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Inhibition of tryptophan hydroxylase by dopamine and the precursor amino acids

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Abstract—Effects of dopamine and its precursor amino acids on the activity of tryptophan hydroxylase were examined. They inhibited the enzyme activity prepared from mastocytoma cells in terms of the biopterin cofactor and the substrate L-tryptophan. In relation to the biopterin, tryptophan hydroxylase was found to have two different kinetics, and dopamine inhibited the activity in a non-competitive way to both the components. Dopamine had the highest affinity to the enzyme, followed by L-DOPA and L-tyrosine, while D-tyrosine did not inhibit the activity. In terms of L-tryptophan, L-tyrosine, L-DOPA and dopamine inhibited the enzyme non-competitively and their affinity to the enzyme was in this order. These results indicate that the indoleamine metabolism may be regulated by catecholamines and their related amino acids in the brain.

Key words: tryptophan hydroxylase; catecholamines; indoleamines; L-tyrosine; L-DOPA; biopterin cofactor

Catecholamines and indoleamines are major monoamine neurotransmitters in the human brain and their metabolism has many common characteristics. The rate-limiting enzymes of their synthesis are TPH* [L-tryptophan, tetrahydropteridine: oxygen oxidoreductase (5-hydroxylating), EC 1.14.16.4,] and TH [tyrosine tetrahydropteridine: oxygen oxidoreductase (5-hydroxylating), EC 1.14.16.2,], respectively. These enzymes require (6*R*)BH_4 as a common cofactor. The hydroxylated products by TH or TPH are decarboxylated by the same enzyme, AADC (EC 1.1.4.28). The catecholamine and indoleamine metabolism have been suggested to interact with each other at the various stages; the transport of the precursor amino acids through the blood–brain barrier, the uptake into the nerve cells and the synthesis and catabolism of the amines. In connection with the biosynthesis, TH was reported to be inhibited by tryptophan derivatives *in vivo* and *in vitro* [1]. 5-Hydroxy-α-methyl-DL-tryptophan was a most potent inhibitor of TH, and 5-HTP inhibited TH, but the D-isomer did not [1]. The inhibition of TH by these amino acids was uncompetitive to the substrate L-tyrosine. In terms of the biopterin cofactor, they inhibit TH in a competitive and noncompetitive way, depending on the biopterin concentration. On the other hand, inhibition of 5-HT synthesis by DOPA and other catechols was also reported in rat brain slices [1]. The inhibition was further proved *in vitro* using TPH samples prepared from rat brainstem and beef pineal, and the inhibition was interpreted to be due to chelating ferrous ion required for the activity [2].

Recently, a series of dopamine-derived tetrahydroisoquinolines have been found to release 5-HT selectively [2]. Out of 6,7-dihydroxy isoquinolines, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), was found to inhibit TPH. The inhibition was non-competitive to L-tryptophan, and in a competitive and non-competitive way to (6*R*)BH_4, dependent on the biopterin concentrations [3]. By comparison of structure–activity relationship, TPH activity was found to be inhibited by 6,7-dihydroxy isoquinolines, but not by tetrahydroisoquinolines without catechol structure [4]. The essential requirement of catechol structure was clearly demonstrated by 6,7-dihydroxy-*N*-cyanomethyl-1,2,3,4-tetrahydroisoquinoline, a dopamine-derivative produced from cigarette smoke [5]. It inhibits TPH activity in a non-competitive way to the substrate, but a β-carboline analog, 6-hydroxy-*N*-cyanomethyl-tetrahydro-β-carboline, is inactive to TPH. These results indicate that catecholamines and their related compounds may affect 5-HT level in the brain and inhibit TPH activity in general.

