A Kinetic Analysis of the Effects of β-Phenylethylamine on the Concentrations of Dopamine and Its Metabolites in the Rat Striatum

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Abstract □ The purpose of this investigation was to determine whether the increase in the dopamine (DA) concentration in the rat striatum after a rapid iv injection of β-phenylethylamine (PEA) can be quantitatively explained by the alteration of the striatum PEA concentration using a constructed DA metabolism model and to examine whether the time courses of the striatum DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentration can be described by this DA metabolism model. The time courses of PEA concentration in plasma and the striatum were determined by gas chromatography–mass spectrometry. The plasma PEA concentration was described by a two-compartment model with nonlinear elimination kinetics. The striatum PEA concentration was about 10 times higher than the plasma PEA concentration. The time course of the striatum PEA concentration was described by a diffusion-limited model including a Michaelis–Menten type transport system from plasma to the striatum and nonlinear elimination from the striatum. The DA concentration in the striatum increased immediately after PEA injection. In contrast, the DOPAC concentration in the striatum decreased immediately. HVA concentration in the striatum increased gradually. Assuming that the enhancement of DA concentration in the striatum after PEA injection is caused by the competitive inhibition of PEA on the reuptake of DA into DA neuronal terminals (and the metabolism from DA to DOPAC is then competitively inhibited by PEA in the DA neuronal terminals), the relationship between the enhancement of DA concentration and PEA concentration in the striatum was analyzed using a constructed DA metabolism model. The enhancement of the DA concentration in the striatum was described quantitatively by this model. Thus, it was clarified that a quantitative relationship between PEA concentration and the enhancement of DA concentration in the striatum is present after PEA injection. However, the time courses of the striatum DOPAC (lower dose) and HVA (time delay) concentrations could not be described by this model. These results indicated that other factors might be necessary to explain the time courses of the DOPAC and HVA concentrations in the striatum after PEA injection, such as the separate evaluation of the effect of PEA on the reuptake of DA into DA neuronal terminals and on the monoamine oxidase-B (MAO-B) activity in the DA neuronal terminals, and the metabolic pathway from DOPAC to HVA.

Introduction

β-Phenylethylamine (PEA) is an endogenous compound and can act as a neuromodulator of dopaminergic responses.[1] PEA has a very complex mode of action in pharmacokinetics and pharmacological and clinical responses. Concerning the pharmacokinetics of PEA, it has been reported that (1) PEA is a lipophilic compound that passes readily through the blood–brain barrier[2] and has a heterogeneous distribution in the mammalian brain in spite of the low endogenous PEA concentration in the brain[3] and (2) the synthesis rate of PEA is quite low,[4] and the turnover rate of PEA in the brain is extremely rapid.[5,6] With respect to the pharmacological responses of PEA, it has been reported that (1) PEA can inhibit the reuptake of dopamine (DA) into DA neuronal terminals and stimulate the release of radiolabeled DA from slices of rat striatum.[7,8] (2) PEA is a typical substrate for monoamine oxidase-B (MAO-B),[9] (3) the effect of PEA on the release of DA and the reduction of its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in rat striatum slices is attributed to the competitive inhibition of PEA on the reuptake of DA into DA neuronal terminals and the inhibition of MAO-B by PEA,[10] (4) the specific PEA receptors or recognition sites are present in the central nervous system,[11,12] and (5) the endogenous PEA concentration in guinea pig striatum was significantly increased by MAO-B inhibitors.[13] As for the clinical responses of PEA, it has been reported that (1) the significant alterations of PEA metabolism are linked to the etiology of schizophrenia[14] and depression,[15] (2) phenylacetic acid (PAA), the main metabolite of PEA, is decreased in the biological fluids of depressed subjects,[15] and (3) the administration of PEA or of its precursor L-phenylalanine improves mood in depressed patients treated with a selective MAO-B inhibitor and may be therapeutic in selected depressed patients.[16] Thus, it is thought that the establishment of the quantitative relationship between the PEA concentration and its pharmacological responses, especially the effect of PEA on the disposition of catecholamines in the central nervous system, is important for the elucidation of the pharmacological mechanism of PEA. However, comprehensive pharmacokinetic studies of PEA and the quantitative studies of the effects of PEA on the disposition of catecholamines have not been performed. A previous study showed that the time courses of the concentrations of DA and its metabolites DOPAC and homovanillic acid (HVA) in the striatum after L-dopa injection can be described quantitatively by a constructed DA metabolism model,[17] which was also able to describe quantitatively the relationship between the striatum chlorpromazine concentration and the alteration of the striatum DA, DOPAC, and HVA concentration in rat after the iv administration of chlorpromazine.[18] The goals of the present investigation were (1) to develop a pharmacokinetic model for PEA in plasma and the striatum, (2) to determine whether the quantitative relationship between PEA concentration and the enlargement of DA concentration in rat striatum is present after PEA injection, and (3) to examine whether the time courses of DOPAC and HVA concentrations in the striatum after PEA injection can be explained by the constructed DA metabolism model.

Experimental Section

Chemicals—β-Phenylethylamine hydrochloride (Sigma Chemicals, St. Louis, MO) was purchased commercially and used without further purification. This drug was dissolved in isotonic sodium chloride solution (Otsuka Pharmaceuticals, Tokyo, Japan) and was administered intravenously. All other chemicals were of reagent grade and were obtained commercially (Wako Pure Chemical Industries, Osaka, Japan).

Animals—Male wistar rats (Sankyo Lab Service Corporation, Inc., Shizuoka, Japan) were used. The rats were separated into two

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groups, one group for measurement of the plasma PEA concentration after PEA injection and the other group for measurement of the PEA, DA, DOPAC, and HVA concentrations in the striatum after PEA injection. The rats were housed individually in metal cages under 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 the rats were then placed in individual plastic metabolic cages. The animals had an indwelling cannula implanted in the right jugular vein 1 day before the experiments.

Animal Experiments for Plasma PEA Concentration—To characterize the PEA disposition in plasma, PEA (10, 25, 50, and 75 mg/kg) was injected rapidly into the right jugular vein. Blood samples (0.2 mL) were collected from the same right jugular vein cannula at 2, 5, 10, 15, 20, 30, 40, 50, and 60 min after PEA injection. The blood was then replaced by injection of an equal volume of citrated blood from a donor rat. The obtained plasma samples were stored at −80 °C until the analysis for plasma PEA concentration.

Animal Experiments for PEA, DA, DOPAC, and HVA Concentration in the Striatum—To characterize the alteration of PEA, DA, DOPAC, and HVA concentrations in the striatum, PEA (25 and 50 mg/kg) was injected rapidly into the right jugular vein. At 2, 5, 10, 15, 20, 30, 45, and 60 min after the dosing, rats were respectively sacrificed by 2 nmol/mL KCl (1 mL) injection and the entire brain was quickly excised, rinsed with cold physiological saline, and dissected into the corpus striatum by a modified method of Glowinski and Inversen.[19] The striatum samples were stored at −80 °C until the analysis for the striatum DA, DOPAC, and HVA concentrations. The remaining striatum filtrate samples (which were used for the striatum DA, DOPAC, and HVA concentrations) were used for the analysis of the striatum PEA concentration using gas chromatography−mass spectrometry (GC−MS) (described below).

Assay Methods for PEA Concentration in Plasma and the Striatum—The PEA concentration in plasma and the striatum was determined by a modification of the GC−MS assay of Kataoka *et al.*[20] with 3-phenyl-1-propylamine (Aldrich Chemicals, Milwaukee, WI) as the internal standard. For the plasma samples, 0.1 mL of the plasma sample was pipetted into a glass tube. To the glass tube was added 0.1 mL of the internal standard solution (3-phenyl-1-propylamine, 500 ng) and 0.05 mL of 50% potassium hydroxide; all were mixed well. The mixture was extracted with 3 mL of *n*-hexane and centrifuged at room temperature at 3000 rpm for 3 min. The aqueous layer was frozen in a chilled methanol bath using a cooler (Eyela Cool ECS-50, Tokyo Rikakikai, Tokyo, Japan). The upper organic layer was then decanted into another glass tube. The organic solvent was evaporated to dryness. To the glass tube containing this residue were added 0.3 mL of water, 0.05 mL of 50% potassium hydroxide, and 0.02 mL of benzenesulfonyl chloride (BSC, Wako) to convert PEA to its *N*-benzenesulfonamide derivative. The mixture was then shaken at 300 rpm (up and down) for 15 min at room temperature. The reaction mixture was extracted with 3 mL of *n*-hexane to remove excess regent and the *N*-benzenesulfonamide derivative of secondary amines. After the hexane extract was discarded, 0.05 mL of 65% potassium hydroxide solution containing 30% methanol was added to the aqueous layer. The aqueous layer was also extracted with 3 mL of *n*-hexane to remove excess regent and *N*-benzenesulfonamide derivative of secondary amines. After the hexane extract was discarded, 0.5 mL of 15% hydrochloric acid was added to the aqueous layer, and the mixture was extracted with 3 mL of *n*-hexane. The aqueous layer was frozen in a chilled methanol bath using the cooler. The upper organic layer was then decanted into another glass tube. The organic solvent was evaporated to dryness, and the residue was dissolved in 0.02 mL of ethyl acetate. One microliter of the ethyl acetate solution was injected into the GC−MS system. For the striatum samples, the remaining striatum filtrate samples which included the internal standard (3-phenyl-1-propylamine, 500 ng) and were used for DA, DOPAC and HVA concentrations (the preparation methods for these filtrate samples are described next) were employed. The extraction and reaction of these striatum filtrate samples were performed by the same procedure as described above.

