Pharmacokinetic and Pharmacodynamic Studies of L-Dopa in Rats. II. Effect of L-Dopa on Dopamine and Dopamine Metabolite Concentration in Rat Striatum

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The purpose of this investigation was to quantitatively describe the time courses of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations in the striatum after L-dopa injection using a constructed dopamine metabolism model. The time courses of dopamine, DOPAC and HVA concentration in the striatum of rats was determined before and after the rapid i.v. injection of 10, 50 and 100 mg/kg using the same animals as in the previous report. The endogenous dopamine, DOPAC and HVA concentrations in the striatum before L-dopa administration were $5.9 \pm 0.7 \mu\text{g}$, $3.6 \pm 0.4 \mu\text{g}$ and $1.0 \pm 0.2 \mu\text{g/g}$, respectively. The dopamine concentration in the striatum increased immediately after L-dopa injection, with the peak concentration ($15.9 \pm 0.5 \mu\text{g/g}$) occurring at 3 min; then it returned to the pre-medication level until 2 h at 100 mg/kg dosing. The time course of dopamine concentration in the striatum was analyzed on a constructed dopamine metabolism model which has a zero-order production rate for the production of dopamine (*i.e.* release from the dopamine neuronal terminals) and two apparent first-order clearance terms, one from L-dopa to dopamine, which was estimated in the previous report, and the other from dopamine to dopamine metabolites (DOPAC and HVA). However, the time course of dopamine concentration in the striatum could not be described by this model. Since the effect of L-dopa on the enlargement of dopamine concentration is known to be attributable to the endogenously released dopamine from the dopamine neuronal terminals, the time course of dopamine concentration in the striatum after L-dopa injection was analyzed on the assumption that the effect of L-dopa on the increase of dopamine concentration is caused not only by the metabolism from L-dopa to dopamine but also by the endogenously released dopamine from dopamine neuronal terminals. The result indicated that the effect of L-dopa on the enlargement of dopamine concentration could be described quantitatively by these assumptions. The DOPAC and HVA concentrations in the striatum also increased gradually after L-dopa injection, with the peak concentration (15.6 ± 2.0 and $6.6 \pm 0.3 \mu\text{g/g}$) occurring at 20 and 90 min, and they then returned to the control level until 4 and 6 h, respectively, at 100 mg/kg dosing. The time course of DOPAC and HVA concentration in the striatum could be reasonably well described by a constructed dopamine metabolism model which has an apparent first-order clearance from dopamine to DOPAC and HVA, and Michaelis–Menten type elimination kinetics of DOPAC and HVA. Thus, it was clarified that the time courses of dopamine, DOPAC and HVA concentration in rat striatum after the i.v. injection of L-dopa can be explained using the dopamine metabolism model. This dopamine metabolism model might be able to be used for the pharmacokinetic–pharmacodynamic analysis of dopaminergic acting drugs.

Keywords L-dopa; dopamine; 3,4-dihydroxyphenylacetic acid (DOPAC); homovanillic acid (HVA); striatum; pharmacokinetics

It has been reported that the effect of L-dopa on the elevation of dopamine concentration is attributed to the following mechanisms: (a) the endogenously released dopamine from the dopamine neuronal terminals *in vitro*¹⁾ and *in vivo*,²⁾ (b) the acceleration of dopamine formation from L-dopa to dopamine,³⁾ (c) the enlargement of dopamine content in the brain.⁴⁾ It is generally agreed that the enhancement of dopamine concentration in the striatum after L-dopa administration is caused not only by the exogenous conversion from L-dopa to dopamine, but also by the endogenously released dopamine from dopamine neuronal terminals. Although several types of clinical fluctuations related to drug treatment have been identified, the most common cause of therapy related fluctuations is an end-of-dose phenomena, often called “wearing-off”.⁵⁾ It has been suggested that the possible cause of these phenomena are caused by both the impaired conversion of L-dopa to dopamine and the reduced capacity for the storage of newly synthesized dopamine in dopaminergic neuronal terminals in Parkinson’s disease striatum. However, comprehensive pharmacokinetic studies on the penetration of L-dopa into

the striatum and the metabolism from L-dopa to dopamine and dopamine metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)) in the striatum have not been performed. In the previous study, it was clarified that the time course of L-dopa concentration in the striatum after the i.v. injection of L-dopa could be described by a basic physiological model, and the apparent metabolism clearance from L-dopa to dopamine in the striatum could be determined. The purpose of this investigation was to describe quantitatively the time courses of dopamine, DOPAC and HVA concentration in the striatum after L-dopa injection using a constructed dopamine metabolism model.

MATERIALS AND METHODS

Chemicals L-Dopa (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was purchased commercially and used without further purification. This drug was dissolved in an isotonic sodium chloride solution (dissolved with the aid of 0.1 N HCl) and was administered intravenously. All other chemicals were of reagent grade and were

obtained commercially (Wako Pure Chemical Industries, Osaka, Japan).