To prove the interaction of indoleamine and catecholamine metabolism, a new *in vivo* method for the simultaneous measurement of *in situ* TH and TPH activity was amended using microdialysis and an AADC-inhibitor, NSD-1015 [6]. After 60 min perfusion of 10–100 μM NSD-1055 in the rat striatum, the level of L-DOPA and 5-HTP reached a plateau. The accumulated L-DOPA and 5-HTP in the extracellular fluid were considered to be an index of *in situ* activity of TH and TPH. By intraperitoneal administration of 5-HTP, the biosynthesis of L-DOPA in the brain was reduced in a dose-dependent way by 5-HTP administrated. Also by L-DOPA injection, the synthesis of 5-HTP in the striatum was reduced markedly. The reduction of 5-HT biosynthesis may be due to inhibition of transport of L-tryptophan into the brain and dopamine cells or direct inhibition of TPH by L-DOPA.

In this article the mechanism of the inhibition of TPH activity by L-DOPA was studied. Using the enzyme sample prepared from the mastocytoma cells, the *in vitro* effects of dopamine and the related amino acids on TPH activity were examined. The interaction of indoleamines and catecholamines is discussed in relation to the possible involvement in the metabolism and regulation of these monoamines under physiological and pathological conditions.

Materials and Methods

The chemicals listed below were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.); antipain, pepstatin A, chymostatin, leupeptin, L- and D-tryptophan, L- and D-DOPA, dopamine, 5-HTP, NSD-1015 (*m*-hydroxybenzylhydrazine) and bovine γ-globulin. PMSF, DTT, β-mercaptoethanol and sodium octanesulfonate were purchased from Nacalai tesque (Kyoto, Japan), and Sephadex G-25 from Pharmacia Fine Chemicals (Uppsala, Sweden). (6*R*)BH_4 was kindly donated by Dr Matsuura (Fujita Health University, Toyoake, Japan). Catalase prepared from bovine liver was purchased from Boehringer

* Abbreviations: TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase; (6*R*)BH_4, (6*R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin; AADC, aromatic L-amino acid decarboxylase; 5-HTP, 5-hydroxy-L-tryptophan; 5-HT, serotonin, 5-hydroxytryptamine; PMSF, phenylmethylsulfonylfluoride; DTT, dithiothreitol.