Mass fragmentography was performed on a Hewlett-Packard 5890 series II gas chromatograph, a 5971 A mass selective detector, and a 7673 autoinjector. The instrument was operated in the electron impact (EI) mode with the interface and ion source at 300 and 280 °C, respectively. The chromatographic column was a capillary column, DB-1301 (cross-linked, J & W Scientific) 15 m × 0.25 mm i.d. The injector port temperature was 220 °C and the helium carrier gas flow rate was 0.8 mL/min. The initial column oven temperature was 200 °C, and the increasing temperature was at 30 °C/min up to 280 °C. The ionization potential was 70 eV. Ion currents at *m*/*z* 261 for PEA (*N*-benzenesulfonamide derivation) and *m*/*z* 275 for 3-phenyl-1-propylamine (*N*-benzenesulfonamide derivation, the internal standard) were recorded by computer (HP Vectra QS/16S) with an MS chemistation program. Retention times were 5.2 and 6.1 min for the *N*-benzenesulfonamide derivative of PEA and 3-phenyl-1-propylamine, respectively. To test the linearity of the calibration graph, various amounts of PEA ranging from 0.25 to 1000 ng for the plasma PEA concentration and from 2.5 to 10000 ng for the striatum PEA concentration were derivatized, respectively. Linear relationships were obtained from both logarithmic plots, and the regression lines for the plasma PEA concentration and the striatum PEA concentration were $\ln y = 0.886 \ln x - 4.419$ ($\gamma = 0.9957$) and $\ln y = 0.895 \ln x - 4.065$ ($\gamma = 0.9938$), respectively, where y is the peak-area ratio and x is the amount of PEA. The coefficient of variation was 5% or less in these concentration ranges for calibration graphs. Detection limits of sensitivity to PEA based on the signal to noise ratio of 3 were determined by injection of diluted standard solutions. The detection limits of the assay for the plasma PEA and the striatum PEA concentrations were about 0.1 ng (1 ng/mL) and 1 ng (10 ng/g), respectively.

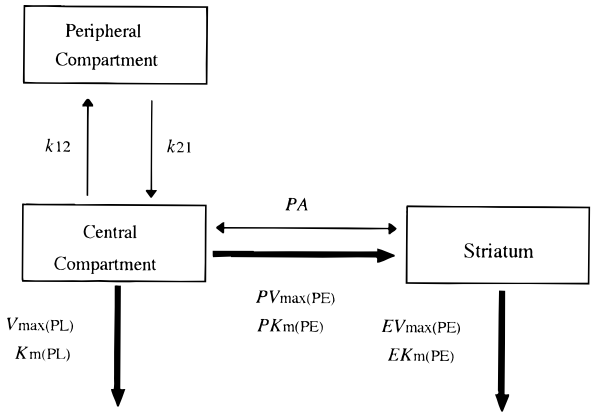

Figure 1—Schematic representation of the pharmacokinetic model for PEA in rat plasma and the striatum.

Assay Methods for DA, DOPAC, and HVA Concentrations in the Striatum—The striatum concentrations of DA, DOPAC, and HVA were determined by a modification of the high-performance liquid chromatographic (HPLC) assay of Murai *et al.*[21] with isoproterenol (Wako) 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 15 000 rpm for 10 s in 500 μL of 0.1 M perchloric acid containing 10 μM 2Na EDTA and isoproterenol (1000 ng) and 3-phenyl-1-propylamine (500 ng, which is the internal standard for the striatum PEA concentration) 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 μm filter (disposable syringe filter unit, dismic-3cp cellulose acetate, ADVANTEC, Tokyo, Japan), and 10 μL of the filtrates was injected onto the HPLC system. The resultant filtrate was loaded onto a reversed-phase HPLC column (Supelcosil LC-18-DB, SUPELCO). The solvent delivery system (L-5000 LC controller and 655A-11 pump, Hitachi, Tokyo, Japan) was equipped with an electrochemical detector (ECD-100, EICOM, Kyoto, Japan) at +0.7 V *vs* an Ag−AgCl reference electrode with an auto sampler (AS-8010, Tosoh, Tokyo, Japan) and with a chromatointegrator (D-2500, Hitachi). A guard column (Supelcosil LC-18-DB, SUPELCO) was placed between the autosampler and the analytical column. The mobile phase was 0.01 M citrate buffer (pH 4.4)−MeOH (90:10 v/v) containing 10 μM 2Na EDTA 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, DA, and internal standard, respectively. To test the linearity of the calibration graph, various amounts of DA, DOPAC, and HVA ranging from 25 to 1000 ng for the striatum DA, DOPAC, and HVA concentrations were prepared. Linear relationships were obtained and the regression lines for the striatum DA, DOPAC, and HVA concentrations were $y = 0.001577x + 0.002426$ ($\gamma = 0.9989$), $y = 0.001326x +$

Table 1—List of Symbols and Definitions

DA	Dopamine
DOPAC	3,4-Dihydroxyphenylacetic acid
HVA	Homovanillic acid
MAO-B	Monoamine oxidase-B
PAA	Phenylacetic acid
PEA	β-Phenylethylamine
C_{DO}	(μg/g) Striatum DA concentration
C_{DO0}	(μg/g) Striatum DA concentration at steady state
C_{HV}	(μg/g) Striatum HVA concentration
C_{HV0}	(μg/g) Striatum HVA concentration at steady state
C_{PA}	(μg/g) Striatum DOPAC concentration
C_{PA0}	(μg/g) Striatum DOPAC concentration at steady state
C_{PE}	(μg/g) Striatum PEA concentration
C_{PL}	(mg/L) Plasma PEA concentration
CL_{DE}	(mL/h) Elimination (conversion) clearance from DA to DA metabolites except DOPAC and HVA in striatum, $CL_{DE} = k_{DE}V_{st}$
CL_{DH}	(mL/h) Metabolism clearance from DA to HVA in striatum, $CL_{DH} = k_{DH}V_{st}$
CL_m	(mL/h) Apparent metabolism clearance from PEA to PAA in striatum, $CL_m = k_{out}V_{st} - PA$
$EK_{m(PE)}$	(μg/g) Striatum PEA concentration at half-maximum elimination rate of PEA in striatum
$EV_{max(PE)'}$	[(μg/g)/h] Maximum elimination rate of PEA in striatum, $EV_{max(PE)'} = EV_{max(PE)}/V_{st}$
$EV_{max(PE)}$	(μg/h) Maximum elimination rate of PEA in striatum, $EV_{max(PE)} = EV_{max(PE)'}V_{st}$
INF_{DO}	[(μg/g)/h] Zero-order production rate of endogenous DA in striatum, $INF_{DO} = k_{0DO}/V_{st}$
$K_{I(PE)}$	(μg/g) Inhibitor constant of PEA for metabolism from DA to DOPAC in striatum
$K_{m(DO)}$	(μg/g) Striatum DA concentration at half-maximum metabolism rate from DA to DOPAC in striatum
$K_{m(HV)}$	(μg/g) Striatum HVA concentrations at half-maximum elimination rate of HVA in striatum
$K_{m(PA)}$	(μg/g) Striatum DOPAC concentration at half-maximum elimination rate of DOPAC in striatum
$K_{m(PL)}$	(mg/kg) Amount of PEA in plasma compartment at half maximum elimination rate of PEA
k_{DE}	(h^{-1}) Elimination (conversion) constant from DA to DA metabolites except DOPAC and HVA in striatum, $k_{DE} = CL_{DE}/V_{st}$
k_{DH}	(h^{-1}) Metabolism constant from DA to HVA in striatum, $k_{DH} = CL_{DH}/V_{st}$
k_{in}	(h^{-1}) Apparent first-order constant of PEA from plasma to striatum, $k_{in} = PA/V_{st}$
k_{out}	(h^{-1}) Apparent first-order constant of PEA from striatum to outside striatum including the apparent metabolism constant from PEA to PAA, $k_{out} = (PA + CL_m)/V_{st}$
k_{0DO}	(μg/h) Zero-order production rate of endogenous DA in striatum, $k_{0DO} = INF_{DO}V_{st}$
k_{12}, k_{21}	(h^{-1}) Intercompartmental transfer rate constant
PA'	(h^{-1}) Apparent diffusion constant of PEA between plasma and striatum, $PA' = PA/V_{st}$
PA	(mL/h) Apparent diffusion clearance of PEA between plasma and striatum, $PA = PA'V_{st}$
$PK_{m(PE)}$	(μg/g) Plasma PEA concentration at half-maximum transport rate of PEA from plasma to striatum
$PV_{max(PE)'}$	[(μg/g)/h] Maximum transport rate of PEA from plasma to striatum, $PV_{max(PE)'} = PV_{max(PE)}/V_{st}$
$PV_{max(PE)}$	(μg/h) Maximum transport rate of PEA from plasma to striatum, $PV_{max(PE)} = PV_{max(PE)'}V_{st}$
t	(h) Time after PEA administration
V_d	(L/kg) Apparent volume distribution of PEA in plasma
V_{st}	(g) Striatum weight
$V_{max(DO)'}$	[(μg/g)/h] Maximum metabolism rate from DA to DOPAC in striatum, $V_{max(DO)'} = V_{max(DO)}/V_{st}$
$V_{max(DO)}$	(μg/h) Maximum metabolism rate from DA to DOPAC in striatum, $V_{max(DO)} = V_{max(DO)'}V_{st}$
$V_{max(HV)'}$	[(μg/g)/h] Maximum elimination rate of HVA in striatum, $V_{max(HV)'} = V_{max(HV)}/V_{st}$
$V_{max(HV)}$	(μg/h) Maximum elimination rate of HVA in striatum, $V_{max(HV)} = V_{max(HV)'}V_{st}$
$V_{max(PA)'}$	[(μg/g)/h] Maximum elimination rate of DOPAC in striatum, $V_{max(PA)'} = V_{max(PA)}/V_{st}$
$V_{max(PA)}$	(μg/h) Maximum elimination rate of DOPAC in striatum, $V_{max(PA)} = V_{max(PA)'}V_{st}$
$V_{max(PL)}$	[(mg/kg)/h] Maximum elimination rate of PEA in plasma
X_1	(mg/kg) Amount of PEA in plasma compartment
X_2	(mg/kg) Amount of PEA in peripheral compartment

