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STUDIES ON THE ACTIVITY OF L-THREO-3,4-DIHYDROXYPHENYLSERINE (L-DOPS) AS A CATECHOLAMINE PRECURSOR IN THE BRAIN

COMPARISON WITH THAT OF L-DOPA

TERUFUMI KATO, NOBUYUKI KARAI, MICHIKO KATSUYAMA, MITSUTAKA NAKAMURA and JUNKI KATSUBE*

Research Laboratories, Sumitomo Pharmaceuticals Co. Ltd., Osakashi, Osakafu 554, Japan

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Abstract—L-Threo-3,4-dihydroxyphenylserine (L-DOPS) was compared with L-3,4-dihydroxyphenylalanine (L-DOPA) with respect to their activities as central amine precursors. The apparent K_m value (the substrate affinity) of L-DOPS for aromatic L-amino acid decarboxylase was nearly equal to that of L-DOPA, whereas the v_{max} value (the rate of decarboxylation) of L-DOPS was much smaller than that of L-DOPA. the penetration of L-DOPS into the brain through the blood–brain barrier was found to be smaller (about one-fourth) than that of L-DOPA but, for an amine precursor, it was still substantial. Unlike L-DOPA, L-DOPS did not cause a marked accumulation of norepinephrine (NE), the corresponding catecholamine in the brain, but nialamide, a monoamine oxidase inhibitor significantly enhanced the L-DOPS-induced rise of NE. Moreover, the brain concentration of 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG), the principal end metabolite of NE, was increased markedly by L-DOPS. These results suggest that L-DOPS may act as an NE precursor in the brain and activate NE neurons by increasing the turnover rate of NE.

It is well known that L-3,4-dihydroxyphenylalanine (L-DOPA), a physiological precursor of dopamine (DA) and also an anti-Parkinsonism agent, is a good substrate of the aromatic L-amino acid decarboxylase, both *in vitro* and *in vivo*, and can produce a marked increase in brain DA level [1–4].

It has also been demonstrated that a synthetic amino acid, DL- or L-threo-3,4-dihydroxyphenylserine (DL- or L-DOPS), is decarboxylated by the same enzyme to yield natural (−)-norepinephrine (NE) both *in vitro* and *in vivo* [1, 4–9]. There has been considerable controversy however, about the capability of L-DOPS as an NE precursor in the central nervous system (CNS). Bartholini *et al.* [1] and Sano *et al.* [9] concluded that L-DOPS should be a poor NE precursor in the CNS, based on the experimental result that the rise of brain NE concentration in rats after the injection of L-DOPS was slight or negligible. In clinical studies, on the other hand, it has been reported that L- or DL-DOPS has a beneficial effect on freezing phenomena or akinesia [10, 11] hypotension [12], or Parkinson's disease, although there have been a couple of papers that described no effect on Parkinson's disease [13] or narcolepsy [14].

Thus, we have attempted to determine whether L-DOPS acts as an NE precursor in the CNS. In this paper, the biochemical actions of L-DOPS in mice or rats have been compared with those of L-DOPA.

MATERIALS AND METHODS

Male dd strain mice, weighing 22–28 g, and male Sprague–Dawley strain rats, weighting 250 g were used.

Experiment I, in vitro

Enzyme preparation. Mice or rats were decapitated, and kidneys and brain removed, weighed and homogenized in 15 or 5 vol. of ice-cold distilled water respectively. The homogenates were centrifuged at 8000 *g* and 4° for 15 min. The supernatant fractions were used for aromatic L-amino acid decarboxylase assay. Protein concentration of enzyme was determined by the method of Lowry *et al.* [15].

Decarboxylation of L-DOPS or L-DOPA. Decarboxylation was carried out according to the method of Fujiwara *et al.* [6]. The reaction was started by the substrate and stopped by the addition of 4 ml of ice-cold 0.4 N perchloric acid with 10 mg of sodium metabisulfite and 200 mg of ethylenediaminetetraacetic acid (EDTA) disodium salt.