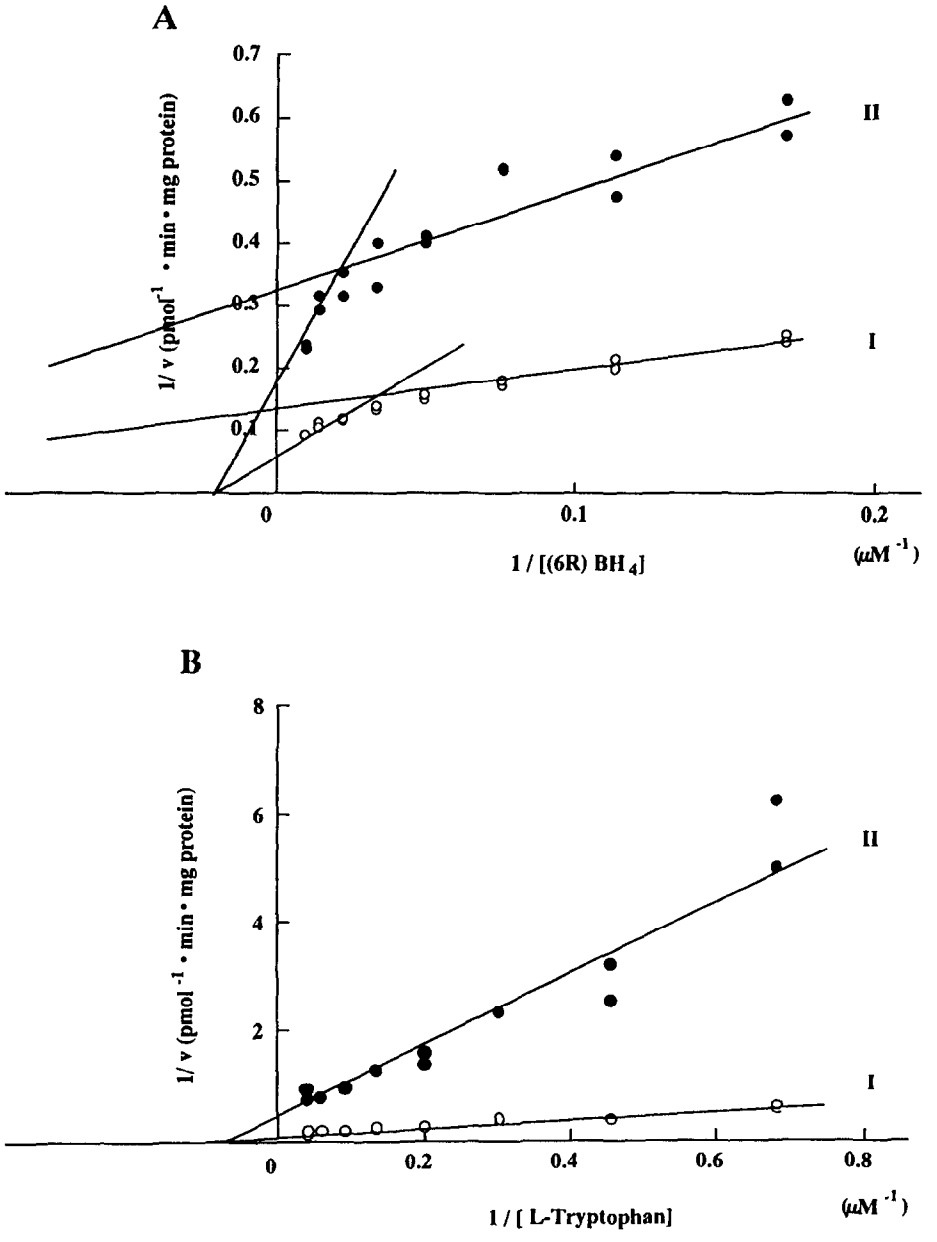

Fig. 1. Effects of L-tyrosine on TPH activity. The enzyme activity was measured with various concentrations of (6*R*)BH_4 and 100 μM L-tryptophan (A), and those of L-tryptophan and 100 μM (6*R*)BH_4 (B). The activity was measured without L-tyrosine (I) and with 100 μM L-tyrosine (II). The reciprocal of the enzyme activity was plotted against that of the concentration of the biopterin cofactor (A) or the substrate (B).

Mannheim (Mannheim, Germany). Organic solvents were of HPLC grade.

The murine mastocytoma cell line P-815, carried in DBA/2 mice [7], was kindly donated by Dr Hosoda (Aichi Cancer Institute, Nagoya, Japan). The ascitic fluid was taken and the cells were gathered by centrifugation at 1000 *g* for 10 min. The cells were washed twice and suspended with Hank's balanced salt solution and stored at −80° until use. The cells (250 mg protein) were diluted with the same volume of the extraction medium; 100 mM Tris-acetate buffer, pH 7.6, containing 2 mM PMSF, 4 mM DTT, 10 μg/mL each of antipain, pepstatin A, chymostatin, and leupeptin. The cell suspension was sonicated and centrifuged at 100,000 *g* for 30 min. The supernatant was passed through a Sephadex G-25 column (2 cm i.d. × 15 cm) equilibrated with 10 mM Tris-acetate buffer, pH 7.6, containing 1 mM PMSF. The fractions were eluted out by the same buffer in a void volume, gathered and used as the enzyme sample.

TPH activity was measured by fluorimetric determination of 5-HTP produced from L-tryptophan in the presence of NSD-1015, an inhibitor of aromatic L-amino acid decarboxylase, according to the previously reported method [8] with slight modification [9]. The enzyme sample (about 60 μg protein) was incubated at 37° for 20 min with 100 μM L-tryptophan in 100 μL of 50 mM HEPES-NaOH buffer,