0.009285 ($\gamma = 0.9983$) and $y = 0.001383x + 0.014338$ ($\gamma = 0.9956$), respectively, where y is the peak-area ratio and x is the amount of DA, DOPAC, or HVA. The coefficient of variation was 3% or less in these concentration ranges for calibration graphs. Detection limits of sensitivity to these substrates (DA, DOPAC, and HVA) based on the signal to noise ratio of 3 were determined by injection of diluted standard solutions. The detection limits of the assay for DA, DOPAC, and HVA amounts were about 2.5, 2.5, and 2.5 ng, respectively.

Pharmacokinetic Analysis for PEA Concentration in Plasma and the Striatum—In order to quantitatively describe the time course of PEA concentration in plasma and the striatum after PEA injection, a pharmacokinetic model for PEA was constructed and is shown in Figure 1. It has been well-recognized that PEA is rapidly and almost quantitatively metabolized to PAA by MAO-B.[6] The time course of PEA concentration in plasma (C_{PL}) after iv administration of PEA was fitted to a two-compartment model with Michaelis-Menten type elimination kinetics as follows.

$$\frac{dX_1}{dt} = k_{21}X_2 - \left(k_{12} + \frac{V_{max(PL)}}{K_{m(PL)} + X_1}\right)X_1 \quad (1)$$

$$\frac{dX_2}{dt} = k_{12}X_1 - k_{21}X_2 \quad (2)$$

$$C_{PL} = X_1/V_d \quad (3)$$

Definitions of the symbols used in all the equations are listed in Table 1. It has been reported that (1) PEA is a lipophilic compound that passes readily through the blood–brain barrier[2] and has a heterogeneous distribution in the mammalian brain in spite of the low endogenous PEA concentration in the brain,[3] (2) PEA is synthesized by the decarboxylation of phenylalanine and this synthesis rate is quite low,[4] and (3) PEA is metabolized via oxidative deamination to PAA by MAO-B very quickly and the turnover rate of PEA in the brain is extremely rapid.[5,6] Therefore, in this analysis, the time course of the striatum PEA concentration was analyzed using a diffusion-limited model including a Michaelis–Menten type transport system from plasma to the striatum and nonlinear elimination from the striatum (Figure 1). The time course of the PEA concentration in the striatum (C_{PE}) can thus be expressed as follows.

$$V_{st}\frac{dC_{PE}}{dt} = PA(C_{PL} - C_{PE}) + \frac{PV_{max(PE)}C_{PL}}{PK_{m(PE)} + C_{PL}} - \frac{EV_{max(PE)}C_{PE}}{EK_{m(PE)} + C_{PE}} \quad (4)$$

Pharmacodynamic Analysis of the Effect of PEA on the Striatum DA, DOPAC, and HVA Concentrations—In order to determine whether the quantitative relationship between PEA concentration and the increase in DA concentration in rat striatum is present after PEA injection and to examine whether the time courses of the DOPAC and HVA concentrations in the striatum after PEA injection can be explained by the above model's assumptions on the effect of PEA on the disposition of catecholamines in the striatum, the DA metabolism model[17] was applied, as shown in Figure 2. It has been reported that (1) MAO exists on the outer surface of mitochondria within the DA neuronal terminals[22] and the reuptake of DA into DA neuronal terminals is performed by the carrier-mediated transport system,[23,24] (2) PEA can inhibit the reuptake of DA into DA neuronal terminals and stimulate the release of radiolabeled DA from slices of rat striatum,[7,8] (3) PEA is a typical substrate for MAO-B,[9] and (4) the effect of PEA on the release of DA and the reduction of DOPAC in rat striatum slices is attributed to the competitive inhibition of PEA on the reuptake of DA into DA neuronal terminals and the inhibition of MAO-B by PEA.[10] Therefore, in the present analysis using the DA metabolism model, it was assumed that (1) the metabolism from DA to DOPAC can be explained by Michaelis–Menten type kinetics, (2) the enhancement of DA concentration in the striatum after PEA injection is caused by the competitive inhibition of PEA on the reuptake of DA into DA neuronal terminals, and (3) the relationship between the alteration of the striatum DA concentration and the striatum PEA concentration can be explained by the competitive inhibition equation. The changes of the striatum DA, DOPAC, and HVA concentrations (C_{DO}, C_{PA}, and C_{HV}) after PEA injection can be expressed by the following equations.

$$V_{st}\frac{dC_{DO}}{dt} = k_{0DO} - \frac{V_{max(DO)}C_{DO}}{K_{m(DO)}(1 + C_{PE}/K_{I(PE)}) + C_{DO}} - CL_{DH}C_{DO} - CL_{DE}C_{DO} \quad (5)$$

$$V_{st}\frac{dC_{PA}}{dt} = \frac{V_{max(DO)}C_{DO}}{K_{m(DO)}(1 + C_{PE}/K_{I(PE)}) + C_{DO}} - \frac{V_{max(PA)}C_{PA}}{K_{m(PA)} + C_{PA}} \quad (6)$$

$$V_{st}\frac{dC_{HV}}{dt} = CL_{DH}C_{DO} - \frac{V_{max(HV)}C_{HV}}{K_{m(HV)} + C_{HV}} \quad (7)$$

At the steady state before PEA administration, the left sides of eqs 5–7 equal zero. Rearrangement of eqs 5, 6, and 7 yield the following eqs 8, 9, and 10, respectively.

$$INF_{DO} = \frac{V_{max(DO)'}C_{DO0}}{K_{m(DO)} + C_{DO0}} + k_{DH}C_{DO0} + k_{DE}C_{DO0} \quad (8)$$

$$V_{max(PA)'} = \frac{V_{max(DO)'}C_{DO0}(K_{m(PA)} + C_{PA0})}{(K_{m(DO)} + C_{DO0})C_{PA0}} \quad (9)$$

$$V_{max(HV)'} = \frac{k_{DH}C_{DO0}(K_{m(HV)} + C_{HV0})}{C_{HV0}} \quad (10)$$

The value of the production rate constant of endogenous DA concentration in the striatum (INF_{DO}) and the value of the maximum elimination rate of DOPAC [$V_{max(PA)'}$] and HVA [$V_{max(HV)'}$] for nonlinear elimination kinetics can be calculated by these equations (eqs 8, 9, and 10, respectively).