Determination of reaction products, NE or DA. After decarboxylation, the mixture was centrifuged at 8000 *g* and 4° for 15 min. The reactive products were purified by the method of Suzuki *et al.* [16], the amounts of NE and DA were determined by high performance liquid chromatography, according to the method of Nagatsu *et al.* [17] with minor modification. The outline is as follows: The reaction solution was mixed with 3,4-dihydroxybenzylamine as an internal standard and 1 M ammonium phosphate buffer, pH 7.5, and applied to a column of boric acid gel (0.5 × 5 cm). L-DOPS or L-DOPA was eluted with 4 ml of 10% sorbitol solution from the boric acid gel column, and NE or DA was eluted with 2 ml of 1.3 M acetic acid in methanol after washing with 60 ml of distilled water and 2 ml of 0.1 N acetic acid in methanol. The eluate was flushed with N_2 gas to dryness, and the residue was dissolved in distilled water. Then the aliquot of the eluate was injected

*Address all correspondence to: Dr. Junki Katsube, Sumitomo Pharmaceuticals Co., Ltd., Research Laboratories, 1–98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554, Japan.

into a high performance liquid chromatograph (Yanaco L-5000, Kyoto, Japan) with an electrochemical detector (Yanaco VMD-101, Kyoto, Japan) (HPLC-ECD) and a Yanapac ODS-A column (Yanaco, Kyoto, Japan).

Experiment II, in vivo

Determination of amino acids or amines. Benserazide or nialamide was given intraperitoneally 1 hr before the intraperitoneal injection of L-DOPS or L-DOPA in mice. At 1 hr after L-DOPS or L-DOPA, blood and brains were collected. Purification and separation of the brain or serum amino acids and amines were carried out according to the method of Suzuki *et al.* [16] and the assay was performed by high performance liquid chromatography-electrochemical detection.

Determination of 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG). MHPG in mouse brain was determined mainly according to the method of Semba and Takahashi [18]. Each brain sample was weighed and homogenized in 2 ml of 0.1 N perchloric acid. After being mixed with 100 ng of *p*-hydroxybenzylalcohol (PHBA), the homogenate was centrifuged at 8000 *g* and 4° for 10 min. The supernatant fraction was shaken with 1 ml of chloroform and centrifuged to remove lipids. Then, to 1 ml of the water phase, was added 1 g of sodium chloride (NaCl) and 300 μl of 2 M potassium acetate (pH 5.7). Free-MHPG was extracted twice with 1.2 ml of ethylacetate. The combined organic phase was washed with 300 μl of 2 M potassium carbonate saturated with NaCl to remove acidic metabolites and flushed with N_2 gas to dryness. The residue was dissolved in 200 μl of distilled water and injected into the HPLC-ECD. The condition of the HPLC-ECD was: column, Yanapac ODS-A; mobile phase, 0.05 M potassium phosphate (pH 5.0) containing 1 mM hexanesulfonate-sodium, 0.1 mM EDTA-potassium and 10% methanol; flow rate, 1.0 ml/min; voltage, 800 mV vs Ag/AgCl. The retention time under this condition was: MHPG, 8 min; PHBA, 15 min.

The total-MHPG including sulfate-conjugated MHPG was also determined in samples hydrolyzed with sulfatase (Type H-1, Sigma, St. Louis, MO, U.S.A.) preliminarily, but total-MHPG levels in mouse brain were almost equal to free-MHPG levels as reported by Warsh *et al.* [19].