pH 7.6, containing 100 μM (6*R*)BH_4, 100 μM β-mercaptoethanol, catalase (40 μg protein) and 0.5 mM NSD-1015. The reaction was terminated by addition of 10 μL of 60% perchloric acid and the sample was mixed, centrifuged at 15,000 g for 10 min, and filtered through a Millipore HV filter (pore size, 0.45 μm). For quantitation of 5-HTP produced, the sample was applied onto a Shimadzu HPLC apparatus, LC6A, connected to a Shimadzu fluorescence detector, FD-500. The column used was a pre-packed reversed-phase column (4 mm i.d. × 150 mm, Cosmosil 5C18-MS, Nacalai tesque, Kyoto, Japan) and the mobile phase was 90 mM sodium acetate–35 mM citric acid buffer, pH 4.35, containing 130 μM disodium EDTA and 2 mM sodium octanesulfonate, to which methanol was added to 11%. The flow rate was 1 mL/min. The fluorescence intensity at 345 nm was measured with excitation at 295 nm. 5-HTP was quantitatively determined by comparison of the peak area with that of a standard. Protein concentration was measured according to Bradford [10] using bovine γ-globulin as standard.

The values of the Michaelis constant, K_m, and the maximal velocity, V_{max}, in terms of the substrate or the cofactor were obtained by Lineweaver–Burk's plot. When there were two different kinetics, two K_m and V_{max} values were calculated by NLS (non-linear square) method, assuming that each component follows the Michaelis–Menten's equation independently. The type and the value of the inhibitor constant, K_i, were also estimated by Lineweaver–Burk's plot.

Results

Kinetic properties of TPH sample used in these experiments are examined. In terms of the substrate, L-tryptophan, the enzyme sample showed a single value of K_m and V_{max}; 8.73 ± 0.85 μM and 38.2 ± 4.9 pmol/min/mg protein, respectively. In terms of the biopterin cofactor, (6*R*)BH_4, TPH was shown to be composed of two components with different kinetic characteristics (Fig. 1A, curve I). One component had a lower value of K_m and V_{max}, 3.44 ± 0.99 μM and 31.7 + 4.1 pmol/min/mg protein, and the other had a higher value of K_m and V_{max}, 796 ± 380 μM and 223 ± 77 pmol/min/mg protein, respectively.

As shown in Fig. 2, the effects of L-DOPA and D-DOPA on TPH activity were examined with a lower substrate (10 μM) and a higher biopterin concentration (100 μM), and *vice versa*. With a low L-tryptophan concentration, L-DOPA inhibited TPH activity more markedly than D-DOPA, and 50% reduction of the activity was obtained with around 100 μM L-DOPA (Fig. 2, I). With a lower concentration of (6*R*)BH_4, the inhibition was more prominent than under the previous conditions, and L-DOPA was a more potent inhibitor than D-DOPA. The activity was reduced to 50% of the control with 1 μM L-DOPA and 100 μM D-DOPA.

The inhibition of TPH activity by L-tyrosine was characterized by plotting the data according to Lineweaver–Burk with various concentrations of L-tryptophan and (6*R*)BH_4. Figure 1 stands for the kinetics of TPH with various concentrations of (6*R*)BH_4 (Fig. 1, A) or of L-tryptophan (Fig. 1, B). The activity was measured in the presence (curve II) and absence (curve I) of 100 μM L-tyrosine. In terms of the biopterin cofactor, L-tyrosine inhibited TPH in a non-competitive way to (6*R*)BH_4 at its lower and higher concentrations. The inhibitor constant, K_i, values for both components with higher affinity (lower K_m value) and lower affinity (higher K_m value) were 16.0 and 306 μM, respectively. In terms of L-tryptophan, L-tyrosine inhibited TPH activity in a non-competitive way. The K_i value of L-tyrosine was found to be 18.5 μM. D-Tyrosine was also incubated with TPH, but no significant effect was observed up to 1 mM in the final concentration. As shown in Fig. 3, dopamine inhibited TPH activity in terms of (6*R*)BH_4 and L-tryptophan, as in the case of L-tyrosine. The K_i value of dopamine was obtained as 7.0 and 39.3 μM for the two components in terms of the biopterin, respectively. Towards L-tryptophan, dopamine had a high K_i value; 94.3 μM. Metabolites of dopamine, 3,4-dihydroxyphenylacetic acid and homovanillic acid, did