Least Squares Model Adaptation—In order to estimate the pharmacokinetic and pharmacodynamic parameters of PEA, the data on the concentrations of PEA (in plasma and the striatum), DA, DOPAC, and HVA (in the striatum) after the iv administration of PEA were fitted to eqs 1–10 by a nonlinear least squares regression program, FKDM,[25] using a digital computer (PC-9821 Ae, NEC, Tokyo, Japan). The inverse value of each datum was used as the weighting value 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}.[26]

Results and Discussion

PEA Concentration in Plasma and the Striatum—PEA is an endogenous compound. It has been reported that the basal endogenous plasma PEA concentration in normal volunteers and the basal rat striatal PEA concentration were 335 ± 255 pg/mL and 2.89 ± 1.03 ng/g, respectively.[27] However, in the present study, the control concentration of PEA in plasma and the striatum before PEA injection could not be determined by GC–MS. The quantitative limit of PEA concentration in plasma and the striatum by GC–MS were about 1 ng/mL and 10 ng/g, respectively. In the analysis of the alterations of the striatum DA concentrations after PEA injection, the competitive inhibition equation was applied (eqs 5 and 6). The striatum PEA concentration (C_{PE}) in eqs 5 and 6 is the difference of the striatum PEA concentration between the control value and the enhancement value. The maximum PEA concentrations in plasma and the striatum after PEA injection were about 20 μg/mL and 100 μg/g, respectively, and these concentration values were extremely high compared with the control values. Therefore, in this study, the control values of plasma and the striatum PEA concentrations before PEA injection were ignored.

Pharmacokinetic Analysis of Plasma PEA Concentration—The time courses of plasma PEA concentration after the iv administration of PEA 10, 25, 50, and 75 mg/kg in the rats are shown in Figure 3a. The data are plotted semilogarithmically as a function of time. The disappearance of the plasma PEA concentration seemed to follow the nonlinear elimination kinetics. In order to confirm whether the disappearance of the plasma PEA concentration can be explained by the nonlinear elimination kinetics, the values of the dose-normalized area under the curve (AUC) of the plasma PEA concentration (AUC/DOSE) were calculated. The values of AUC/DOSE at 10, 25, 50, and 75 mg/kg iv were 8.43×10^{-3}, 29.30×10^{-3}, 65.71×10^{-3} and 77.68×10^{-3} ((μg/mL)h)/(mg/kg), respectively. The values of AUC/DOSE were increased with the increase in the dose of PEA. These results indicated that the assumption of the nonlinear elimination kinetics is necessary to explain the pharmacokinetic behavior of the plasma PEA concentration after PEA injection. In the case of the lowest dose (10 mg/kg), the disappearance of plasma PEA concentration followed a two-exponential curve. Therefore, in this analysis, the two-compartment model with the nonlinear elimination kinetics was used to explain the plasma PEA concentration after PEA injection (Figure 1). The solid lines in Figure 3a represent the calculated values. The pharmacokinetics of PEA in plasma could be described quantitatively using this model. The pharmacokinetic parameters (k_{12}, k_{21}, $V_{max(PL)}$, $K_{m(PL)}$, and V_d) were computed by the nonlinear least squares method and are listed in Table 2.

Pharmacokinetic Analysis of the Striatum PEA Concentration—The time course of PEA concentration in the striatum after iv administration of PEA (25 and 50 mg/kg) is shown in Figure 3b. The striatum PEA concentration was about 10 times higher than the plasma PEA concentration. First, the time course of the striatum PEA concentration was analyzed using the hybrid model[28] in which the striatum compartment is independently connected with the plasma compartment by the apparent diffusion clearance. The changes in the striatum PEA concentration can be expressed as follows.

$$V_{st}\frac{dC_{PE}}{dt} = PAC_{PL} - PAC_{PE} - CL_{m}C_{PE} \quad (11)$$

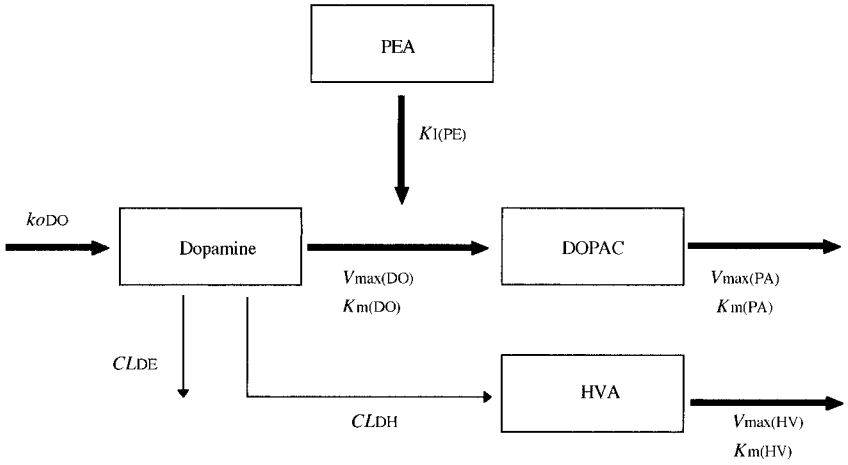

Figure 2—Schematic representation of pharmacodynamic models for PEA in DA, DOPAC, and HVA concentrations in rat striatum.

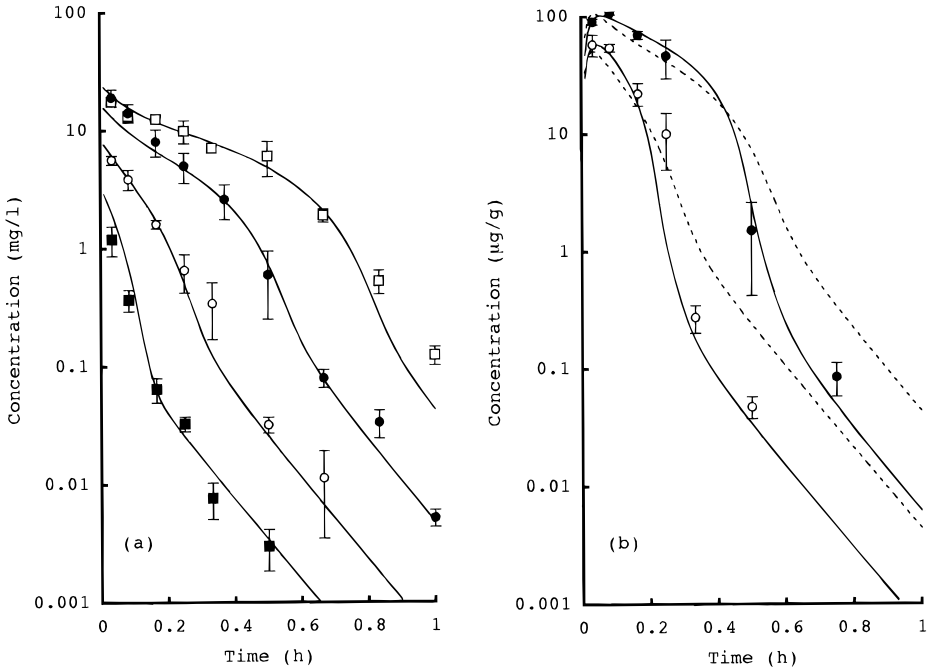

Figure 3—Time course of PEA concentrations in rat plasma and the striatum after the intravenous administration of PEA: (a) plasma PEA concentration: □, 75 mg/kg ($n = 3$); ●, 50 mg/kg ($n = 3$); ○, 25 mg/kg ($n = 3$); and ■, 10 mg/kg ($n = 3$). (b) the striatum PEA concentration: ●, 50 mg/kg ($n = 3$) and ○, 25 mg/kg ($n = 3$). The plotted points represent the observed data. The plasma PEA concentrations at 50 and 60 min (25 mg/kg) and at 40, 50, and 60 min (10 mg/kg) could not be detected by GC–MS. The striatum PEA concentration at 60 min (50 mg/kg) could not be detected by GC–MS. The solid lines represent the calculated values using eqs 1–4 in the text. The dotted lines represent the calculated values using eq 12 in the text. Each experimental point is shown as the mean ± SD.

Equation 11 was simplified and the following equation was given:

$$\frac{dC_{PE}}{dt} = k_{in}C_{PL} - k_{out}C_{PE} \qquad (12)$$

The dotted lines in Figure 3b represent the calculated values using eq 12. The time course of the striatum PEA concentration could not be described by this equation. The estimated values of k_{in} and k_{out} were 584.62 ± 392.98 h^{-1} and 71.13 ± 48.26 h^{-1}, respectively. The value of k_{in} (PA/V_{st}) was greater than the value of $k_{out}((PA + CL_m)/V_{st})$. Moreover, the value of k_{in} was about 16 times greater than the value of the serum flow rate constant to the brain[29] ($Q_{BR}' = 37.2\ h^{-1}$: $Q_{BR}' = Q_{BR}/V_{BR}$; $Q_{BR} = 64.728$ mL/h, $V_{BR} = 1.74$ mL[30]). These results indicated that the time course of the striatum PEA concentration after PEA injection cannot be described by the general diffusion-limited model.