Drug preparation

L-DOPS was synthesized in the laboratory of Sumitomo Pharmaceuticals Co. Ltd., Osaka, Japan. L-DOPS or L-DOPA (Nakarai, Kyoto, Japan) was suspended in 0.5% methylcellulose solution for *in vivo* study and dissolved in 0.01 N hydrochloride solution for *in vitro* study. Nialamide (Sigma) was dissolved in a minimum quantity of 0.1 N hydrochloride solution made up to volume with distilled water. Benserazide hydrochloride (Hoffmann-La Roche, Basel, Switzerland) was dissolved in distilled water.

RESULTS

In vitro *study*

As shown in Table 1, the substrate affinity (apparent K_m values) of L-DOPS with aromatic L-amino acid decarboxylase prepared from mouse kidney and brain was almost equal to that for L-DOPA. On the other hand, the maximum velocity (V_{max} values) of decarboxylation of L-DOPS was much smaller than that of L-DOPA. As in the mouse, in the rat apparent K_m values of L-DOPS with kidney and brain enzymes were almost equal to those for L-DOPA, and v_{max} values for L-DOPS decarboxylation were much smaller than those of L-DOPA.

In vivo *study*

L-DOPA (400 and 600 mg/kg, i.p.) markedly increased brain DA concentrations and, at 600 mg/kg, slightly but significantly increased the brain NE levels. In contrast, L-DOPS at 400 mg/kg produced a slight but not significant, and at 800 mg/kg (i.p.) a slight but significant, increase in brain NE levels, whereas brain DA concentrations were not increased at all by L-DOPS (Table 2).

As shown in Table 3, the serum amino acid levels after 400 mg/kg (i.p.) of L-DOPS and L-DOPA were 59.8 ± 8.1 and 12.8 ± 0.09 μg/ml respectively; that of L-DOPS was about 5-fold higher than that of L-DOPA. In contrast, the brain amino acid levels due to the same dose (400 mg/kg, i.p.) of L-DOPS and L-DOPA were almost equal, being 1.89 ± 0.09 and 2.43 ± 0.37 μg/g respectively. When the same doses (400 mg/kg, i.p.) of L-DOPS and L-DOPA were administered simultaneously, the brain and serum concentrations of both amino acids were enhanced

Table 1. Apparent K_m and V_{max} values of aromatic amino acid decarboxylation of L-DOPS and L-DOPA by supernatant fractions of mouse kidney, mouse brain, rat kidney and rat brain homogenates

Drugs	Apparent K_m (mM)				V_{max} (nmol/mg protein/min)			
	Brain		Kidney		Brain		Kidney	
	Rat	Mouse	Rat	Mouse	Rat	Mouse	Rat	Mouse
L-DOPS	1.3	0.4	1.2	0.4	0.08	0.08	0.50	0.07
L-DOPA	2.0	0.3	2.0	0.4	8.3	1.9	33	1.8
L-DOPS/L-DOPA					1/104	1/24	1/66	1/26

Apparent K_m values (the substrate affinity) and V_{max} values (the rat of decarboxylation) of L-DOPS or L-DOPA were obtained by Lineweaver–Burk plots.

Table 2. Concentrations of norepinephrine and dopamine in the brain after injection of L-DOPS or L-DOPA into mice that were, and were not pretreated with benserazide

Drugs	Dose (mg/kg, i.p.)	N	Brain concentration (μg/g) Norepinephrine		Dopamine	
Control		5	0.67 ± 0.03	(100)	1.20 ± 0.03	(100)
L-DOPS	400	5	0.76 ± 0.04	(113)	1.22 ± 0.08	(102)
	800	5	0.85 ± 0.03*	(127)	1.19 ± 0.03	(99)
L-DOPA	400	5	0.79 ± 0.07	(118)	2.19 ± 0.17*	(183)
	600	5	0.81 ± 0.08*	(121)	2.30 ± 0.19*	(191)
L-DOPS + benserazide	400 1	5	0.73 ± 0.03	(109)	1.23 ± 0.06	(102)
L-DOPA + benserazide	400 1	5	0.62 ± 0.05	(93)	3.69 ± 0.30*,†	(307)

L-DOPS or L-DOPA was injected intraperitoneally 1 hr after intraperitoneal injection of benserazide. The animals were killed 1 hr after L-DOPS or L-DOPA N = number of animals used. Values are expressed as means ± SEM. Numbers in parentheses are the percents of control.

