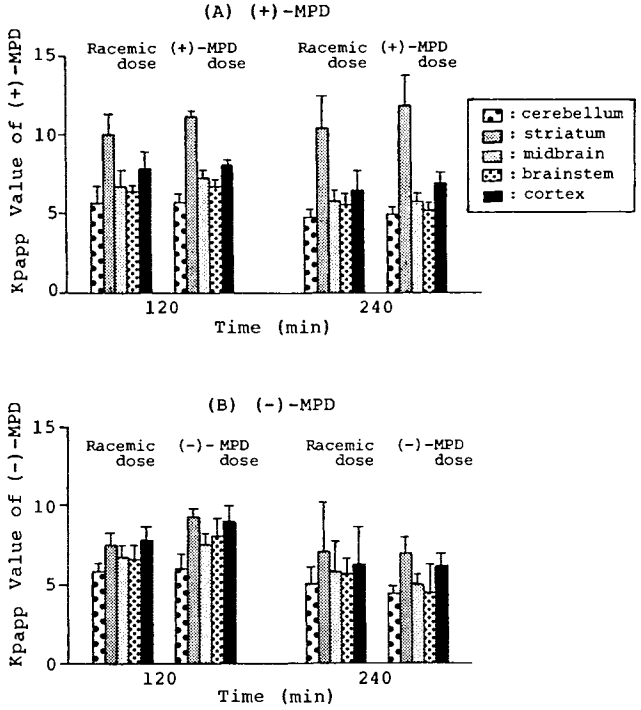

Fig. 4. Comparative $K_{P_{app}}$ values of (+)-MPD in regional brains at 120 and 240 min after intravenous administration of (+)-MPD (1 mg/kg) or racemic MPD (2 mg/kg) (A) and comparative $K_{P_{app}}$ values of (−)-MPD in regional brains after intravenous administration of (−)-MPD to rats (1 mg/kg) or racemic MPD (2 mg/kg) (B). Each bar represents the mean ± SD of four to six experiments.

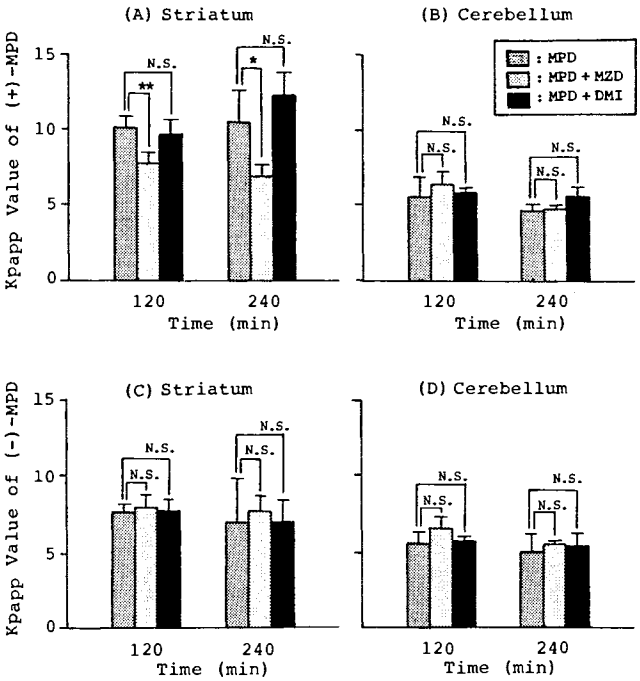

Fig. 5. Effect of mazindol (MZD; 8 mg/kg dose) and desipramine (DMI; 8 mg/kg dose) on $K_{P_{app}}$ values of (+)-MPD in striatum (A) and cerebellum (B) and (−)-MPD in striatum (C) and cerebellum (D) after intravenous administration of racemic MPD (2 mg/kg dose) to rats. Each bar represents the mean ± SD of three to six experiments. (*) $P < 0.05$ and (**) $P < 0.01$; N.S., not significant (Student's t test).

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