Animal Experiments Inbred male Wistar rats (Sankyo Lab Service Corporation, Inc., Shizuoka, Japan) were used in this investigation. Before the investigation, the rats were housed individually in metal cages in an environment of controlled temperature (22–24 °C) and alternating 12 h light (7 a.m.—7 p.m.) and dark cycles. Food and water were withdrawn in the morning on the day of the experiment, and these rats were placed in individual plastic metabolic cages before the experiment. The rats used for the pharmacokinetic studies of dopamine, DOPAC and HVA in the striatum had indwelling cannula implanted in the right jugular vein 1 d before the experiment. To characterize the disposition of dopamine, DOPAC and HVA in the striatum using a constructed dopamine metabolism model, L-dopa 10, 50 and 100 mg/kg was injected rapidly into the right jugular vein. Before drug administration, and at 1, 2, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after the dosing. Immediately after blood collection each rat was sacrificed by 2 mmol/ml KCl (1 ml) injection and their whole brains were quickly excised, rinsed with cold physiological saline and dissected into the corpus striatum according to a modification of the method of Glowinski and Iversen.⁶⁾ The striatum samples were stored at –80 °C until analysis.

Assay Methods for Dopamine, DOPAC and HVA The striatum concentrations of dopamine, DOPAC and HVA were determined by a modification of the high-performance liquid chromatographic assay of Murai *et al.*⁷⁾ with isoproterenol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as the internal standard. The striatum samples (0.05 g) were put into glass test tubes and homogenized with a polytron homogenizer (PT 10-35, Kinematica, Switzerland) at 15000 rpm for 10 s in 500 μ l of 0.1 M perchloric acid containing 10 μ M EDTA 2Na and isoproterenol for the precipitation of protein. After centrifugation at 4000 rpm for 10 min at 4 °C, the clear supernatants were filtered through a 0.45 μ m filter (disposable syringe filter unit, dsmic-3cp cellulose acetate, Advantec, Tokyo, Japan), and 10 μ l of the filtrates were injected onto the HPLC system. The resultant filtrate was loaded onto a reversed-phase HPLC column (Supelcosil LC-18-DB, Supelco, Inc., PA, U.S.A.). The solvent delivery system (L-5000 LC controller and 655A-11 pump, Hitachi, Ltd., Tokyo, Japan) was equipped with an electrochemical detector (ECD-100, Eicom Co., Kyoto, Japan) at +0.7 V vs. an Ag-AgCl reference electrode with an auto sampler (AS-8010, Tosoh, Co., Tokyo, Japan) and with a chromato-integrator (D-2500, Hitachi, Ltd., Tokyo, Japan). A guard column (Supelcosil LC-18-DB, Supelco, Inc., PA, U.S.A.) was placed between the auto sampler and the analytical column. The mobile phase was 0.01 M citrate buffer (pH 4.4)–MeOH (90:10 v/v) containing 10 μ M EDTA 2Na and 0.5 mM sodium 1-octanesulfonate, and the flow rate was 1.0 ml/min. Retention times were 9, 24, 27 and 45 min for DOPAC, HVA, dopamine and internal standard, respectively.

Pharmacokinetic Analysis In order to quantitatively describe the time course of dopamine, DOPAC and HVA concentration in the striatum after L-dopa injection, the

Fig. 1. Diagrammatic Representation of Pharmacodynamic and Pharmacokinetic Models for L-Dopa, Dopamine, DOPAC and HVA in Rat Striatum

dopamine metabolism model was constructed and is shown in Fig. 1.

The time course of dopamine concentration in the striatum was analyzed on the constructed dopamine metabolism model which has a zero-order production rate for the creation of dopamine (*i.e.* release from the dopamine neuronal terminals) and two apparent first-order constant terms, one from L-dopa to dopamine, the other from dopamine to dopamine metabolites (DOPAC and HVA). The changes in the concentration of dopamine in the striatum could be expressed as follows:

$$V_{st} \frac{dC_{DO}}{dt} = k_{0DO} + CL_m C_{LD} - (CL_{DP} + CL_{HV}) C_{DO} \quad (1)$$

where V_{st} is the weight of the striatum (g), and C_{LD} and C_{DO} represent the concentrations of L-dopa and dopamine in the striatum (μ g/g), respectively. k_{0DO} is the zero-order production rate of endogenous dopamine in the striatum (μ g/h). CL_m represents the metabolism clearance from L-dopa to dopamine in the striatum (ml/h). CL_{DP} and CL_{HV} represent the metabolism clearance from dopamine to DOPAC and from dopamine to HVA (ml/h), respectively. Since the effect of L-dopa on the enlargement of dopamine concentration is known to be attributable to the endogenously released dopamine from the dopamine neuronal terminals, the time course of dopamine concentration in the striatum after L-dopa injection was analyzed on the assumption that the effect of L-dopa on the increase of dopamine concentration is caused not only by the metabolism from L-dopa to dopamine but also the endogenously released dopamine from dopamine neuronal terminals. It was assumed that the relationship between striatum L-dopa concentration and the pharmacologic effect of L-dopa at dopamine neuronal terminals can be described by the Emax model. These assumptions were expressed as following equations:

