Pharmacodynamic Modeling for Change of Locomotor Activity by Methylphenidate in Rats

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Purpose. The locomotive activity changes after intravenous (i.v.) administration of methylphenidate (MPD) in rats were pharmacodynamically analyzed.

Methods. MPD concentration in plasma, MPD concentration and dopamine (DA) level in striatal dialysate collected by microdialysis method, and the locomotor activity after i.v. administration of MPD (2, 5 and 10 mg/kg doses) were used for the analysis.

Results. The transport of MPD from plasma to the interstitial fluid in the brain could be expressed by the linear two-compartment model. The clockwise hysteresis between the MPD concentration and the DA level in the dialysate could be explained by the pharmacodynamic model considering Michaelis-Menten type reuptake process of the extracellular DA into the terminal of the dopaminergic nerve and its competitive inhibition by the extracellular MPD. The inhibition constant (K_i) of MPD for DA reuptake was estimated to be 41.3 ± 73.8 nM (mean ± SE), which was closely consistent with the in vitro value after correction with dialysis recovery. The relationship between DA level in dialysate and locomotor activity was expressed by the Emax model considering two contrary effects, hyperkinesia and stereotypy. The bi-phasic locomotor activity-time profiles after high dose of MPD could be represented by this model.

Conclusions. The developed model made it possible to explain the tolerance in DA increase and the complicated locomotive change induced by MPD, and may be useful for other DA reuptake inhibitors, such as amphetamine and methamphetamine.

KEY WORDS: methylphenidate; rats; locomotor activity; pharmacodynamic modeling; Emax model.

INTRODUCTION

Methylphenidate [*threo*-(+)/(−)-methyl-2-phenyl-2-(2′-piperidyl) acetate; MPD], a central nervous system (CNS) stimulant, is used for the treatment of narcoleptic and essential hypersomniac patients, or children with attention deficit disorder (1–5). The pharmacological activities of MPD as a CNS stimulant are caused by the increase of endogenous DA level in extracellular space in the striatum due to the inhibition of DA reuptake by MPD (6,7).

We reported (8) that (+)-MPD was distributed stereoselectively in the striatum of the brain after intravenous (i.v.) administration of racemic MPD to rats, which was due to stereoselective binding to the DA reuptake sites in this region. Further, we determined the extracellular MPD concentration in the striatum after i.v. administration of MPD by the microdialysis method. The DA level increased rapidly and thereafter declined in parallel to the plasma concentration in our previous study (9). We also found a clockwise hysteresis between MPD concentration and DA level in striatal dialysate. Concerning the behavioral pharmacological effects, a positive correlation was found between the locomotor activity (ambulation distance) and the extracellular DA level after i.v. administration of MPD at the low dose (2 mg/kg) to rats, while the locomotor activity decreased after administration of the higher doses (5 and 10 mg/kg). This bi-phasic phenomenon may be due to the two contrary effects, hyperkinesia and stereotypy (9). Although similar complicated changes in locomotor activities have been reported with other CNS stimulants such as amphetamine and methamphetamine (10,11), the relationship between DA level in the striatum and locomotor activity after administration of CNS stimulants has not yet been pharmacodynamically investigated.

In the present study, we developed a pharmacodynamic model for MPD-induced change in locomotive activity to clarify the complicated relationship among the disposition of MPD in plasma, the extracellular concentrations of MPD and DA in the striatum, and locomotive activity after i.v. administration of DA reuptake inhibitors.

MODEL DEVELOPMENT

The data of the concentration of MPD in plasma (8,9) and striatal dialysate (9), the striatal DA level (9), and locomotor activity (ambulation distance) (9) after i.v. administration of racemic MPD (2, 5, and 10 mg/kg) to Male Wistar rats were used for the analysis. The details of the experimental conditions were described in the previous reports. All analysis was conducted using racemic MPD concentrations. DA level in the dialysate was expressed as the ratio to the basal level determined before administration of MPD.

The pharmacokinetic—pharmacodynamic model of MPD was developed in consideration of DA release and reuptake in the dopaminergic nerve terminal in the striatum of rat (Fig. 1).