*$P < 0.05$, compared with control (Student's t-test).

†$P < 0.05$, compared with L-DOPA alone (Student's t-test).

significantly when compared with the cases where each amino acid was administered alone. After pretreatment with benserazide (1 mg/kg, i.p.), the concentrations of L-DOPA in both serum and brain were increased markedly by 182 and 117%, respectively, when compared with L-DOPA alone, whereas those of L-DOPS were increased by only 52 and 59%, respectively. As shown in Fig. 1, the serum L-DOPS concentrations were not increased further by benserazide at high doses (10 and 30 mg/kg i.p.) whereas the serum L-DOPA concentrations continued to increase markedly in a dose-dependent manner at the same high doses.

The ratio of brain concentration to serum concentration at 1 hr after intraperitoneal injection of various doses of L-DOPS or L-DOPA was determined, from the correlation plots, to be 0.04 for L-DOPS and 0.17 for L-DOPA (Fig. 2).

The increase in brain DA concentration after L-DOPA injection (400 mg/kg, i.p.) was increased further by benserazide (1 mg/kg, i.p.), whereas the slight increase in brain NE concentration by L-DOPS (400 mg/kg, i.p.) was not enhanced by benserazide (Table 2). By pretreatment with nialamide (30 mg/kg, i.p.), however, the slight increase in the brain NE level by L-DOPS was enhanced significantly (Fig. 3). In addition, at various doses of L-DOPS (50–800 mg/kg, i.p.), the levels of MHPG, a principal end metabolite of NE in the brain, were increased in a dose-dependent manner (Fig. 4).

Table 3. Concentrations of L-DOPS and L-DOPA in serum and brain after injection of L-DOPS and/or L-DOPA following pretreatment with benserazide

Drugs	Dose (mg/kg, i.p.)	N	Serum L-DOPS (μg/ml)	Serum L-DOPA (μg/ml)	Brain L-DOPS (μg/g)	Brain L-DOPA (μg/g)
L-DOPS	400	5	59.8 ± 8.1		1.89 ± 0.09	
L-DOPA	400	5		12.8 ± 0.09		2.43 ± 0.37
L-DOPS + L-DOPA	400 400	5	96.2 ± 24.1*	24.1 ± 2.7*	2.92 ± 0.57*	4.36 ± 0.69*
L-DOPS + benserazide	400 1	5	90.9 ± 9.1*		3.00 ± 0.14*	
L-DOPA + benserazide	400 1	5		36.1 ± 3.4*		5.28 ± 0.46*

L-DOPS or L-DOPA was injected intraperitoneally 1 hr after the injection of benserazide. The animals were killed 1 hr after L-DOPS or L-DOPA. Values are expressed as mean ± SEM. N = number of animals used.

*$P < 0.05$, compared with amino acid alone (Student's t-test).