$$R-LD = \frac{E-LD C_{LD}^*}{K-LD + C_{LD}^*} \quad (2)$$

$$V_{st} \frac{dC_{DO}}{dt} = k_{0DO} + R-LD + CL_m C_{LD} - (CL_{DP} + CL_{HV}) C_{DO} \quad (3)$$

where R-LD is the pharmacologic effect of L-dopa on the striatum dopamine production. C_{LD}^* is the difference in concentration of L-dopa in the striatum between the control value and the enhancement value (μ g/g). E-LD is the maximum effect of the L-dopa on the striatum dopamine production (μ g/h); $E-LD' = E-LD/V_{st}$ (μ g/g/h). K-LD is the L-dopa concentration in the striatum at half of the maximum effect (μ g/g). At steady state, the left sides

of Eqs. 2 and 3 equal zero. Rearrangement of Eq. 3 yields the following equation:

$$\text{INFDO} = (k_{\text{DP}} + k_{\text{HV}})C_{\text{DOO}} - k_{\text{m}}C_{\text{LDO}} \quad (4)$$

where INFDO is the zero-order production rate of endogenous dopamine concentration in the striatum ($\mu\text{g/g/h}$); $\text{INFDO} = k_{\text{DOO}}/V_{\text{st}}$. C_{DOO} and C_{LDO} are the dopamine and L-dopa concentrations in the striatum at steady state ($\mu\text{g/g}$), respectively. k_{DP} and k_{HV} represent the metabolism constant from dopamine to DOPAC and from dopamine to HVA (h^{-1}), respectively; $k_{\text{DP}} = CL_{\text{DP}}/V_{\text{st}}$ and $k_{\text{HV}} = CL_{\text{HV}}/V_{\text{st}}$. k_{m} represents the metabolism constant from L-dopa to dopamine in striatum (h^{-1}); $k_{\text{m}} = CL_{\text{m}}/V_{\text{st}}$. The value of the production rate constant of endogenous dopamine concentration in the striatum can be calculated by Eq. 4.

The time courses of DOPAC and HVA concentration in the striatum were analyzed using the constructed dopamine metabolism model which has apparent first-order clearance from dopamine to DOPAC and HVA, and using the Michaelis–Menten type elimination kinetics of DOPAC and HVA. The changes in the concentrations of DOPAC and HVA in the striatum could be expressed as follows in Eqs. 5 and 6, respectively:

$$V_{\text{st}} \frac{dC_{\text{PA}}}{dt} = CL_{\text{DP}}C_{\text{DO}} - \frac{V_{\text{max}}(\text{PA})C_{\text{PA}}}{K_{\text{m}}(\text{PA}) + C_{\text{PA}}} \quad (5)$$

$$V_{\text{st}} \frac{dC_{\text{HV}}}{dt} = CL_{\text{HV}}C_{\text{DO}} - \frac{V_{\text{max}}(\text{HV})C_{\text{HV}}}{K_{\text{m}}(\text{HV}) + C_{\text{HV}}} \quad (6)$$

where V_{st} is the volume of the striatum (g), and C_{DO} , C_{PA} and C_{HV} represent the concentrations of dopamine, DOPAC and HVA in the striatum ($\mu\text{g/g}$), respectively. CL_{DP} and CL_{HV} represent the metabolism clearance from dopamine to DOPAC and from dopamine to HVA (ml/h), respectively. $V_{\text{max}}(\text{PA})$ and $V_{\text{max}}(\text{HV})$ represent the maximum elimination rate of DOPAC and HVA for Michaelis–Menten kinetics (ml/h), respectively. $K_{\text{m}}(\text{PA})$ and $K_{\text{m}}(\text{HV})$ represent the DOPAC and HVA concentrations in the striatum at half the maximum elimination rate of DOPAC and HVA for Michaelis–Menten kinetics ($\mu\text{g/g}$), respectively. At steady state, the left sides of Eqs. 5 and 6 equal zero. Rearrangement of Eqs. 5 and 6 yield the following Eqs. 7 and 8, respectively:

$$V_{\text{max}}(\text{PA})' = k_{\text{DP}}C_{\text{DOO}}(K_{\text{m}}(\text{PA}) + C_{\text{PAO}})/C_{\text{PAO}} \quad (7)$$