Karoum *et al.* reported that the administration of deuterated PEA leads to an unexpected elevation in brain endogenous PEA concentration, and they speculated that the enhancement of brain endogenous PEA concentration may be the result of competitive inhibition of endogenous PEA metabolism by the deuterated PEA.[31] These results suggested that the separated pharmacokinetic analyses for exogenous and endogenous PEA concentration in the striatum are necessary to explain the time course of the striatum PEA concentration after PEA injection. However, the exogenous and endogenous PEA concentration in the striatum were not measured separately in the present study. Therefore, the Michaelis−Menten type transport system from plasma to the striatum was assumed to explain the time course of the striatum PEA concentration after PEA injection (eq 4). Moreover, it has been demonstrated that the oxidative deamination of PEA by MAO-B is the main route of catabolism of PEA in rat brain.[5,6] Therefore, in the present analysis, it was

Table 2—Values of Pharmacokinetic Parameters of PEA in Rats, Obtained from Computer Fitting of Plasma and the Striatum Concentration Data after Intravenous Administration of PEA (10, 25, 50, and 75 mg/kg)

k_{21} (h^{-1})	5.91 ± 3.93^a
k_{12} (h^{-1})	8.62 ± 4.53^a
$V_{max(PL)}$ [(mg/kg)/h]	97.39 ± 10.59^a
$K_{m(PL)}$ (mg/kg)	1.39 ± 0.87^a
V_d (L/kg)	2.97 ± 0.47^a
PA' (h^{-1})	45.94 ± 17.81^a
$PV_{max(PE)'}$ (μg/g)/h	10213 ± 4075^a (84.3×10^{-3} M/h)^b
$PK_{m(PE)}$ (μg/g)	10.97 ± 13.12^a (90.5×10^{-6} M)^b
$EV_{max(PE)'}$ (μg/g)/h	1037 ± 1396^a (8.6×10^{-3} M/h)^b
$EK_{m(PE)}$ (μg/g)	1.45 ± 3.47^a (12.0×10^{-6} M)^b
V_{st} (g)	0.082^c
PA (mL/h)	3.77^d
$PV_{max(PE)}$ (μg/h)	837.5^d
$EV_{max(PE)}$ (μg/h)	85.0^d

[a] The pharmacokinetic parameters of PEA in plasma and the striatum were estimated by fitting the data to eqs 1–4 using the computer program FKDM. All values are expressed as the mean ± SD of the estimated parameter. [b] The values of these parameters were expressed using molar concentrations. [c] The value of this parameter was fixed to the value obtained from the L-dopa study.[17] [d] The values of these parameters were calculated by the following equations: $PA = PA'V_{st}$, $PV_{max(PE)} = PV_{max(PE)'}V_{st}$, and $EV_{max(PE)} = EV_{max(PE)'}V_{st}$.

assumed that the disappearance of the striatum PEA concentration can be explained by the nonlinear elimination kinetics (eq 4). The analysis of the striatum PEA concentration was performed using eq 4. The solid lines in Figure 3b represent the calculated values. The time course of the striatum PEA concentration after PEA injection can be described by the constructed pharmacokinetic model for PEA (Figure 1). The pharmacokinetic parameters [PA, $PV_{max(PE)}$, $PK_{m(PE)}$, $EV_{max(PE)}$, and $EK_{m(PE)}$] were computed by the nonlinear least squares method and are listed in Table 2. Although the constructed pharmacokinetic model for the striatum PEA concentration in the present study is not a unique model for explaining the kinetic behavior of PEA in the striatum, this pharmacokinetic model was a useful compartment model for explaining the disposition of PEA in the striatum after PEA injection. The estimated value of the apparent diffusion constant between plasma and the striatum (PA') was 45.94 h^{-1}, as shown in Table 2. The value of PA' was almost same as the value of the serum flow rate constant to the brain ($Q_{BR}' = 37.2$ h^{-1}). This result indicated that the time course of the striatum PEA concentration after PEA injection can be described using the serum flow rate to the brain.

Pharmacodynamic Analysis of the Effect of PEA on the Striatum DA Concentration—The time course of the striatum DA concentration before and after the iv administration of PEA 25 and 50 mg/kg is shown in Figure 4. The DA concentration in the striatum increased immediately after PEA injection, with the peak concentration (8.0 ± 0.3 μg/g) occurring at 2 min; then it returned to the premedication level until 45 min at 50 mg/kg dosing. In order to determine whether the quantitative relationship between the striatum PEA concentration and the increase in the striatum DA concentration is present after PEA injection, the DA metabolism model[17] was applied (Figure 2) and the analyses were performed using eqs 5−10. The solid lines in Figure 4 represent the calculated values. The time course of the striatum DA concentration after PEA injection was reasonably well-described using this constructed DA metabolism model. Thus, it was clarified that the quantitative relationship between the striatum PEA concentration and the enhancement of the striatum DA concentration is present after PEA injection. The parameters [$K_{I(PE)}$, $V_{max(DO)'}$, $K_{m(DO)}$, and k_{DE}] for PEA and DA were computed by the nonlinear least squares method. The parameter k_{DH} was fixed to the obtained values of the L-dopa study,[17] and the other parameter (INF_{DO}) was calculated by eq 8 and the values of these parameters are listed in Table 3.

The endogenous DA, DOPAC, and HVA concentrations in the striatum before PEA injection were 6.18 ± 0.13, 2.23 ± 0.25, and 0.59 ± 0.04 μg/g (mean ± SD), respectively, and these values are consistent with the report of the previous L-dopa study[17] (DA, 5.9 ± 0.7 μg/g; DOPAC, 3.6 ± 0.4 μg/g; and HVA, 1.0 ± 0.2 μg/g). The values of the parameters for the production rate constant of endogenous DA concentration in the striatum (INF_{DO}), and the maximum elimination rate of DOPAC [$V_{max(PA)'}$] and HVA [$V_{max(HV)'}$] can be calculated by eqs 8, 9, and 10, respectively. Since the values of these parameters [INF_{DO}, $V_{max(PA)'}$, and $V_{max(HV)'}$] contain the values of the endogenous DA, DOPAC, and HVA concentrations (C_{DO0}, C_{PA0}, and C_{HV0}), the values of these parameters are changed by the alterations of the alues of C_{DO0}, C_{PA0}, and C_{HV0}. Therefore, in the present study, the values of C_{DO0}, C_{PA0}, and C_{HV0} were used with the obtained values of the L-dopa study. The values of DA, DOPAC, and HVA concentrations in Figures 4 and 5 were the recalculated values using the control values from the L-dopa study and the enhanced values of the present study. In Table 3, although the values of k_{DH}, $K_{m(PA)}$, $K_{m(HV)}$, $V_{max(HV)'}$, CL_{DH}, and $V_{max(HV)}$ are the same as the values of the L-dopa study, the values of INF_{DO}, $V_{max(PA)'}$, k_{0DO}, $V_{max(DO)}$, and $V_{max(PA)}$ are different from those of the L-dopa study. These differences were dependent on the differences (modifications) of the DA metabolism model in the L-dopa study and the PEA study.

In the L-dopa study,[17] in order to construct a simple model for DA metabolism and to obtain the stable values of the parameters for DA and DOPAC, it was assumed that the metabolism from DA to DOPAC can be explained by the first-order clearance term. In contrast, in the PEA study, it was assumed that the metabolism from DA to DOPAC can be explained by Michaelis−Menten type kinetics and that the inhibition of PEA on the reuptake of DA into DA neuronal terminals can be explained by the competitive inhibition equation (eqs 5 and 6). These assumptions were based on the pharmacological mechanism of the effect of PEA on the DA disposition in the central nervous system. Moreover, in the L-dopa study,[17] the elimination (conversion) parameter from DA to DA metabolites except DOPAC and HVA (CL_{DE}) was not assumed. In order to confirm whether the time course of the striatum DA concentration after PEA injection can be explained without this conversion parameter (CL_{DE}), the analysis was performed using the following equation (eq 13).

$$V_{st}\frac{dC_{DO}}{dt} = k_{0DO} - \frac{V_{max(DO)}C_{DO}}{K_{m(DO)}(1 + C_{PE}/K_{I(PE)}) + C_{DO}} - CL_{DH}C_{DO} \quad (13)$$

However, the calculation was unsuccessful; the parameters for DA concentration could not be estimated by this equation. This result indicated that the elimination parameter (CL_{DE}) is necessary to describe the time course of the striatum DA concentration after PEA injection.