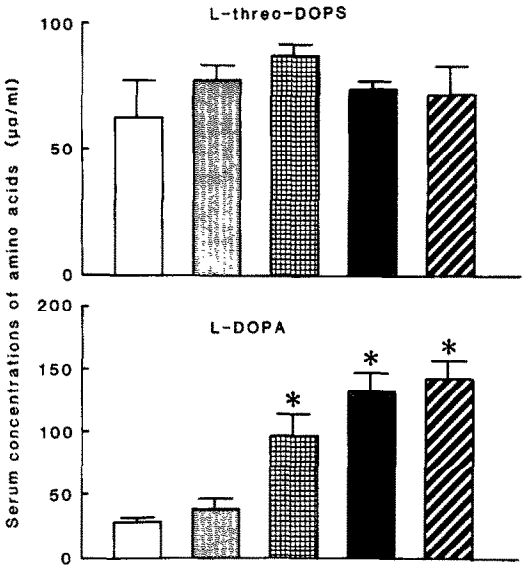

Fig. 1. Effect of benserazide on serum concentration of L-DOPS or L-DOPA in mice. L-DOPS or L-DOPA (400 mg/kg) was injected intraperitoneally 1 hr after the intraperitoneal injection of benserazide. The animals were killed 1 hr after L-DOPS or L-DOPA. Key: (□) amino acid alone; (▩) amino acid + benserazide (0.1 mg/kg); (▦) amino acid + benserazide (1 mg/kg); (■) amino acid + benserazide (10 mg/kg); and (▨) amino acid + benserazide (30 mg/kg). Values are means ± SEM, N = 5 (numbers of animals used). An asterisk (*) indicates $P < 0.05$, compared with amino acid alone (Student's t-test).

DISCUSSION

It is well known that L-DOPA causes a marked increase in brain DA concentration [1–4]. In contrast, it has been reported that the increase in brain NE concentration after administration of L-DOPS is very slight [1, 4, 9].

As shown in Table 2, our experiments also confirmed that the enhancement brain NE concentration by L-DOPS was slight even at high doses, in contrast to the marked enhancement of brain DA concentration by L-DOPA. We undertook this study in order to clarify why the rise of brain NE concentration after L-DOPS injection is so small compared to the rise of brain DA concentration after L-DOPA.

The enzymatic parameters of aromatic L-amino acid decarboxylation of both L-DOPS and L-DOPA were measured using the same enzyme preparations (Table 1). Apparent K_m and V_{max} values for L-DOPS and L-DOPA of kidney and brain were higher in the rat than in the mouse. The difference between rat and mouse decarboxylation was due, most likely, to differences in the aromatic L-amino acid decarboxylase activities of the tissues in the species used, since Rahman *et al.* [20] reported that decarboxylase activity of serum is three times greater in the rat than in the mouse. In our experiment, apparent K_m values for L-DOPS were found to be almost equal to those of L-DOPA, although L-DOPS has been reported to have a relatively low affinity for the enzyme, compared with 5-hydroxytryptophan (5-HTP) or L-DOPA [1, 8]. This finding is also supported by our other experiment in which a combination of L-DOPS and L-DOPA significantly increased the serum con-