$$V_{\text{max}}(\text{HV})' = k_{\text{HV}}C_{\text{DOO}}(K_{\text{m}}(\text{HV}) + C_{\text{HVO}})/C_{\text{HVO}} \quad (8)$$

where $V_{\text{max}}(\text{PA})'$ and $V_{\text{max}}(\text{HV})'$ represent the maximum elimination rate of DOPAC and HVA for Michaelis–Menten kinetics ($\mu\text{g/g/h}$), respectively; $V_{\text{max}}(\text{PA})' = V_{\text{max}}(\text{PA})/V_{\text{st}}$ and $V_{\text{max}}(\text{HV})' = V_{\text{max}}(\text{HV})/V_{\text{st}}$. $K_{\text{m}}(\text{PA})$ and $K_{\text{m}}(\text{HV})$ represent the DOPAC and HVA concentrations in the striatum at half the maximum elimination rate of DOPAC and HVA for Michaelis–Menten kinetics ($\mu\text{g/g}$), respectively. C_{DOO} , C_{PAO} and C_{HVO} are the dopamine, DOPAC and HVA concentrations in the striatum at steady state ($\mu\text{g/g}$), respectively. k_{DP} and k_{HV} represent the metabolism constant from dopamine to DOPAC and from dopamine to HVA (h^{-1}), respectively; $k_{\text{DP}} = CL_{\text{DP}}/V_{\text{st}}$ and $k_{\text{HV}} = CL_{\text{HV}}/V_{\text{st}}$. The value of the maximum elimination

rate of DOPAC and HVA for Michaelis–Menten kinetics can be calculated by Eqs. 7 and 8, respectively.

In order to estimate the pharmacokinetic and pharmacodynamic parameters of E-LD', K-LD, k_{DP} , k_{HV} , $K_{\text{m}}(\text{PA})$ and $K_{\text{m}}(\text{HV})$, the data on the concentrations of dopamine, DOPAC and HVA in the striatum after the i.v. administration of L-dopa were fitted to Eq. 2 though Eq. 8 by a nonlinear least squares regression program, FKDM,⁽⁸⁾ using a digital computer (PC-9821 Ae, NEC Corp., Tokyo, Japan). The inverse value of each data was used as the weighting value ($1/W = 1$) of the least squares method. Convergency was assumed to be complete when the iteration for the relative change in the sum of weighted squares was less than 10^{-6} .⁽⁹⁾ The values of INFDO and $V_{\text{max}}(\text{PA})'$ and $V_{\text{max}}(\text{HV})'$ were calculated by Eqs. 4, 7 and 8.

RESULTS

Dopamine Concentrations in the Striatum The time course of dopamine concentration in the striatum before and after the i.v. administration of L-dopa 100 mg/kg in the same animals as in the previous report are shown in Fig. 2. The endogenous dopamine in the striatum before L-dopa administration was $5.9 \pm 0.7 \mu\text{g/g}$. The dopamine concentration in the striatum increased immediately after L-dopa injection, with the peak concentration ($15.9 \pm 0.5 \mu\text{g/g}$) occurring at 3 min, and a return to the pre-medication level until 2 h at 100 mg/kg dosing. The time course of dopamine concentration in the striatum was analyzed using the dopamine metabolism model. The dotted line in Fig. 2 represents the calculated values which were obtained using Eq. 1. It was clarified that the enhancement of dopamine concentration in the striatum after the i.v. administration of L-dopa cannot be described by the apparent first-order clearance from L-dopa to dopamine which was estimated in the previous report. Since the effect of L-dopa on the enlargement of dopamine concentration is known to be attributable to the endogenously released dopamine from the dopamine neuronal terminals, the time course of dopamine concentration in the striatum after the L-dopa injection was analyzed on the assumption that the effect of L-dopa on the increase in dopamine concentrations was caused not only by the metabolism from L-dopa to dopamine but also to the endogenously released dopamine from dopamine neuronal terminals. The solid lines in Fig. 2 represent the calculated values which were obtained using Eqs. 2 and 3. The results indicated that the effect of L-dopa on the enlargement of dopamine concentration can be described quantitatively by these assumptions. The pharmacokinetic and pharmacodynamic parameters (E-LD', K-LD, k_{DP} and k_{HV}) were computed by the nonlinear least squares method, and INFDO was calculated using Eq. 4. See Table I.