Pharmacokinetic Model of MPD

The linear two-compartment open model was used for the analysis of the plasma concentration of MPD after i.v. administration. Equation 1 was fitted to the plasma concentrations (Cp) by the least squares method to determine the pharmacokinetic parameters (8,9).

$$Cp(t) = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t) \quad (1)$$

where A (ng/ml), B (ng/ml), α (h^{-1}) and β (h^{-1}) are hybrid pharmacokinetic parameters. The time course of MPD concentration in dialysate (C_d, ng/ml) was expressed as Eq. 2,

$$V_s \cdot \frac{dC_d}{dt} = C \cdot V_p \cdot k_1 - C_d \cdot V_s \cdot k_2 \quad (2)$$

where V_p (ml) and V_s (ml) are the distribution volumes of MPD in plasma and striatal extracellular compartments, respectively.

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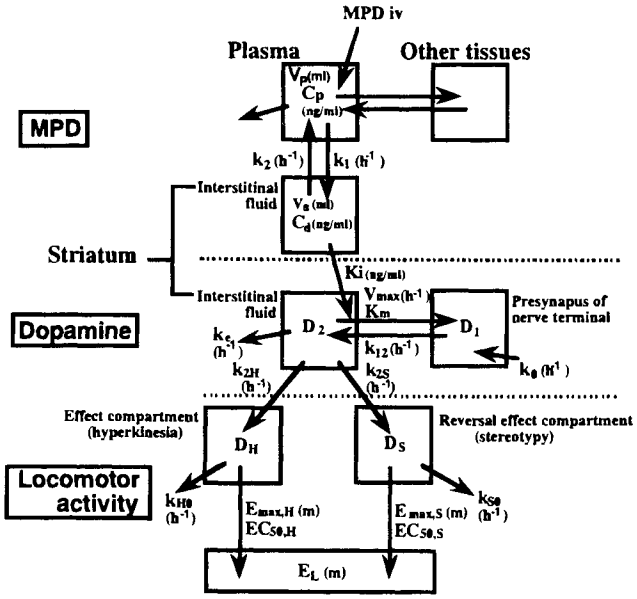

Fig. 1. Pharmacokinetic-pharmacodynamic model of MPD and DA in dopaminergic nerve terminal in striatum of rat, linked with pharmacological change of behavior.

The k_1 (h^{-1}) and k_2 (h^{-1}) are the rate constants from plasma to the striatal dialysate and that for the opposite direction, respectively. The C_d is the concentration of MPD in dialysate collected from the microdialysis probe inserted into the rat brain striatum. Since the diffusion clearance of MPD through the probe membrane obtained by previous in vitro recovery experiments (9) was sufficiently high, MPD concentration in dialysate was assumed to be parallel with the true extracellular concentration. Each parameter in Eq. 2 was obtained by simultaneous fitting to C_d values after three doses of MPD, using the plasma concentration-time profile at each dose as an input function.

Relationship Between MPD Concentration and DA Level in Striatal Dialysate

For the analysis of the clockwise hysteresis between MPD concentration and DA level in striatal dialysate, a model considering the competitive inhibition of DA reuptake by MPD was constructed with following assumptions:

(1) The DA in the dopaminergic nerve terminals (D_1) is synthesized with the zero order rate process (k_0, h^{-1}), and released into the extracellular space with the first order rate process (k_{12}, h^{-1}).

(2) The reuptake process of DA from striatal extracellular DA (D_2) to dopaminergic nerve terminal is Michaelis-Menten type transport, and the degradation of DA follows first order rate process (k_e, h^{-1}).

(3) Extracellular MPD in the striatum competitively inhibits the reuptake of DA. With these assumptions, the time courses of D_1 and D_2 are represented by Eqs. 3 and 4, respectively,

$$\frac{dD_1}{dt} = k_0 - k_{12} \cdot D_1 + \frac{V_{max} \cdot D_2}{K_m \cdot \left(1 + \frac{C_d}{K_i}\right) + D_2} \quad (3)$$

$$\frac{dD_2}{dt} = k_{12} \cdot D_1 - \frac{V_{max} \cdot D_2}{K_m \cdot \left(1 + \frac{C_d}{K_i}\right) + D_2} - k_e \cdot D_2 \quad (4)$$

where V_{max} (h^{-1}), K_m and K_i (h^{-1}) are the maximal reuptake rate, Michaelis constant and inhibition constant for DA reuptake, respectively. Each parameter was calculated by simultaneous fitting to the DA level after three doses of MPD, using C_d at each dose as an input function. DA level was represented as the ratio to the basal level.