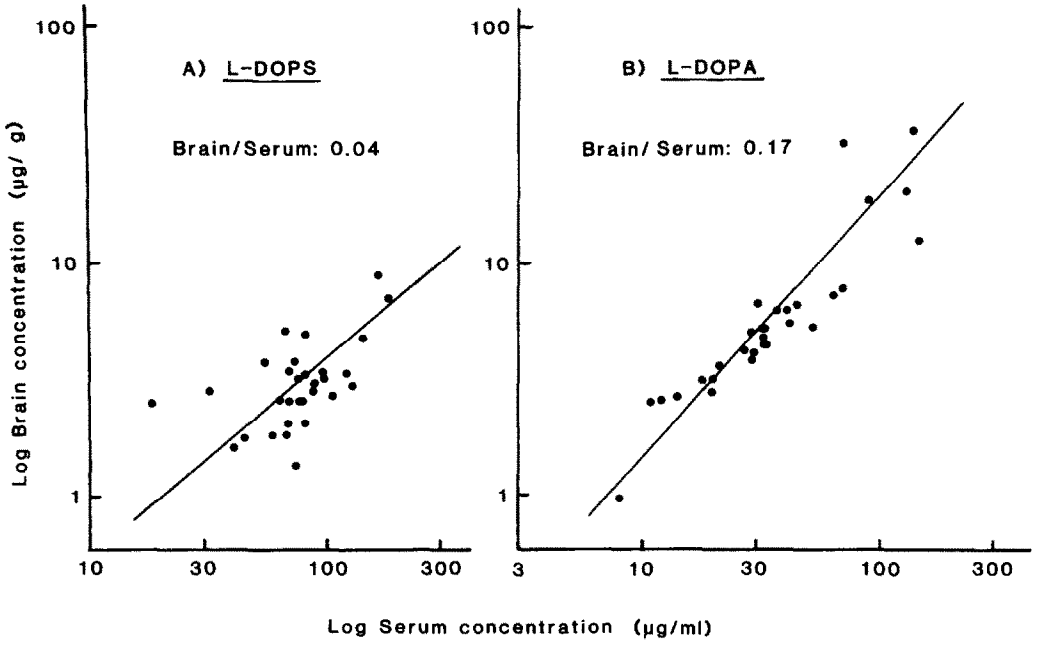

Fig. 2. Correlation between brain and serum concentrations of L-DOPS and L-DOPA. Various doses of L-DOPS or L-DOPA were injected intraperitoneally. The animals were killed 1 hr after L-DOPS or L-DOPA.

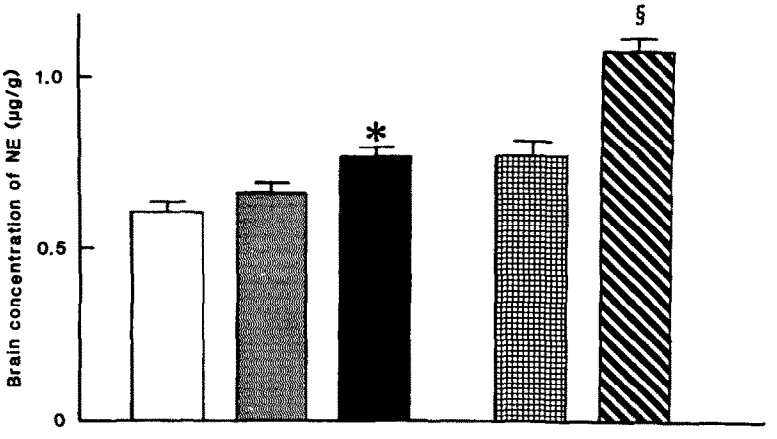

Fig. 3. Effect of L-DOPS on NE levels in mouse whole brain. Nialamide (30 mg/kg) was injected intraperitoneally 1 hr before intraperitoneal injection of L-DOPS. The animals were killed 1 hr after L-DOPS. Key: (□) control; (▩) L-DOPS (400 mg/kg); (■) L-DOPS (800 mg/kg); (▦) nialamide and (▨) L-DOPS (400 mg/kg) + nialamide. Values are means ± SEM, N = 5 (numbers of animals used). Symbols: (*) $P < 0.05$, compared with control; and (§) $P < 0.05$, compared with nialamide (Student's *t*-test).

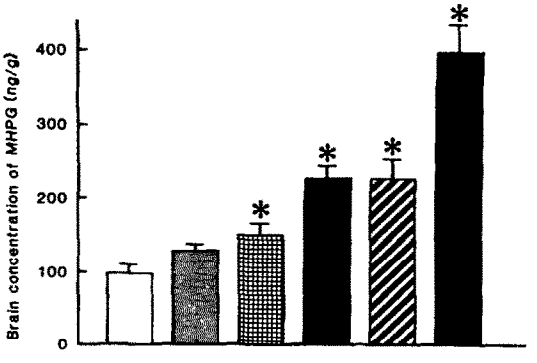

Fig. 4. Effect of L-DOPS on the MHPG levels in mouse whole brain. L-DOPS was injected intraperitoneally. The animals were killed 1 hr after L-DOPS. Key: (□) control; (▩) L-DOPS (50 mg/kg); (▦) L-DOPS (100 mg/kg); (■) L-DOPS (200 mg/kg); (▨) L-DOPS (400 mg/kg); and (■) L-DOPS (800 mg/kg). Values are means ± SEM, N = 5 (numbers of animals used). An asterisk (*) indicates $P < 0.05$, compared with control (Student's *t*-test).