Horn examined the effect of DA and PEA on the inhibition of [^{3}H]DA uptake into the rat corpus striatum homogenate.[32] The values of IC_{50} (which represent the concentration of inhibitor required to produce a 50% inhibition of the uptake of [^{3}H]DA) of DA and PEA were 3.5×10^{-7} and 1.4×10^{-6} M, respectively. In order to compare these values with the data obtained in the present study, the values of IC_{50} of DA and PEA were calculated using the obtained values of $K_{m(DO)}$ and $K_{I(PE)}$ (which represent the striatum DA concentration at half the maximum metabolism rate from DA to DOPAC and the inhibitor constant (concentration) of PEA for the metabolism from DA to DOPAC) in this analysis. The calculated values

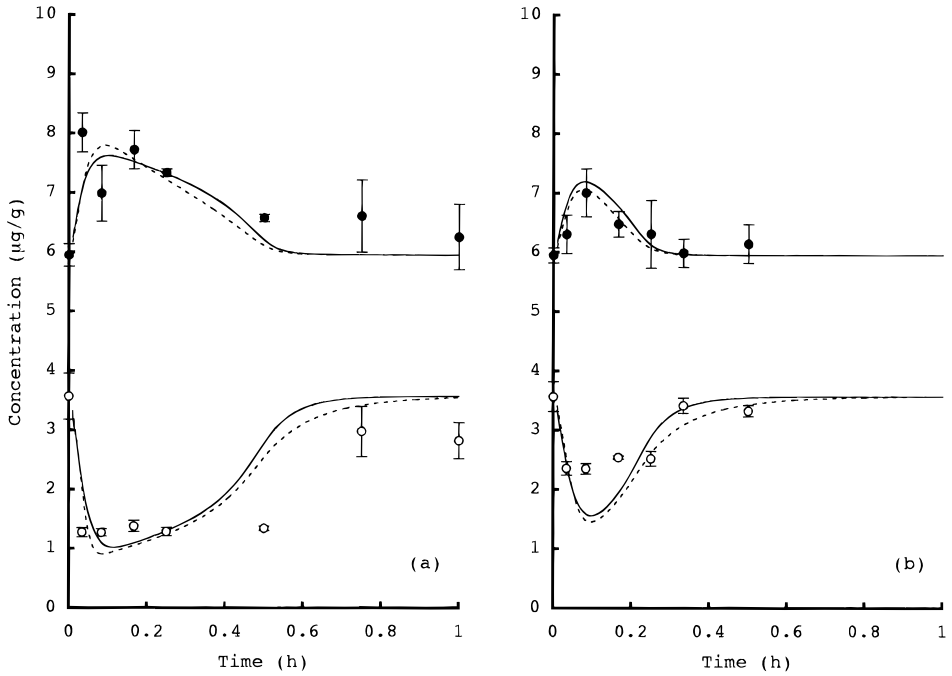

Figure 4—Time courses of DA and DOPAC concentration in the striatum before and after intravenous administration of PEA: (a) 50 mg/kg ($n = 3$); (b) 25 mg/kg ($n = 3$); ●, DA concentration; ○, DOPAC concentration. The plotted points represent the observed data. The solid lines represent the calculated values by eqs 5–10 using the values of the parameters which were obtained in the L-dopa study[17] in the text. The dotted lines represent the calculated values by eqs 5–10 using the values of the parameters which were estimated by the nonlinear least squares method. Each experimental point is shown as the mean ± SD.

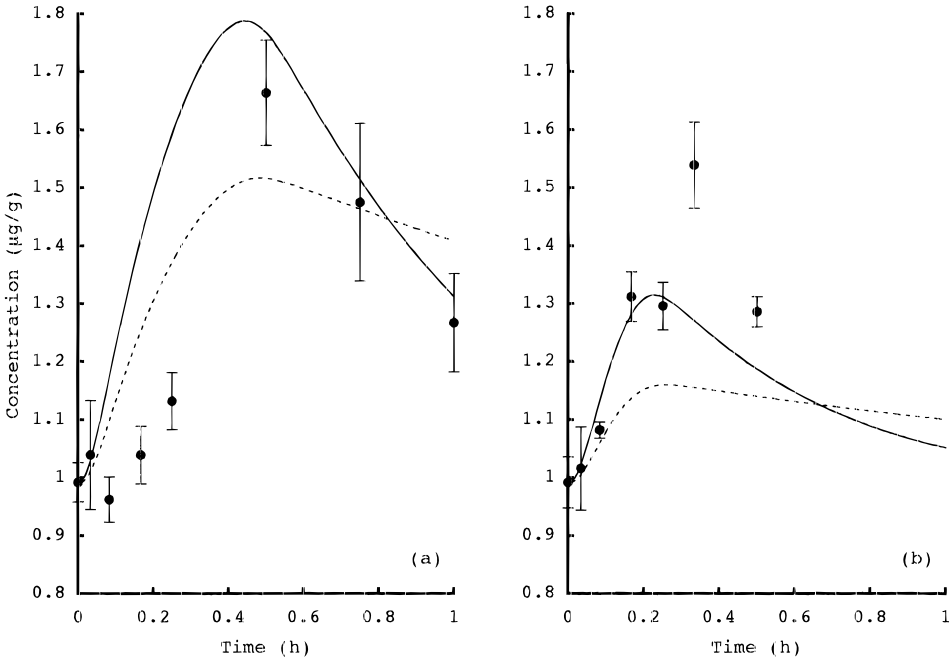

Figure 5—Time course of HVA concentration in the striatum before and after intravenous administration of PEA: (a) 50 mg/kg ($n = 3$), (b) 25 mg/kg ($n = 3$). The plotted points represent the observed data. The solid lines represent the calculated values by eqs 5–10 using the values of the parameters which were obtained in the L-dopa study[17] in the text. The dotted lines represent the calculated values by eqs 5–10 using the values of the parameters which were estimated by the nonlinear least squares method. Each experimental point is shown as the mean ± SD.

of IC_{50} of DA and PEA were 0.14×10^{-9} and 1.80×10^{-9} M, respectively. These calculated values of IC_{50} [$K_{m(DO)}$ and $K_{I(PE)}$] of DA and PEA were consistent with the values of IC_{50} of DA and PEA in Horn's study. These results indicated that the calculation of the IC_{50} values using this DA metabolism model might be a useful method for the evaluation of the inhibitor's ability on the DA uptake into the DA neuronal terminals.

Effect of PEA on the DOPAC and HVA Concentration in the Striatum—The time courses of the striatum DOPAC and HVA concentration before and after PEA injection are shown in Figures 4 and 5, respectively. The striatum DOPAC concentration after PEA injection (50 mg/kg) decreased immediately and recovered to the premedication level 45 min later. The HVA concentration in the striatum increased gradually after the PEA injection. In order to determine whether the time courses of DOPAC and HVA concentrations after PEA injection can be described by this DA metabolism model, the analyses were performed using eqs 5−10. The solid

Table 3—Values of Parameters for PEA, DA, DOPAC, and HVA in Rats, Obtained from Computer Fitting and Calculating of Striatum Concentration Data after Intravenous Administration of PEA (25 and 50 mg/kg)