When the drug was free at the steady state, Eq. 5 and Eq. 6 were induced from Eq. 3 and Eq. 4, respectively.

$$k_o = k_{12} \cdot D_1 - V_{max} \cdot D_2/(K_m + D_2) \quad (5)$$

$$k_e \cdot D_2 = k_{12} \cdot D_1 - V_{max} \cdot D_2/(K_m + D_2) \quad (6)$$

Therefore, k_o was equal to $k_e \cdot D_2$. As D_2 was defined to 1 before the administration of MPD, k_o became equal to k_e.

Relationship Between DA Level in Striatal Dialysate and Locomotor Activity

For the analysis of the relationship between DA level in the striatal dialysate and locomotor activity (ambulation distance), the Emax model considering two effect compartments for hyperkinesia and the reversal behavior was developed, since a high dose of MPD seemed to reduce ambulation distance, which may be due to stereotypy (9). The locomotor activity was assumed to be the difference between both activities. The DA levels in the effect compartment for hyperkinesia (D_H) and reversal behavior (D_S) were represented by Eqs. 7 and 8,

$$\frac{dD_H}{dt} = k_{2H} \cdot D_2 - k_{H0} \cdot D_H \quad (7)$$

$$\frac{dD_S}{dt} = k_{2S} \cdot D_2 - k_{S0} \cdot D_S \quad (8)$$

where k_{2H} (h^{-1}) and k_{H0} (h^{-1}) are transfer rate constants from extracellular DA to the effect compartment for hyperkinesia and the elimination rate from that, respectively, and k_{2S} and k_{S0} are those for the reversal effect. The Emax model was assumed for the relationship between DA concentrations and the effect for hyperkinesia (E_H) and reversal effect (E_S),

$$E_H = \frac{E_{max,H} \cdot D_H}{EC_{50,H} + D_H} \quad (9)$$

$$E_S = \frac{E_{max,S} \cdot D_S}{EC_{50,S} + D_S} \quad (10)$$

where $E_{max,H}$ (m), $EC_{50,H}$, $E_{max,S}$ (m) and $EC_{50,S}$ are the E_{max} and EC_{50} for hyperkinesia and the reversal effect, respectively. The locomotive activity (E_L) was represented as the difference between E_H and E_S, and E_L was assumed to be zero if $E_H < E_S$ (Eq. 11).

$$E_L = E_H - E_S \quad (E_H \geqq E_S)$$

$$E_L = 0 \quad (E_H < E_S) \quad (11)$$

Equations 5–11 were simultaneously fitted to the ambulation distances after three different doses of MPD, using DA level in dialysate at each dose as an input function.

RESULTS

Pharmacokinetics of MPD

The concentration-time profiles of MPD in plasma and pharmacokinetic parameters after i.v. administration of MPD

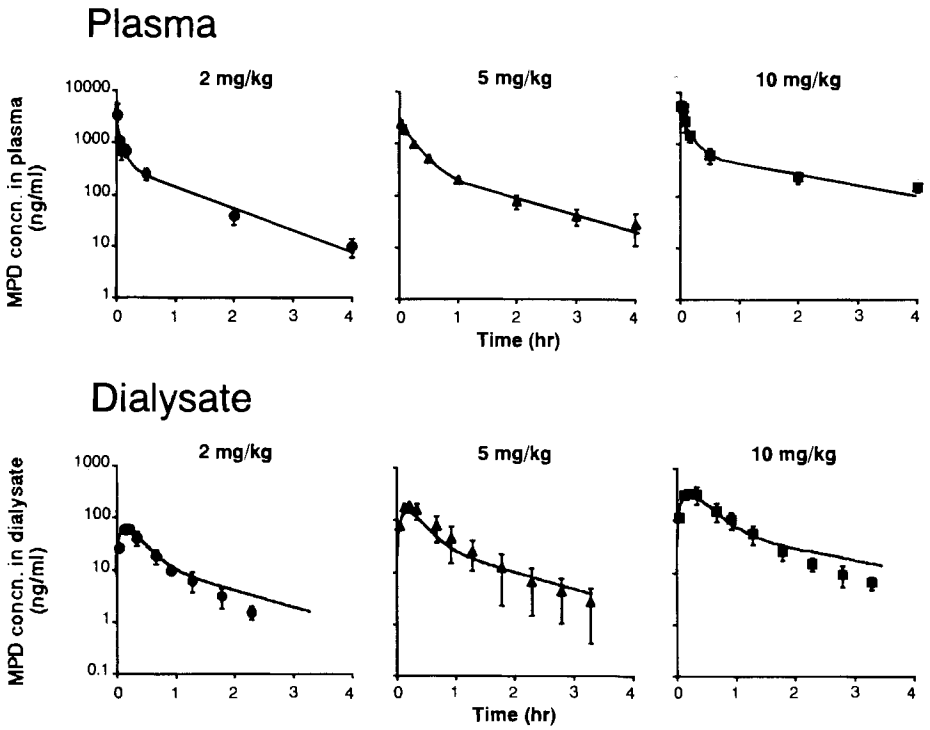

Fig. 2. Time-concentration profiles of MPD in plasma (upper) and striatal dialysate (lower) after intravenous administration of MPD (2, 5 and 10 mg/kg dose) to rats. Each point represents the mean ± SD with n = 4. Each solid line represents the simulation curve using estimated parameters.