DOPAC and HVA Concentrations in the Striatum The time course of DOPAC and HVA concentration in the striatum before and after the i.v. administration of L-dopa 100, 50 and 10 mg/kg are shown in Figs. 3 and 4, respectively. The endogenous DOPAC and HVA concentrations in the striatum before L-dopa administration were $3.6 \pm 0.4 \mu\text{g/g}$ and $1.0 \pm 0.2 \mu\text{g/g}$, respectively. The DOPAC

Fig. 2. Time Courses of Dopamine Concentration in the Striatum before and after Intravenous Administration of L-Dopa

(a) 100 mg/kg ($n=3$), (b) 50 mg/kg ($n=3$), (c) 10 mg/kg ($n=3$). The upper and lower panels represent the same data in the striatum but on a different time scale. The plotted points represent the observed data and the dotted line and the solid line represent the calculated values using Eq. 1 and 3, respectively, in the text. Each experimental point shown represents the mean $\pm$ S.D.

and HVA concentrations in the striatum increased gradually after L-dopa injection, with the peak concentration ($15.6 \pm 2.0 \mu\text{g}$ and $6.6 \pm 0.3 \mu\text{g/g}$) occurring at 20 and 90 min and returning to the control level until 4 and 6 h, respectively, at 100 mg/kg dosing. The time courses of DOPAC and HVA concentration in striatum were analyzed using the dopamine metabolism model. The solid lines in Figs. 3 and 4 represent the calculated values which were obtained using Eqs. 5 and 6. The results indicated that the time course of DOPAC and HVA concentrations in the striatum after i.v. injection of L-dopa can be described quantitatively by the dopamine metabolism model. The pharmacokinetic parameters ($K_m(\text{PA})$ and $K_m(\text{HV})$) were computed by the nonlinear least squares method, and $V_{\max}(\text{PA})'$ and $V_{\max}(\text{HV})'$ were calculated by Eqs. 7 and 8 and are listed in Table I.

DISCUSSION

Prolonged treatment using L-dopa is often associated with various side effects, including a gradual decline in efficacy.¹⁰⁾ This side effect is closely related to alterations of the availability of L-dopa in the striatum. However, comprehensive pharmacokinetic studies on the penetration of L-dopa into the striatum and the metabolism from L-dopa to dopamine and dopamine metabolites in the striatum have not been performed. In the previous report, it was clarified that the time course of L-dopa concentration in rat striatum can be described by a physiologically based

pharmacokinetic model, and the diffusion clearance of L-dopa from plasma to the striatum and the apparent metabolism clearance from L-dopa to dopamine in the striatum can be determined using this model.¹¹⁾ The purpose of this investigation was to develop a dopamine metabolism model for dopamine, DOPAC and HVA in the striatum and to quantitatively describe the time course of dopamine, DOPAC and HVA concentration in the striatum after the i.v. injection of L-dopa using the dopamine metabolism model.

In the present study, a simple kinetic model for dopamine, DOPAC and HVA concentration in the striatum was constructed. Several kinetic models have been used to explain the disposition of dopamine and dopamine metabolites in the striatum.¹²⁾ There are two major pathways responsible for the catabolism of dopamine in the striatum. The deamination and *o*-methylation of dopamine is caused by monoamine oxidase (MAO) and catechol-*o*-methyltransferase (COMT), respectively. Westerink and Korf showed that dopamine is predominantly metabolized to DOPAC and that this metabolite is partly removed from the brain and partly *o*-methylated to HVA.¹³⁾ Westerink and Spaan demonstrated that about 80% of HVA is formed from DOPAC and 20% from 3-methoxytyramine in rat striatum.¹⁴⁾ It has been well presented that there are other pathways responsible for the eliminations of dopamine in the striatum, such as the conversion from dopamine to norepinephrine by dopamine β -hydroxylase¹⁵⁾ and the re-uptake of dopamine

TABLE I. Values of Pharmacokinetic and Pharmacodynamic Parameters of L-Dopa, Dopamine, DOPAC and HVA in Rats Obtained from Computer Fitting and Calculating of Striatum Concentration Data after Intravenous Administration of L-Dopa 100, 50 and 10 mg/kg

C_{LD0}	($\mu\text{g/g}$) ^{a)}	38.8×10^{-3}
C_{DO0}	($\mu\text{g/g}$) ^{b)}	5.9 ± 0.7 (6)
C_{PA0}	($\mu\text{g/g}$) ^{b)}	3.6 ± 0.4 (6)
C_{HV0}	($\mu\text{g/g}$) ^{b)}	1.0 ± 0.2 (6)
k_m	(h^{-1}) ^{c)}	12.93
E-LD'	($\mu\text{g/g/h}$) ^{d)}	134.29 ± 13.38
K-LD	($\mu\text{g/g}$) ^{d)}	0.276 ± 0.089
k_{DP}	(h^{-1}) ^{d)}	17.56 ± 2.714
k_{HV}	(h^{-1}) ^{d)}	2.128 ± 0.330
INFDO	($\mu\text{g/g/h}$) ^{e)}	116.67
K_m (PA)	($\mu\text{g/g}$) ^{d)}	3.002 ± 0.484
K_m (HV)	($\mu\text{g/g}$) ^{d)}	0.255 ± 0.046
$V_{\max}$ (PA)	($\mu\text{g/g/h}$) ^{e)}	192.41
$V_{\max}$ (HV)	($\mu\text{g/g/h}$) ^{e)}	15.915
V_{st}	(g) ^{b)}	0.082
CL_m	(ml/h) ^{f)}	1.060
k_{ODO}	($\mu\text{g/h}$) ^{f)}	9.567
E-LD	($\mu\text{g/h}$) ^{f)}	11.01
CL_{DP}	(ml/h) ^{f)}	1.440
CL_{HV}	(ml/h) ^{f)}	0.175
$V_{\max}$ (PA)	($\mu\text{g/h}$) ^{f)}	15.78
$V_{\max}$ (HV)	($\mu\text{g/h}$) ^{f)}	1.305