centration of both amino acids, indicating that both amino acids may act as competitive decarboxylase inhibitors of the other (Table 3). On the other hand, V_{max} values (the rate of decarboxylation) of L-DOPS were found to be much smaller than those of L-DOPA (about 1/25 to 1/100). Such a marked difference between L-DOPS and L-DOPA is likely to be an important factor in determining their activities as catecholamine precursors, since the rate of an enzymatic reaction is reported to correlate with the value of V_{max}/K_m when the substrate concentration is below K_m [21, 22].

Distribution of each amino acid was measured after administration of 400 mg/kg, i.p. In the serum, the L-DOPS concentration was about five times higher than the L-DOPA concentration, but they were nearly equal in the brain. The lower serum L-DOPA concentration compared with L-DOPS is thought to be due to its faster decarboxylation in the periphery. This view is supported by the experiment with benserazide, a peripheral decarboxylase inhibitor. The serum L-DOPA concentrations were markedly and dose-dependently increased (about 5.5-fold) by pretreatment with benserazide. In contrast, the serum L-DOPS concentration was increased slightly (by only about 50%) with 1 mg/kg of benserazide and was not increased further at higher doses (10 and 30 mg/kg, i.p.) (Fig. 1). These results suggest that the inhibitory effect of benserazide on decarboxylation is greater in L-DOPA because of its fast decarboxylation. Bartholini *et al.* [23] reported that L-DOPS has a poor penetration through the blood–brain barrier and a slow decarboxylation rate, since the injection of L-DOPS into a cerebral ventricle led to a significant increase of brain NE concentration whereas after intraperitoneal injection of L-DOPS, the increase in brain NE was slight. To compare tha penetration of L-DOPS to that of L-DOPA, the ratio of the amino acid concentration in the brain to that in the serum was determined from correlation plots (Fig. 2), and the ratio of L-DOPS concentrations was found to be smaller (1/4) than that of L-DOPA concentrations. The data presented here, therefore, indicate that the penetration of L-DOPS is lower that that of L-DOPA, but it still seems substantial as a precursor in the brain.

In contrast, Bartholini *et al.* [1] reported that the small rise of brain NE by DL-DOPS is not enhanced, but diminished, by pretreatment with benserazide (50 mg/kg, i.p.), whereas the rise of brain DA by L-DOPA was enhanced markedly by the same dose of benserazide. Furthermore, they also reported that benserazide markedly increases the parenchymal fluorescence after L-DOPA but does not modify the DOPS-induced weak parenchymal fluorescence. Based on these results, they suggested that the small rise of the brain NE concentrations by L-DOPS would mainly be due to the moiety formed in the

capillary walls and not to the one formed in the brain parenchyma. In our experiment as well, the rise of the brain NE level by L-DOPS was not enhanced by pretreatment with benserazide (1 mg/kg, i.p.).

The enhancing effect of a decarboxylase inhibitor (DCI) on the brain catecholamine level is thought to be associated with the rise of the serum amino-acid level induced by inhibition of extracerebral decarboxylation with a DCI. On the other hand, it has been reported in a couple of papers that intracerebral decarboxylation of L-DOPA in rats is partly inhibited by extracerebral administration (i.p.) of benserazide at high doses such as 10 mg/kg [24], 15 mg/kg [25] and 200 mg/kg [26]. Thus, it is suggested that an appropriate dose of benserazide as a DCI should be the minimum amount that would induce a maximum rise in serum amino acid levels.