Characteristics	Fixed[a]	Estimated[b]	L-Dopa Study[c]
C_{DO0} (μg/g)	5.9[d] (38.5 × 10^{-6} M)[e]	5.9[d]	5.9
C_{PA0} (μg/g)	3.6[d] (21.4 × 10^{-6} M)[e]	3.6[d]	3.6
C_{HV0} (μg/g)	1.0[d] (5.5 × 10^{-6} M)[e]	1.0[d]	1.0
$V_{max(DO)'}$ [(μg/g)/h]	124.27 ± 36.86[f] (0.81 × 10^{-3} M/h)[e]	1506 ± 3872[f] (9.8 × 10^{-3} M/h)[e]	(k_{DP} = 17.56 h^{-1})[g]
$K_{m(DO)}$ (μg/g)	0.021 × 10^{-3} ± 0.126 × 10^{-3} [f] (0.14 × 10^{-9} M)[e]	0.0012 ± 0.0049[f] (7.8 × 10^{-9} M)[e]	—
$K_{I(PE)}$ (μg/g)	0.218 × 10^{-3} ± 1.290 × 10^{-3} [f] (1.8 × 10^{-9} M)[e]	0.305 ± 1.459[f] (2.5 × 10^{-6} M)[e]	—
k_{DE} hr^{-1}	38.28 ± 12.80[f]	37.01 ± 13.03[f]	–
k_{DH} hr^{-1}	2.128[d]	1.116 ± 0.951[f]	2.128
$K_{m(PA)}$ μg/g	3.002[d] (17.9 × 10^{-6} M)[e]	0.062 ± 0.170[f] (0.37 × 10^{-6} M)[e]	3.002
$K_{m(HV)}$ μg/g	0.255[d] (1.40 × 10^{-6} M)[e]	0.116 ± 0.396[f] (0.64 × 10^{-6} M)[e]	0.255
INF_{DO} (μg/g)/hr	364.78[h] (2.38 × 10^{-3} M/h)[e]	1733[h] (11.3 × 10^{-3} M/h)[e]	116.67 (0.761 × 10^{-3} M/hr)[e]
$V_{max(PA)'}$ (μg/g)/hr	228.77[h] (1.36 × 10^{-3} M/h)[e]	1532[h] (9.11 × 10^{-3} M/h)[e]	192.41 (1.14 × 10^{-3} M/hr)[e]
$V_{max(HV)'}$ (μg/g)/hr	15.915[h] (87.36 × 10^{-6} M/h)[e]	7.421[h] (40.74 × 10^{-6} M/h)[e]	15.915
k_{0DO} (μg/h)	29.912[i]	142.1[i]	9.567
CL_{DE} (mL/h)	3.139[i]	3.035[i]	–
CL_{DH} (mL/h)	0.174[i]	0.0915	0.174
$V_{max(DO)}$ (μg/h)	10.190[i]	123.54[i]	(CL_{DP} = 1.44 mL/h)[g]
$V_{max(PA)}$ (μg/h)	18.759[i]	125.67[i]	15.78
$V_{max(HV)}$ (μg/h)	1.305[i]	0.609[i]	1.305
SS values[j]			
DA (50 mg/kg)	1.611	1.651	–
DA (25 mg/kg)	0.448	0.251	–
DOPAC (50 mg/kg)	3.772	2.935	–
DOPAC (25 mg/kg)	1.151	1.448	–
HVA (50 mg/kg)	0.410	0.164	–
HVA (25 mg/kg)	0.085	0.218	–
Total SS	7.477	6.668	–
AIC	84.45	86.10	–

[a] The values of the parameters [k_{DH}, $K_{m(HV)}$, and $K_{m(PA)}$] for HVA and DOPAC were fixed to the obtained values of the L-dopa study.[17] [b] The values of the parameters [k_{DH}, $K_{m(HV)}$, and $K_{m(PA)}$] for HVA and DOPAC were estimated by fitting the data using FKDM. [c] The values of these parameters were obtained in the L-dopa study.[17] [d] The values of these parameters were fixed to the values obtained from the L-dopa study.[17] [e] The values of these parameters were expressed using molar concentrations. [f] The values of these parameters were estimated by fitting the data using the computer program FKDM. All values are expressed as the mean ± SD of the estimated parameters. [g] The values of these parameters were the metabolic constant (clearance) from DA to DOPAC in the L-dopa study. [h] The values of these parameters were calculated by eqs 8, 9, and 10 in the text, respectively. [i] The values of these parameters were calculated by the following equations, respectively: $k_{0DO} = INF_{DO}V_{st}$, $CL_{DE} = k_{DE}V_{st}$, $CL_{DH} = k_{DH}V_{st}$, $V_{max(DO)} = V_{max(DO)'}V_{st}$, $V_{max(PA)} = V_{max(PA)'}V_{st}$, $V_{max(HV)} = V_{max(HV)'}V_{st}$. [j] The values of sum of squares (SS) and Akaike's information criterion (AIC) on DA, DOPAC, and HVA data.

lines in Figures 4 and 5 represent the calculated values. The time courses of the striatum DOPAC concentration at 30 (50 mg/kg), 5, and 10 min (25 mg/kg) and the lag time of the striatum HVA concentration could not be described by this DA metabolism model. The parameters [$K_{m(PA)}$, k_{DH}, and $K_{m(HV)}$] for DOPAC and HVA were fixed to the obtained values of the L-dopa study,[17] and the other parameters [$V_{max(PA)'}$ and $V_{max(HV)'}$] were calculated by eqs 9 and 10; the values of these parameters are listed in Table 3.

In order to examine the effects of PEA on the metabolism rate from DA to HVA (k_{DH}) and on the elimination rate of DOPAC and HVA [$K_{m(HV)}$ and $K_{m(PA)}$], and to confirm whether the time courses of the striatum DOPAC and HVA concentrations can be explained by the reestimated values of these parameters [k_{DH}, $K_{m(HV)}$, and $K_{m(PA)}$], the analyses were performed using eqs 5−10. The dotted lines in Figures 4 and 5 represent the calculated values. There was no significant difference between the paths of the solid lines and the dotted lines. In spite of the reestimation of these parameters, the time courses of the striatum DOPAC and HVA concentrations could not be explained, and there were notable differences between the fixed and estimated values of the parameters (Table 3). These results suggested that other assumptions are necessary to explain the time courses of the striatum DOPAC and HVA concentrations after PEA injection. The values of the reestimated parameters, sum of square (SS), and Akaike's information criterion (AIC)[33] are listed in Table 3. In order to clarify the reasons for the differences between the observed data and the calculated values of the striatum DOPAC and HVA concentrations, the direct relationship between the striatum PEA concentration and DA, DOPAC, and HVA concentrations was examined; these relationships are shown in Figure 6. The solid and dotted lines represent the calculated values of 50 and 25 mg/kg using the DA metabolism model, respectively. The arrows on the solid lines show the progress of time. Concerning the relationship between the striatum DA concentration and the striatum PEA concentration (Figure 6a), it was clarified that the anticlockwise hysteresis relationship between the alteration of the striatum DA concentration (pharmacological response of PEA) and the striatum PEA concentration is present after PEA injection. This result indicated that the effect compartment analysis[34] or another assumption is necessary to quantitatively describe this anticlockwise hysteresis relationship. The constructed DA metabolism model was able to describe the time course of the striatum DA concentration after PEA injection, indicating that the DA metabolism model is a useful compartment model for the explanation of this anticlockwise hysteresis relationship.

Figure 6b represents the relationship between the alteration of the DOPAC concentration and the striatum PEA concentration after PEA injection. In spite of the increase in the striatum PEA concentration, the striatum DOPAC concentrations were almost constant values (about 1.3 μg/g at 50 mg/kg dosing and about 2.4 μg/g at 25 mg/kg dosing). This response of the striatum DOPAC concentration seemed to be an on−off response. It has been reported that the reduction of the striatum DOPAC concentration after PEA injection is caused by the competitive inhibition of PEA not only on the reuptake of DA into DA neuronal terminals[7,8] but also on the

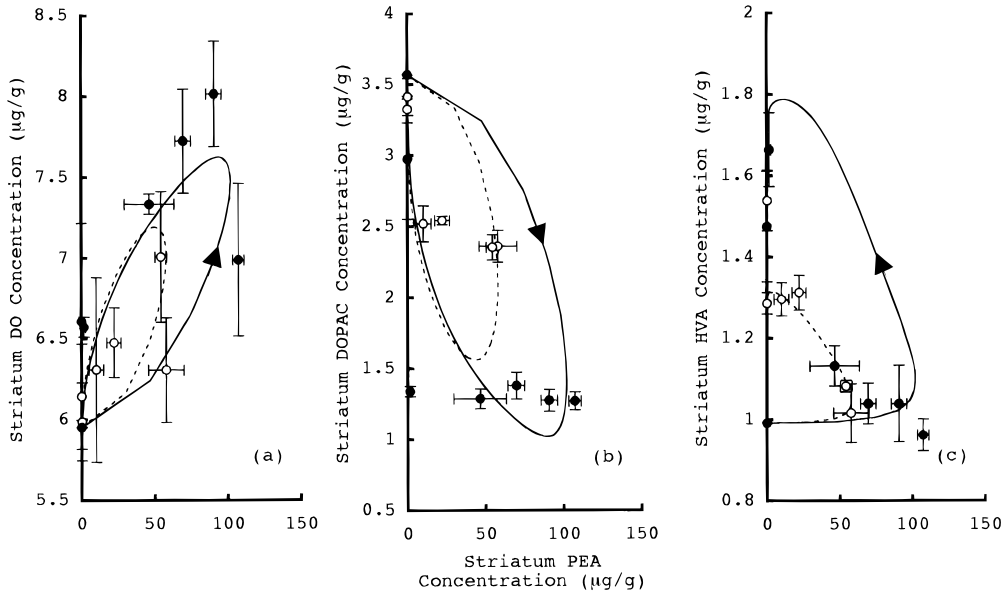

Figure 6—Relationship between the striatum PEA concentration and the striatum DA, DOPAC, and HVA concentration after intravenous administration of PEA: (a) PEA *vs* DA concentration ($n = 3$); (b) PEA *vs* DOPAC concentration ($n = 3$); (c) PEA *vs* HVA concentration ($n = 3$); ●, 50 mg/kg; ○, 25 mg/kg. The plotted points represent the observed data. Each experimental point is shown as the mean ± SD. The solid (50 mg/kg) and dotted (25 mg/kg) lines represent the calculated values. The allows on the solid lines show the progress of time.

metabolism from DA to DOPAC (the inhibition of PEA on MAO-B activity) in the DA neuronal terminals.[9] Moreover, it has been well-recognized that PEA is rapidly and almost quantitatively metabolized to PAA by MAO-B.[6] Thus, the MAO-B activity contributed to not only the metabolism from DA to DOPAC but also the metabolism from PEA to PAA. The discrepancy between the observed striatum DOPAC concentrations and the calculation values using the DA metabolism model might be caused by these complex effects of PEA on the disposition of catecholamines in the striatum. These results suggested that other assumptions such as the separate evaluation of the effect of PEA on the reuptake of DA into DA neuronal terminals and on MAO-B activity in the DA neuronal terminals might be necessary to explain the time course of the striatum DOPAC concentration after PEA injection.