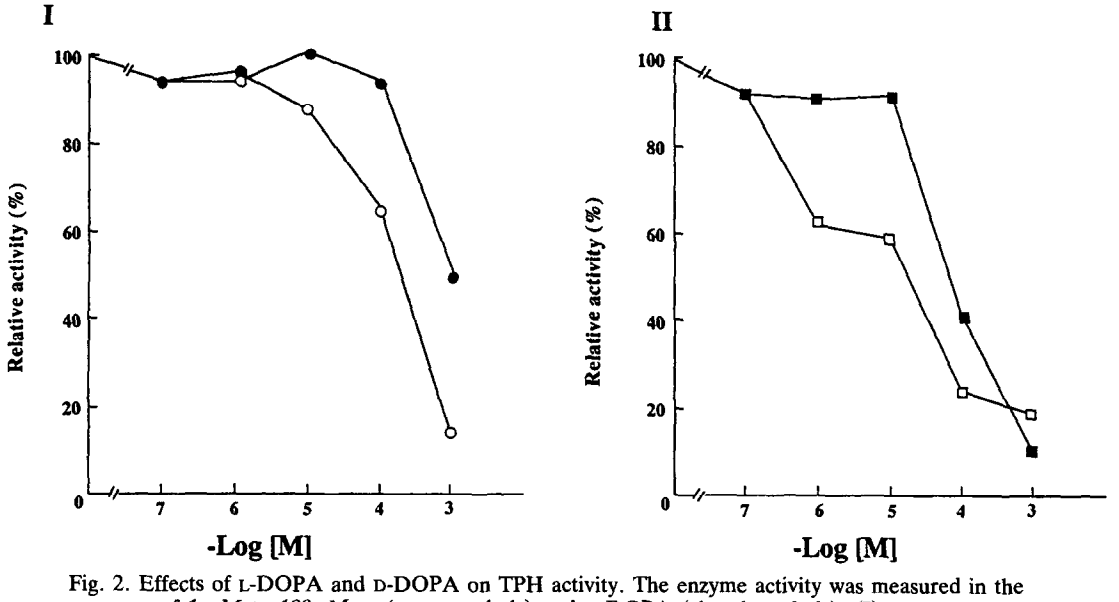

Fig. 2. Effects of L-DOPA and D-DOPA on TPH activity. The enzyme activity was measured in the presence of 1 mM to 100 nM L- (open symbols) and D-DOPA (closed symbols). (I) represents the enzyme activity measured with low L-tryptophan (10 μM) and high (6*R*)BH_4 (100 μM) concentration, and (II) represents that measured with high substrate (100 μM) and low biopterin (10 μM) concentration. Each spot shows the mean of the data obtained by duplicate measurements of two experiments.