Table 1. Pharmacokinetic Parameters After Intravenous Administration of MPD

Parameters	Dose(mg/kg)		
	2	5	10
A(ng/ml)	1051.7	2379.5	5462.0
B(ng/ml)	171.2	379.4	738.2
$\alpha(h^{-1})$	4.49	4.86	3.68
$\beta(h^{-1})$	0.722	0.733	0.541

(2, 5 and 10 mg/kg dose) are shown in Fig. 2 (upper panel) and Table 1, respectively. Dose dependency was not observed, and the elimination half-life was approximately 1 h within the dose range in this study. The concentration of MPD in the dialysate rapidly elevated after i.v. administration, reached the maximum at 15 min, and declined in parallel with the plasma concentration thereafter (Fig. 2, lower panel). Eq. 2 was fitted to the C_d at each dose to estimate the $k_1 \cdot V_P/V_S$ and k_2 by the simultaneous nonlinear least squares method (Table 2). The MPD concentration-time profiles in dialysate correlated well with the observed values.

Table 2. Brain Distribution Kinetic Parameters After Intravenous Administration of MPD

Parameters	Estimated values (mean ± Standard error)
$k_1 \cdot V_p/V_s$	0.830 ± 0.073 hr^{-1}
k_2	8.025 ± 0.832 hr^{-1}

Relationship Between MPD Concentration and DA Level in Striatal Dialysate

Figure 3 shows the DA level in striatal dialysate-time profiles and the fitting lines after i.v. administration of MPD. The estimated parameters are listed in Table 3. The DA level in the striatal dialysate rapidly increased as MPD concentration in the dialysate increased, and reached the maximum at 5–10 min. However, DA level returned to the basal level before MPD in dialysate was eliminated completely. Since the rate of decline of DA level was faster than that of MPD in dialysate, a clockwise hysteresis was observed in the relationship between MPD concentration and DA level in the striatal dialysate as shown in Fig. 4.

Relationship Between DA Level in Striatal Dialysate and Locomotor Activity

The locomotor activity-time profiles after i.v. administration of three doses of MPD is shown in Fig. 5. At the low dose of 2 mg/kg of MPD, both the DA level in the striatum and the

Table 3. DA Disposition Parameters After Intravenous Administration of MPD

Parameters	Estimated values (mean ± Standard error)
k_e	29.13 ± 18.95 hr^{-1}
k_{12}	4.232 ± 2.330 hr^{-1}
K_m	1.268 ± 0.810
V_{max}	504.6 ± 350.1 hr^{-1}
K_i	9.635 ± 17.22 ng/ml (41.3 ± 73.8 nM)

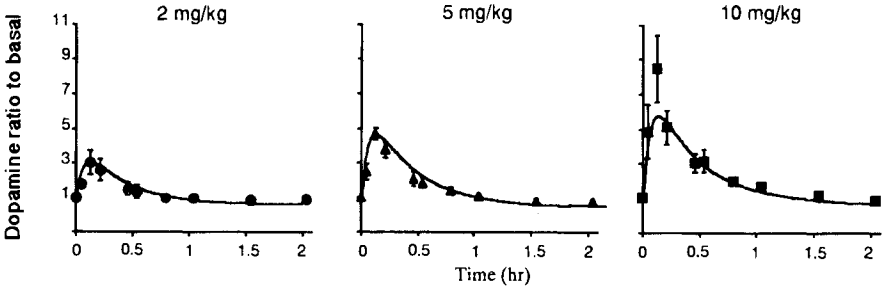

Fig. 3. Time-DA level in striatal dialysate profiles after intravenous administration of MPD (2, 5 and 10 mg/kg dose) to rats. Each point represents the mean ± SD with n = 4. Each solid line represents the simulation curve using estimated parameters.