Figure 6c represents the relationship between the alteration of the HVA concentration and the striatum PEA concentration after PEA injection. In spite of the increase in the striatum PEA concentration, the striatum HVA concentrations did not increase. This result indicated that here is no direct relationship between the alteration of HVA concentration and that of PEA concentration in the striatum. Westerink and Korf showed that DA is predominantly metabolized to DOPAC and that this metabolite is partly removed from the brain and partly o-methylated to HVA.[35] Westerink and Spaan demonstrated that about 80% of HVA is formed from DOPAC and 20% from 3-methoxytyramine in rat striatum.[36] These findings indicated that the metabolic pathway from DOPAC to HVA might be necessary to explain the time courses of the striatum DOPAC and HVA concentrations after PEA injection. However, the ratio of the metabolism from DOPAC to HVA was not measured in the present study. If the DA metabolism model including the metabolism parameters from DOPAC to HVA are constructed, this DA metabolism model will be complicated and the estimation of these parameters might be very difficult. Therefore, in this analysis, the metabolism parameters from DOPAC to HVA were not assumed.

Conclusion

The time courses of the plasma and the striatum PEA concentrations after PEA injection were well-explained by the two-compartment model with nonlinear elimination kinetics and the diffusion-limited model including a Michaelis−Menten type transport system from plasma to the striatum and the nonlinear elimination from the striatum, respectively. The time courses of the striatum DA, DOPAC, and HVA concentrations after PEA injection were analyzed using the DA metabolism model. The alteration of the striatum DA concentration after PEA injection could be described quantitatively by the assumption that the enhancement of the striatum DA concentration is caused by the competitive inhibition of PEA on the reuptake of DA into DA neuronal terminals. However, the time courses of the striatum DOPAC and HVA concentrations could not be described by this DA metabolism model. From these results, the following circumstances were clarified: (1) A quantitative relationship between PEA concentration and the enhancement of DA concentration in the striatum is present after PEA injection. (2) Since there is no quantitative relationship between the increase in DA concentration and the reduction of DOPAC concentration in the striatum, another assumption such as the separate evaluation of the effect of PEA on the reuptake of DA into DA neuronal terminals and on MAO-B activity in the DA neuronal terminals might be necessary to explain the time course of the striatum DOPAC concentration. (3) The assumption of the metabolic pathway from DOPAC to HVA might be necessary to explain the time courses of the striatum DOPAC and HVA concentrations after PEA injection. Thus, although high doses of PEA were used in the present study, the results obtained contribute to the elucidation of the pharmacological mechanism of PEA on the disposition of catecholamines in the striatum.

References and Notes

1. Berry, M. D.; Scarr, E.; Zhu, M.-Y.; Paterson, I. A.; Juorio, A. V. *Br. J. Pharmacol.* **1994**, *113*, 1159−1166.
2. Oldendorf, W. H. *Am. J. Physiol.* **1971**, *221*, 1629−1639.

3. Juorio, A. V. Brain β-phenylethylamine: localization, pathways, and interrelation with catecholamines. In Progress in catecholamine research, part B: Central aspects. Neurology and Neurobiology, Vol. 42B, eds. Sandler, M., Dahlstrom, A., Belmaker, R. H., Eds.; Alan Liss: New York, 1988; pp 433−437.
4. Yu, P. H. Gen. Pharm. **1994**, *25*, 1527−1539.
5. Paterson, I. A.; Juorio, A. V.; Boulton, A. A. *J. Neurochem*. **1990**, *55*, 1827−1837.
6. Durden, D. A.; Philips, S. R. *J. Neurochem*. **1980**, *34*, 1725−1732.
7. Dyck, L. E. *Brain. Res. Bull*. **1984**, *12*, 23−28.
8. Parker, E. M.; Cubeddu, L. X. *J. Pharmacol. Exp. Ther*. **1988**, *245*, 199−210.
9. Tang, H. Y. T.; Neff, N. H. *J. Pharmacol. Exp. Ther*. **1973**, *187*, 365−371.
10. Tyce, G. M.; Rorie, D. K. *Life. Sci*. **1985**, *37*, 2439−2448.
11. Hauger, R. L.; Skolnick, P.; Paul, S. M. *Eur. J. Pharmacol*. **1982**, *83*, 147−148.
12. Paetsch, P. R.; Greenshaw, A. J. *Cell. Mol. Neurobiol*. **1993**, *13*, 203−215.
13. Juorio, A. V.; Paterson, I. A.; Zhu, M.-Y. *Eur. J. Pharmacol*. **1994**, *254*, 213−220.
14. O'Reilly, R. L.; Davis, B. A. *Prog. Neuro-Psychopharmacol. Biol. Psychiat*. **1994**, *18*, 63−75.
15. Davis, B. A.; Boulton, A. A. *Prog. Neuro-Psychopharmacol. Biol. Psychiat*. **1994**, *18*, 17−45.
16. Sabelli, H. C.; Javaid, I. J. *J. Neuropsychiatry. Clin. Neurosci*. **1995**, *7*, 6−14.
17. Sato, S.; Koitabashi, T.; Koshiro, A. *Biol. Pharm. Bull*. **1994**, *17*, 1622−1629.
18. Sato, S.; Koitabashi, T.; Koshiro, A. *Biol. Pharm. Bull*. **1995**, *18*, 990−995.
19. Glowinski, J.; Iversen, L. L. *J. Neurochem*. **1966**, *13*, 655−669.
20. Kataoka, H.; Ohrui, S.; Miyamoto, Y.; Makita, M. *J. Chromatogr*. **1992**, *578*, 120−123.
21. Murai, S.; Saito, H.; Masuda, Y.; Itoh, T. *J. Neurochem*. **1988**, *50*, 473−479.
22. Pearce, L. B.; Roth, J. A. *Arch. Biochem. Biophys*. **1983**, *224*, 464−472.
23. Coyle, J. T.; Snyder, S. H. *J. Pharmacol. Exp. Ther*. **1969**, *170*, 221−231.
24. DeMet, E. M.; Vosmer, G.; Halaris, A. E. *J. Neurochem*. **1981**, *36*, 917−923.
25. Berman, M.; Shahn, E.; Weiss, M. F. *Biophys. J*. **1962**, *2*, 275−287.
26. Marquardt, D. W. *J. Soc. Ind, Appl. Math*. **1963**, *11*, 431−441.
27. Huebert, N. D.; Schwach, V.; Richter, G.; Zreika, M.; Hinze, C.; Haegele, K. D. *Anal. Biochem*. **1994**, *221*, 42−47.
28. Sato, S.; Koitabashi, T.; Koshiro, A. *Biol. Pharm. Bull*. **1994**, *17*, 1616−1621.
29. Sato, S.; Koshiro, A. *Biol. Pharm. Bull*. **1995**, *18*, 593−599.
30. Itoh, T.; Sawada, Y.; Lin, T.; Iga, T.; Hanano, M. J. *Pharmacobio-Dyn*. **1988**, *11*, 289−308.
31. Karoum, F.; Speciale, S. G. Jr.; Chuang, L.-W.; Wyatt, R. J. *J. Pharmacol. Exp. Ther*. **1982**, *223*, 432−439.
32. Horn, A. S. *Br. J. Pharm*. **1973**, *47*, 332−338.
33. Yamaoka, K.; Nakagawa, T.; Uno, T. *J. Pharmacokinet. Biopharm*. **1978**, *6*, 165−175.
34. Sheiner, L. B.; Stanski, D. R.; Vozeh, S.; Miller, R. D.; Ham, J. *Clin. Pharmacol. Ther*. **1979**, *25*, 358−371.
35. Westerink, B. H. C.; Korf, J. *Eur. J. Pharmacol*. **1976**, *37*, 249−255.
36. Weaterink, B. H. C.; Spaan, S. J. *J. Neurochem*. **1982**, *38*, 342−347.

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