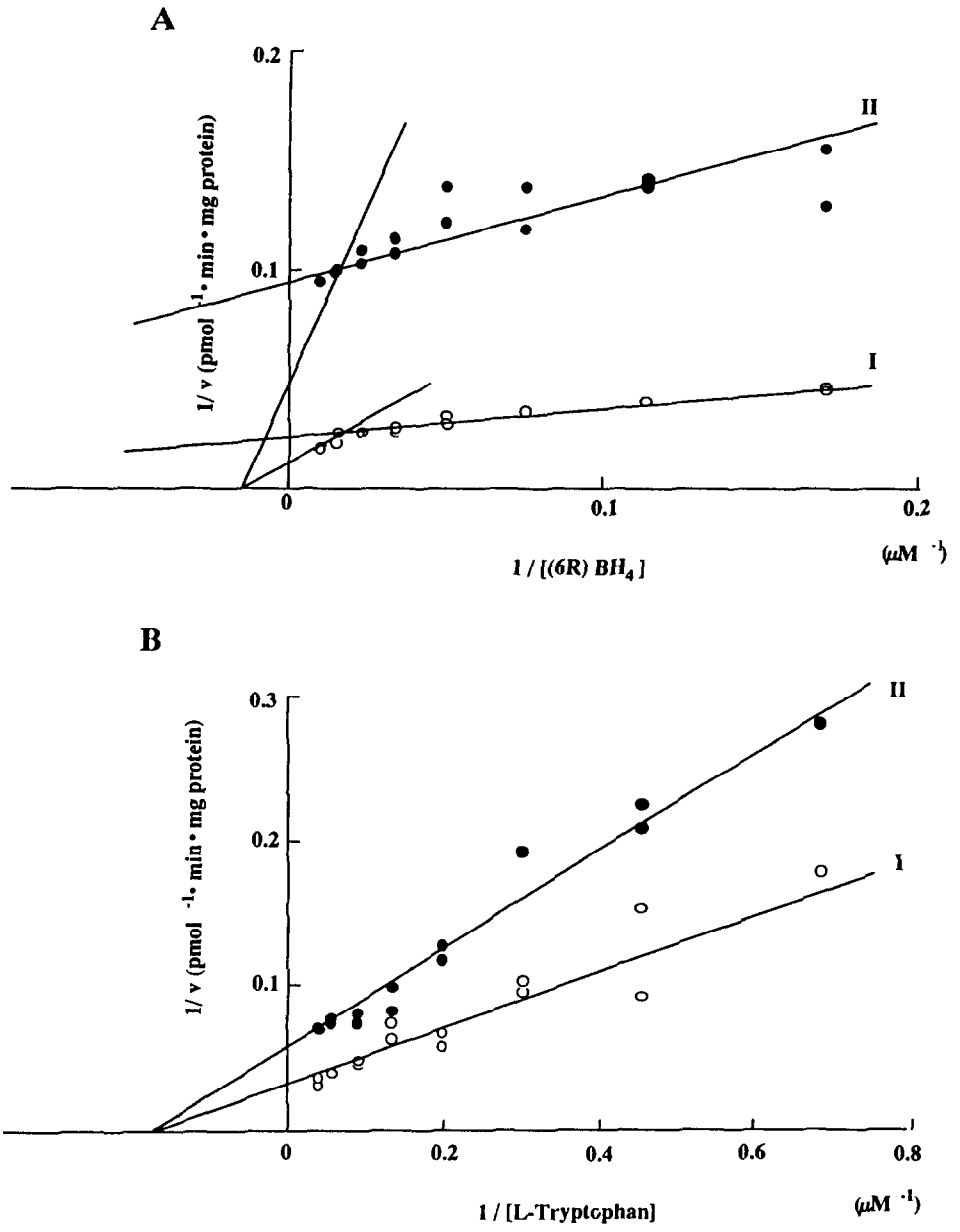

Fig. 3. Effect of dopamine on TPH activity. The conditions for the measurement of the activity and of the effect of dopamine are the same as described in Fig. 1. The dopamine concentration was 100 μM.

not inhibit TPH activity up to their concentrations of 1 mM.

Table 1 summarizes the effects of L- and D-tyrosine, L- and D-DOPA and dopamine on TPH activity. The most potent inhibitor of TPH in terms of (6*R*)BH_4 was dopamine, followed by L-tyrosine, L-DOPA. D-Tyrosine did not inhibit TPH and D-DOPA was a weak inhibitor. In terms of L-tryptophan, L-DOPA and L-tyrosine were potent inhibitors, and dopamine was less potent. The type of inhibition by these compounds was non-competitive to the biopterin cofactor and the substrate.

Discussion

As factors regulating TPH activity in the brain, the concentration of the substrate L-tryptophan and the biopterin cofactor have been proposed. Indeed, in the brain the biopterin concentration is around 3 μM under normal conditions [11], which is almost the same as the K_m value of a high-affinity component, 3.44 μM. The concentration is under saturation for another biopterin-requiring enzyme, tyrosine hydroxylase [12]. On the other hand, TPH activity in the brain is more dependent on the substrate concentration than that of (6*R*)BH_4. The interaction of dopamine and related compounds to serotonin synthesis has been previously reported by *in vivo* and *in vitro* experiments [1, 13, 14]. The inhibition of the activity of TPH solubilized from rat brainstem was non-competitive in terms of biopterin cofactor and the inhibition was claimed to be by chelating ferrous ion [13]. However, the results reported in this paper show that the inhibition potential of L-tyrosine and L-DOPA are clearly higher than D-isomers, which suggests that TPH can distinguish their steric configuration and that the inhibition may not be due