In the case of L-DOPS, the rise of the serum L-DOPS level was found to be much smaller, compared to that of L-DOPA and, thus, the enhancing effect of a DCI on the CNS actiivty of L-DOPS is thought to be essentially small. Furthermore, as shown in Fig. 1, the serum L-DOPS level was maximally increased with a small amount of benserazide (around 1 mg/kg), whereas the maximum increase of the serum L-DOPA level was achieved with a fairly large amount (more than 30 mg/kg). Thus, it is suggested that the effective dose of benserazide for L-DOPS should be a small amount and that the 50 mg/kg i.p. of benserazide which was employed in the experiment of Bartholini *et al.* would be inappropriate as a DCI for L-DOPS decarboxylation.

The metabolic pathway of endogeneous NE has been studied extensively in several laboratories; indications are that monoamine oxidase (MAO) should be a primary metabolic enzyme and MHPG should be a principal end metabolite in the brain [27–29]. In view of such a dynamic metabolic system, we measured both the NE concentration after administration of L-DOPS in combination with an MAO inhibitor (MAO-I), nialamide, and the brain MHPG concentration after administration of L-DOPS alone in mice. The rise of the brain NE concentration was enhanced significantly by the combination of L-DOPS with nialamide (Fig. 3). This result is compatible with those of previous studies done with DL- or L-DOPS in combination with MAO-I in normal or NE-depleted mice or rats [4, 30, 31]. Moreover, the brain MHPG concentration was increased by L-DOPS in a dose-dependent manner and more markedly than the brain NE concentration; several hundred % versus several ten % (Fig. 4). Such a marked increase of the brain MHPG level by DL- or L-DOPS has also been reported by Edwards and Rizk [32] and Semba and Takahashi [18]. Edwards and Rizk [32], however, suggested that the rise of the brain MHPG level might be attributed mostly to the rise of MHPG level formed in the capillary walls, based on the fact that the rise was diminished by benserazide (50 mg/kg, i.p.). The amount of benserazide used in their investigation, however, seems to be too much, taking our experiments into consideration. In one of our experiments, it was found that L-DOPS (400 mg/kg, i.p.) caused marked increases of MHPG concentration in both brain (260%) and serum (700%), and the rise in serum diminished markedly by pretreatment with benserazide (reduction by 31 and 62% of control at 0.3 and 1.0 mg/kg, i.p., respectively), whereas the rise in the brain was not modified [108 and 105% vs control) by the same doses of benserazide*. It is suggested from these data, therefore, that the L-DOPS-induced rise in brain MHPG concentration would be mostly of central origin, and that the turnover of NE would be enhanced markedly by L-DOPS although accumulation of the brain NE by L-DOPS was small.

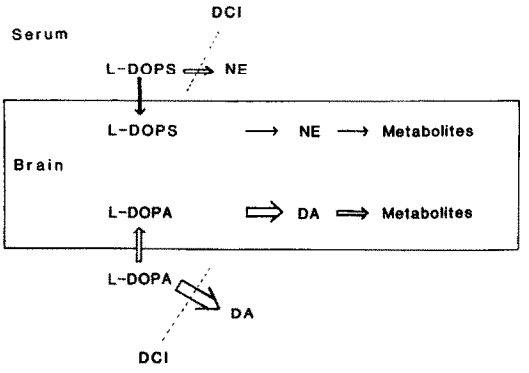

Fig. 5. Scheme of the difference of the activity as an amine precursor in the CNS between L-DOPS and L-DOPA.

In summary, the difference between activities of L-DOPS and L-DOPA amine precursors in the CNS can be explained by the scheme shown in Fig. 5. Much slower decarboxylyation of L-DOPS in the brain may cause almost no accumulation of NE under normal catabolic conditions, but instead a significant increase in metabolites such as MHPG and, in the peripheral system its slower decarboxylation, may minimize the economizing effect of benserazide, a DCI.

In conclusion, L-DOPS is thought to activate NE neurons in the CNS by increasing the turnover rate of NE; this is one possible mode of action for its clinical usefulness on Parkinson's disease.

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* The details of these results have been submitted to another journal.

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