a) In the previous report, the value of this parameter was calculated by the following equation: $C_{stss} = k_0 / CL_m \times k_{in} / k_{out}$. b) The values of these parameters were measured in this study, and the number of animals used is indicated in parentheses. c) In the previous report, the value of this parameter was calculated by the following equation: $k_m = k_{out} - k_{in}$. d) The pharmacokinetic parameters for L-dopa (E-LD' and K-LD) and the pharmacodynamic parameters for dopamine, DOPAC and HVA (k_{DP} , k_{HV} , K_m (PA) and K_m (HV)) in the striatum were estimated by fitting the data to Eqs. 2, 3, 5 and 6, respectively, using the computer program FKDM. All values are expressed as the mean $\pm$ S.D. of the estimated parameters. e) The values of these parameters were calculated by Eqs. 4, 7 and 8, respectively. f) The values of these parameters were calculated by the following equations, respectively. $CL_m = k_m \times V_{st}$, $k_{ODO} = INFDO \times V_{st}$, $E-LD = E-LD' \times V_{st}$, $CL_{DP} = k_{DP} \times V_{st}$, $CL_{HV} = k_{HV} \times V_{st}$, $V_{\max}$ (PA) = $V_{\max}$ (PA)' $\times V_{st}$, $V_{\max}$ (HV) = $V_{\max}$ (HV)' $\times V_{st}$.

into dopamine neuronal terminals by the carrier-mediated transport system.¹⁶⁾ However, in this study, in order to construct a simple model for dopamine metabolism and to obtain stable values of the parameters for dopamine and dopamine metabolites (DOPAC and HVA), the metabolism parameter from DOPAC to HVA and the conversion parameters from dopamine to norepinephrine or other dopamine metabolites and the elimination parameter regarding the re-uptake of dopamine into dopamine neuronal terminals were not assumed. If the data of norepinephrine or other dopamine metabolite concentrations in the striatum or dopamine concentrations in dopamine neuronal terminals after L-dopa administration are obtained, the dopamine metabolism model will be reconstructed and the obtained value of INFDO by Eq. 4 will be altered. Thus, the constructed dopamine metabolism model in this study is not a unique model for explaining the kinetic behavior of dopamine and dopamine metabolites in the striatum.

Dedek *et al.* showed that most of the DOPAC and the HVA is eliminated from the brain in a conjugated form.¹⁷⁾ Therefore, in this study, it was assumed that the elimination of DOPAC and HVA from rat striatum can be explained by Michaelis-Menten type elimination kinetics. It has been well recognized that MAO exists on the outer surface of mitochondria within the dopamine neuronal terminals.¹⁵⁾ It can be considered that the metabolism from dopamine to DOPAC and from dopamine to HVA might be explained by Michaelis-Menten type kinetics. The Michaelis-Menten equation ($V_{\max} C / (K_m + C)$) can be rearranged to the following equation, $V_{\max}' C / (1 + K_m' C)$: $V_{\max}' = V_{\max} / K_m$ and $K_m' = 1 / K_m$. Analysis of the metabolism from dopamine to DOPAC

Fig. 3. Time Courses of DOPAC Concentration in the Striatum before and after Intravenous Administration of L-Dopa

(a) 100 mg/kg ($n=3$), (b) 50 mg/kg ($n=3$), (c) 10 mg/kg ($n=3$). The plotted points represent the observed data and the solid lines represent the calculated values using Eq. 5 in the text. Each experimental point shown represents the mean $\pm$ S.D.

Fig. 4. Time Courses of HVA Concentration in the Striatum before and after Intravenous Administration of L-Dopa

(a) 100 mg/kg ($n=3$), (b) 50 mg/kg ($n=3$), (c) 10 mg/kg ($n=3$). The plotted points represent the observed data and the solid lines represent the calculated values using Eq. 6 in the text. Each experimental point shown represents the mean $\pm$ S.D.

and from dopamine to HVA was performed using the rearranged Michaelis-Menten equation. The values of K_m became almost zero. Therefore, in this analysis, it was assumed that the metabolism from dopamine to DOPAC and HVA can be explained by first-order clearance terms.