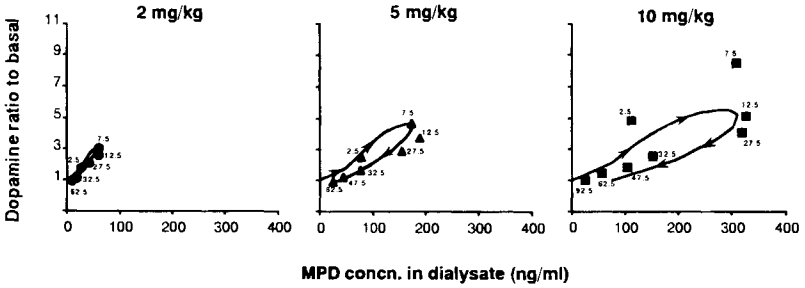

Fig. 4. Relationship between MPD concentration and DA level in the striatal dialysate after intravenous administration of MPD (2, 5 and 10 mg/kg dose) to rats. Each point represents the mean value with n = 4. Each solid line represents the simulation curve using estimated parameters. The numbers in figure are expressed the time (min) after administration.

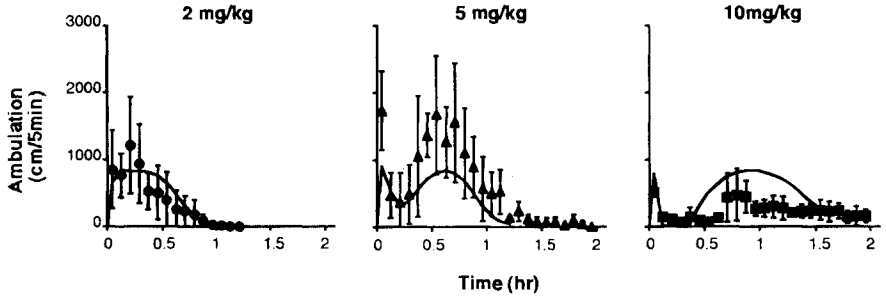

Fig. 5. Time-ambulation distance after intravenous administration of MPD (2, 5 and 10 mg/kg dose) to rats. Each point represents the mean ± SD with n = 4. Each solid line represents the simulation curve using estimated parameters.

locomotive activity rapidly increased, and both declined to basal level within 1 hr. On the other hand, the locomotive activity transiently increased, then rapidly decreased within 20 min, and increased again to reach the maximum at about 30 min after 5 mg/kg of MPD administration. Further, marked change in ambulation distance was not observed after 10 mg/kg of MPD, though the DA level in the dialysate increased dose-dependently. Such a complicated behavioral change after MPD administration was analyzed by simultaneous fitting of Eqs. 5–11 to estimate each parameter, using DA level in the dialysate after three doses of MPD as an input function (Table 4). we could demonstrate the bi-phasic phenomenon in the locomotive activity-time profiles by this model (Fig. 5).

Table 4. Pharmacodynamic Parameters for Locomotive Change After Intravenous Administration of MPD

Parameters	Estimated values (mean ± Standard error)
k_{2H}	32.7 ± 379.9 hr^{-1}
k_{2S}	6.15 ± 15.61 hr^{-1}
k_{L0}	40.2 ± 65.8 hr^{-1}
k_{S0}	18.6 ± 14.4 hr^{-1}
$EC_{50,H}$	1.027 ± 0.617
$EC_{50,S}$	4.52 ± 50.48
$E_{max,H}$	9.74 ± 3.09 m
$E_{max,S}$	32.8 ± 249.1 m

DISCUSSION

It is known that MPD inhibits DA reuptake at the dopaminergic nerve terminals (6,7) and the resulting elevation of DA level is related to the pharmacological activity of MPD. We have investigated the pharmacodynamics of MPD in rats (9) and humans (12), and demonstrated the stereoselectivity of MPD in pharmacokinetics and pharmacological activity. The DA level in the striatum increased after i.v. administration of MPD, which may probably be due to the inhibition of DA reuptake mainly by (+)-MPD.

Increase of DA level was positively correlated to the (+)-MPD concentration in the striatum with a clockwise hysteresis. Several pharmacodynamic models have been reported (4,13–15) for the clockwise hysteresis to explain the pharmacodynamics of drugs which exhibit acute tolerance or desensitization. In the models for pharmacodynamics of cocaine (13) and nicotine (15), the effect compartment model involving hypothetical antagonistic metabolites was considered. In the case of furosemide (14), the effect of the drug was assumed to decrease time-dependently. Recently, we have developed the tolerance model considering receptor desensitization for the pharmacodynamic analysis of muscle contraction change induced by cholinesterase inhibitors (16). In the present study, we could explain this clockwise relationship between MPD concentration and DA level in striatal dialysate by considering the depletion of DA in the dopaminergic neuron terminal.