Table 1. Effects of dopamine and the precursor amino acids on kinetics of TPH

In terms of substrate L-tryptophan	K_i (μM)	
L-Tyrosine	18.5 ± 0.51	
D-Tyrosine	NI	
L-DOPA	17.0 ± 3.3	
D-DOPA	76.1 ± 21.6	
Dopamine	94.3 ± 13.8	
In terms of cofactor (6*R*)BH_4	**K_i (μM)**	**K_i (μM)**
L-Tyrosine	16.2 ± 11.8	305.9 ± 76.8
D-Tyrosine	NI	
L-DOPA	80.3 ± 45.3	88.7 ± 30.3
D-DOPA	609.5 ± 133.3	201.9 ± 32.3
Dopamine	7.01 ± 0.01	39.3 ± 6.2

Each value represents the mean ± SD of duplicate measurements of three experiments. The kinetic data of the enzyme activity in terms of L-tryptophan were determined with 100 μM (6*R*)BH_4. The kinetic data of the enzyme activity in terms of (6*R*)BH_4 were determined with 100 μM L-tryptophan. Type of inhibition was non-competitive to the substrate and the biopterin cofactor.
NI, no inhibition.

to chelating iron. In addition, TPH sample used in these experiments does not require ferrous ion for the activity.

Recently benserazide, 2-[(2, 3, 4-trihydroxypheny)-methyl]hydrazine, which is a catechol and an AADC inhibitor, was found to inhibit TPH in competition with the biopterin cofactor and in a non-competitive way to the substrate, L-tryptophan [15]. On the other hand, food-derived heterocyclic amines, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole inhibit TPH in competition to the substrate and non-competitive way to the biopterin [16]. 6,7-Dihydroxyisoquinolines, such as salsolinol, inhibit TPH in a similar way to heterocyclic amines [3]. In terms of the substrate and cofactor, dopamine and related amino acids have different intensities of inhibition. Dopamine has the highest affinity to TPH in terms of biopterin, while L-tyrosine and L-DOPA are more potent than dopamine in terms of the substrate. The presence of the side chain may affect the binding of these agents to the active site of TPH. TPH may bind L-tyrosine and L-DOPA near to the binding site of L-tryptophan. On the other hand, dopamine and salsolinol without the side chain may be bound at or near to the biopterin binding site. The increase in the length of the side chain reduced the inhibition in relation to biopterin, as shown with *N*-cyanomethylisoquinoline [5].

In the case of L-DOPA therapy to the patients with Parkinson's disease, TPH activity may be inhibited by L-DOPA and L-tyrosine, which are enhanced markedly by L-DOPA administration. In the cerebrospinal fluid of parkinsonian patients, the concentrations of tyrosine and DOPA were found to increase to 33 ± 19 μM and 100 ± 61 nM from 14 ± 7 μM and non-detectable level in the control, respectively [17]. Considering that these amino acids are more concentrated in the cells, the inhibition of TPH may occur in the brain. Thus the effects of catecholamines and the related compounds on 5-HT biosynthesis should be taken into account in the case of L-DOPA therapy. It might be relevant to the observation that in the patients with Parkinson's disease 5-HT contents are decreased in the brain, and may elicit some clinical psychiatric symptoms, such as insomnia, depressive state, uneasiness and anxiety. These results indicate that the metabolism of catecholamines and indoleamines interact with each other in the brain under physiological and pathological conditions.

†Department of Biosciences
Nagoya Institute of Technology
‡Department of Neurology
Nagoya University School of Medicine
Nagoya
§Department of Food and Nutrition
Konan Women's College
Konan
‖Department of Psychiatry
Gifu Prefecture Tajimi Hospital
Tajimi, Gifu
Japan
¶Unite de Neuropharmacologie
Universitet de Paris XI
Orsay Cedex
France

MAKOTO NAOI*†
WAKAKO MARUYAMA‡
TSUTOMU TAKAHASHI§
MIYUKI OTA‖
HASAN PARVEZ¶

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* Corresponding author: Department of Biosciences, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466, Japan. Tel. 52-732-211, Ext. 2367; FAX 52-732-9872.

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