The control concentrations of dopamine and DOPAC in the striatum were 5.9 ± 0.7 $\mu\text{g/g}$ and 3.6 ± 0.4 $\mu\text{g/g}$, respectively, and these values are consistent with the report of Ehrenstrom and Johansson (dopamine: 4.4 ± 0.5 $\mu\text{g/g}$, DOPAC: 6.5 ± 1.1 $\mu\text{g/g}$).¹⁸⁾ In order to describe the disposition of dopamine in the striatum after the i.v. administration of L-dopa, the dopamine metabolism model was constructed. However, the enhancement of dopamine concentration in the striatum after the i.v. administration of L-dopa could not be described by the apparent first-order clearance from L-dopa to dopamine which was estimated in the previous report. Snyder *et al.* have examined the effects of L-dopa on the endogenous dopamine efflux from superfused striatal slices prepared from adult male rats, and it was clarified that L-dopa increases the spontaneous release of dopamine.^{1b)} Several reports have suggested that L-dopa increases dopamine release both *in vitro*¹⁾ and *in vivo*.²⁾ Keller *et al.* have examined the effect of L-dopa on the *in vivo* release of dopamine in rat striatum using carbon fiber voltammetry, and it was demonstrated that the effect of L-dopa on the dopamine released by nigrostriatal bundle stimulation was reduced by 94 % in animals pretreated with intraventricular 6-hydroxydopamine.^{2a)} Therefore, in this analysis, it was assumed that the enlargement of dopamine concentration in the striatum after the i.v. injection of

TABLE II. Values of Calculated Peak Time, Increased Dopamine Concentration and Their Concentration Ratio by Metabolism from L-Dopa to Dopamine and by Release from Dopamine Neuronal Terminals after Intravenous Administration of L-Dopa 100, 50 and 10 mg/kg^{a)}

		100 mg/kg	50 mg/kg	10 mg/kg
Peak time	(h)	0.14	0.14	0.12
Dopamine conc. by metabolism	($\mu\text{g/g}$) ^{b)}	0.93	0.46	0.08
Dopamine conc. by releasing	($\mu\text{g/g}$)	6.30	5.07	2.11
Dopamine conc. ratio by metabolism and releasing	(%)	85.2	90.9	96.2

a) The dopamine concentrations were the difference in concentration of dopamine in the striatum between the calculated control value and the enhanced value.

b) The calculated values of dopamine concentration were obtained using Eq. 1.

L-dopa is caused by the endogenously released dopamine from dopamine neuronal terminals. The ratio of the enlargement of dopamine concentration by the metabolism from L-dopa to dopamine and by the endogenously released dopamine from dopamine neuronal terminals was calculated at the peak time and is shown in Table II. From these results and Fig. 2, it was demonstrated that the greater part (about 90%) of the enlargement of dopamine concentration after the i.v. injection of L-dopa is caused by the endogenously released dopamine from dopamine neuronal terminals. These results suggested that the therapy-related fluctuations after chronic treatment using L-dopa is closely related to a reduction of the capacity to store of newly synthesized dopamine in the dopaminergic

neuronal terminals in Parkinson's disease patients.

Many types of models such as the linear model, the log-linear model, the Emax model and the sigmoid Emax model (Hill's equation) have been suggested to quantitatively describe the relationship between pharmacologic intensity and the concentration of a drug.¹⁹⁾ The linear model, the log-linear model, the Emax model and the sigmoid Emax model were expressed using the following Eqs. 9, 10, 11, and 12, respectively.