The standard errors of the K_m and K_i values in Table 3 were quite large, which may be because the concentration of MPD in dialysate was much higher than the K_i value, therefore the reuptake of DA may be completely inhibited. DA concentration also increased so high that the reuptake process was saturated in this experimental condition, preventing precise estimation of K_m. Given, however, that this process is saturated and is competitively inhibited by MPD, the Michaelis-Menten type reuptake process was assumed for DA disposition. The estimated K_i value is consistent with the value reported in the previous in vitro study (281 nM, 17, 720 nM, 18), if the data are corrected with recovery in microdialysis probe (9,19).

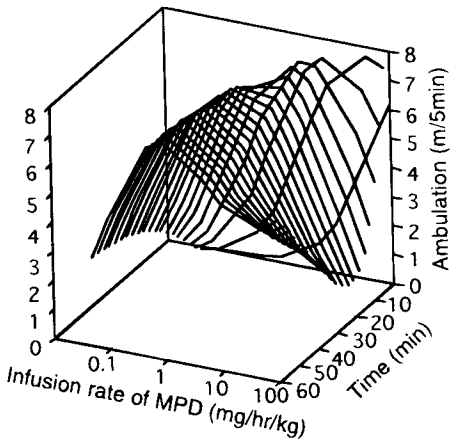

Fig. 6. Simulation of the drug effect during constant infusion of MPD to rats. X-axis: Time, Y-axis: Infusion rate of MPD, Z-axis: Ambulation distance as drug effect. Solid lines represent the relationships between infusion rate of MPD and ambulation every 3 min from 0 to 60 min for infusion.

Since behavioral change after MPD administration showed bi- or tri-phasic effects on locomotive activity in rats, the pharmacodynamics of MPD was clearly complex. In order to explain MPD's complicated pharmacological characteristics, the Emax model was developed for considering two contrary effect compartments for change of locomotive activity. Similar complicated pharmacodynamic behavior has been reported for other CNS stimulants, such as amphetamine and methamphetamine (10,11). Since these drugs are also DA reuptake inhibitors, application of a similar model for pharmacodynamic analysis may be useful.

In this study, only locomotion data was used for concentration-effect relationship analysis. By applying the Emax model to analyze both hyperactivity and stereotyped behaviors, one possible explanation of dose-dependent, bi-phasic behavioral change after MPD administration was obtained. An alternative models with fewer parameters (e.g. linear model) may be more appropriate, since the locomotion data alone may be insufficient to estimate 6 parameters for the incompatible behaviors.

Figure 6 shows the relationship among infusion dose, ambulation distance, and time after infusion of MPD. The ambulation distance was simulated using the developed pharmacokinetic-pharmacodynamic model, if various i.v. doses of MPD are infused constantly within the range of 0.05–100 mg/h/kg. The effect seemed larger with increase of dose within the range of 0.05–0.5 mg/h/kg. The effect increased dose dependently until 6 min after infusion, taking on a bell shape (i.e. ceiling effect) with one peak after 9 min. These results show that the complicated pharmacological effect of MPD after i.v. infusion can also be predicted over a wide dose range. It has been reported that the ceiling effect occurs with larger doses of buprenorphine, an analgesic, since this drug has both agonistic and antagonistic properties (20). In the case of MPD, binding to the receptor may explain the two types of locomotor activity, hyperkinesia and the reversal effect possibly due to the stereotyped activity of the drug. Consequently, the locomotor activity (E_L) obtained by subtracting stereotyped activity (E_S) from the hyperkinesia activity (E_H) may lead to the ceiling effect, since the ED_{50} in the E_S may be larger than that in the E_S. Furthermore, the measurement of stereotyped behavior is necessary for the detailed analysis of the relationships between DA level and locomotor activity.

In conclusion, we developed a unified explanation of the processes between administration of MPD and the observed changes in behavior: transfer of MPD into the brain, inhibition of DA reuptake, change in locomotion activity. Acute tolerance of DA increase induced by MPD can be explained by the depletion of DA at the dopaminergic nerve terminal in this model. The developed model can also represent the complicated effect of MPD on locomotive activity, and may possibly be applicable to the pharmacodynamic analysis of other DA reuptake inhibitors.

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