$$R-LD = sC_{LD}^* \quad (9)$$

$$R-LD = s' \ln(C_{LD}^*) + I \quad (10)$$

$$R-LD = \frac{E-LDC_{LD}^*}{K-LD + C_{LD}^*} \quad (11)$$

$$R-LD = \frac{E-LDC_{LD}^{*a}}{K-LD^a + C_{LD}^{*a}} \quad (12)$$

Where s and s' represent the proportional constants for the pharmacologic response of L-dopa on the production rate of endogenous dopamine in the striatum. I represents the intercept on the relationship between drug concentration and the pharmacologic response on the log-linear model. E-LD and K-LD are the maximum effects of L-dopa and the L-dopa concentrations in the striatum at half the maximum effect, respectively. a is the Hill's constant of L-dopa. The effect of L-dopa on the enlargement of dopamine concentration in the striatum was analyzed using these models. In the log-linear model, when the drug concentrations (C_{LD}^*) were equal to zero, the pharmacologic effect of L-dopa (R-LD) could not be estimated. Thus, the log-linear model was not suitable for analyzing the relationship between the concentration and the pharmacological response of L-dopa on the dopamine metabolism model. The estimated values of the pharmacokinetic and pharmacodynamic parameters and sum of square (SS) and Akaike's information criterion (AIC)²⁰⁾ on the linear model, the Emax model and the sigmoid Emax model are shown in Table III. The absolute values of the SS of dopamine data and DOPAC data were almost the same on the linear model, the Emax model and the sigmoid Emax model. However, the absolute values of the SS of HVA data on the linear model were bigger than that of either the Emax model or the sigmoid Emax model. Thus, the time course of dopamine, DOPAC and HVA concentration in the striatum after L-dopa injection could be explained by both the Emax model and the sigmoid Emax model. In Table III, the values of the pharmacodynamic parameters of E-LD, K-LD and a were as follows; E-LD = 364.9 ± 378.8 , K-LD = 4.621 ± 11.74 and $a = 0.623 \pm 0.142$, respectively. The values of AIC ($IW=1$) on the Emax model and the sigmoid Emax model were 384.1 and 380.6, respectively. These results suggest that the sigmoid Emax model, rather than the Emax model, is more suitable for describing the effect of L-dopa on the enlargement of dopamine concentration in rat striatum. However, in the sigmoid Emax model, the values of S.D. of E-LD and K-LD were bigger than that of the Emax model. It can be considered that the bigger obtained values of S.D. of E-LD and K-LD are caused by the number of parameters on the sigmoid Emax model (the Emax model

TABLE III. Values of Pharmacokinetic, Pharmacodynamic Parameters, Sum of Squares and AIC of L-Dopa, Dopamine, DOPAC and HVA in Rats Obtained from Computer Fitting and Calculating of Striatum Concentration Data on Linear Model, Emax Model and Sigmoid Emax Model after Intravenous Administration of L-Dopa 100, 50 and 10 mg/kg

		Linear model	Emax model	Sigmoid Emax model
s	($\mu\text{g/g/h}$)	74.5 ± 5.48	—	—
E-LD'	($\mu\text{g/g/h}$)	—	134.29 ± 13.38	364.89 ± 378.81
K-LD	($\mu\text{g/g}$)	—	0.276 ± 0.089	4.621 ± 11.743
a		—	—	0.623 ± 0.142
k_{DP}	(h^{-1})	10.97 ± 1.54	17.56 ± 2.714	16.25 ± 2.339
k_{HV}	(h^{-1})	2.277 ± 0.39	2.128 ± 0.330	2.290 ± 0.360
INFDO	($\mu\text{g/g/h}$)	78.351	116.67	109.86
K_m (PA)	($\mu\text{g/g}$)	1.570 ± 0.292	3.002 ± 0.484	2.642 ± 0.419
K_m (HV)	($\mu\text{g/g}$)	0.145 ± 0.030	0.255 ± 0.046	0.260 ± 0.047
V_{max} (PA)	($\mu\text{g/g/h}$)	94.026	192.41	168.33
V_{max} (HV)	($\mu\text{g/g/h}$)	15.538	15.915	17.209
SS values ^{a)}				
Dopamine (100 mg/kg)		114.0	113.4	105.4
Dopamine (50 mg/kg)		40.4	23.6	27.0
Dopamine (10 mg/kg)		7.9	5.5	4.4
DOPAC (100 mg/kg)		112.4	113.8	98.5
DOPAC (50 mg/kg)		53.3	43.5	35.6
DOPAC (10 mg/kg)		5.7	9.3	11.2
HVA (100 mg/kg)		8.0	1.3	2.4
HVA (50 mg/kg)		11.2	0.9	1.3
HVA (10 mg/kg)		1.0	0.2	0.5
Total SS ($IW=1$) ^{b)}		36.8	31.4	29.8
AIC ($IW=1$) ^{b)}		399.3	384.1	380.6

a) The absolute values of sum of square (SS) on dopamine, DOPAC and HVA data. b) The value of total SS and AIC were obtained using the weighting value ($IW=1$).

has two parameters and the sigmoidal Emax model has three parameters). The difference between the values of AIC on the Emax model and sigmoid Emax model was very small. It seemed that the shape of the relationship between the disposition and pharmacological response of L-dopa on the dopamine metabolism system are more close to the shape of a hyperbolic curve rather than a sigmoidal curve. Therefore, in this study, the Emax model was adopted for the effect of L-dopa on dopamine concentrations in the striatum. However, analysis of the effect of L-dopa on the dopamine metabolism model using the sigmoidal Emax model yields results which cannot be denied. The effect of L-dopa on dopamine, DOPAC and HVA concentration in rat striatum were reasonably described by both the Emax model and the sigmoid Emax model.

In conclusion, the calculation of dopamine, DOPAC and HVA concentration in the striatum after the i.v. administration of L-dopa was performed using the constructed dopamine metabolism model. The time courses of dopamine, DOPAC and HVA concentrations in the striatum were well explained by the dopamine metabolism models. This dopamine metabolism model might be able to be used for the analysis of the effect of dopaminergic acting drugs on dopamine, DOPAC and HVA concentrations in the striatum.

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