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Mechanism of Intestinal Absorption and Brain Uptake of L-5-Hydroxytryptophan in Rats, as compared to Those of L-3,4-Dihydroxyphenylalanine[1]

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L-5-Hydroxytryptophan (5-HTP) and L-3,4-dihydroxyphenylalanine (DOPA) were comparatively investigated of their mechanisms of intestinal absorption, metabolism in the intestine and liver tissues and mechanisms of passage through the blood-brain barriers, by means of *in vitro* and/or *in situ* techniques using rat tissues. The corresponding D-isomers were also investigated for comparison. L-5-HTP, but not the D-isomer, was found to be absorbed from the intestine by an active transport mechanism in the same way as L-DOPA. *In vitro* studies using rat brain cortex slices proved that both L-5-HTP and L-DOPA, but not their D-isomers, are taken up by the brain tissues by an active transport mechanism which is saturable, energy-dependent and competitive. There was no substantial difference between L-5-HTP and L-DOPA in the rate of intestinal absorption and that of brain uptake. It was indicated, however, that L-5-HTP was decarboxylated in the intestine to a much less extent than L-DOPA during the absorption and the decarboxylation activity of rat intestine and liver homogenates was found to be about four and seven times higher for L-DOPA than for L-5-HTP, respectively. The latter results suggest that the oral dose of L-5-HTP can be much more easily transferred into the circulating blood without being suffered from decarboxylation in the peripheral organs than L-DOPA, resulting in a more efficient penetration of L-5-HTP into the brain as a serotonine precursor.

Keywords——5-HTP; DOPA; optical isomer; intestinal absorption; brain uptake; active transport; drug metabolism

Since it was found that[3] the brain and CSF serotonine and 5-hydroxyindole-3-acetic acid levels were decreased appreciably in depressant patients, the oral use of L-5-hydroxytryptophan (L-5-HTP) as a precursor of brain serotonine has been tested clinically.[4] It has been well established that[5] L-3,4-dihydroxyphenylalanine (L-DOPA) is effective for Parkinsonism as a precursor of brain dopamine. We reported in a previous paper[6] that an active transport mechanism is involved in the intestinal absorption of L-DOPA and that L-DOPA is decarboxylated to a considerable extent during the intestinal absorption. It was also demonstrated in rats that[7] the brain uptake of radioactivity was detected autoradiographically only when the oral dose of L-DOPA-^{14}C was increased to over 50 mg/kg, because of the decarboxylation at the peripheral organs including the intestine and liver. When L-5-HTP-^{14}C was administered orally to rats, on the contrary, the brain uptake of

1) This paper was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.
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6) H. Shindo, T. Komai, and K. Kawai, *Chem. Pharm. Bull.* (Tokyo), **21**, 2031 (1973).
7) H. Shindo, N. Miyakoshi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).

radioactivity was observed[8] even at a low dose as 10 mg/kg. In the present investigation, therefore, the absorption and metabolism of L-5-HTP in the rat intestine and the mechanism of brain uptake are studied *in vitro* in comparison with those of L-DOPA, using ^{14}C-labeled D- and L-5-HTP and 5-hydroxytryptamine (5-HT).

Material and Method

Materials——D- and L-5-HTP-3-^{14}C were purchased from the New England Nuclear Corporation, Boston, U.S.A. Their specific activities were 28.7 and 28.2 μCi/mg, respectively, and the radiochemical purity was ascertained to be over 98% by thin-layer chromatography. The optical purity was also over 98% for both isomers by means of reverse isotope dilution method with non-radioactive D- and L-standards. 5-HT-3-^{14}C creatinine sulfate with specific activity of 14.3 μCi/mg was purchased from the Radiochemical Center, Amersham, England. Standard samples of D- and L-5-HTP were supplied from Kyowa Hakko Co., Ltd and that of 5-HT was purchased from Sigma Chemicals Co. Other chemicals were of reagent grade and used without further purification.

Experiments on Excretion after Oral Administration to Rats——Male rats of Wistar-Imamichi strain weighing 200 to 220 g were used after fasting for 16 hr. D- and L-5-HTP-^{14}C was administered orally at the dose of 5 mg (72 μCi)/kg, and an equivalent dose (9.5 mg (42 μCi)/kg) of 5-HT-^{14}C, as an aqueous solution by a stomach tube. The animals were placed in an individual metabolic cage and food and water was given *ad lib.* The urine and feces were collected separately at the intervals of 6, 24 and 48 hr periods after the administration. The feces were homogenized in two volumes of water and extracted twice with 4% $HClO_4$ in Polytron homogenizer (Kinematica GMBH, Switzerland). The combined extracts and the urine samples were assayed for radioactivity.

***In Situ* Experiments on Absorption from Rat Intestine**——Male rats of Wistar-Imamichi strain weighing about 200 g were used after fasting for 16 hr. After anesthetizing with ether, the intestine was exteriorized through a central mid-line incision and an acute loop of about 8 cm long was prepared from the upper part of the jejunum by ligature of both ends. Aqueous solution (0.5 ml) of the test compound was injected into the lumen of the loop with a syringe, the loop was returned and the incision was sutured. After a given time, the loop was removed and the contents were washed out five times each with 2 ml ice-cold saline. It was ascertained that the last washing contained no radioactivity. The combined washings were centrifuged after adding an equal volume of 7% $HClO_4$. The residues were extracted with 4% $HClO_4$ and the combined supernatant was assayed for radioactivity. The tissue was homogenized in 4 to 5 volumes of 4% $HClO_4$ and centrifuged. The residues were reextracted and the combined supernatant was assayed. The recovery of radioactivity by the extraction procedure was ascertained to be over 98% by determining the radioactivity of the final residue after solubilizing with NaOH. The amount of the drug taken into the blood stream from the loop was then obtained by subtracting the residual amount in the lumen and that retained in the tissue from the dose amount and was referred as the amount truly absorbed.

The pH of the extracts were adjusted to 5.0—5.5 with 2N KOH and after allowing to stand at 0° overnight the precipitates were removed by filtration. The solution was concentrated to dryness under a reduced pressure and the residue was dissolved in a small amount of 50% 10^{-3}N HCl in ethanol. The metabolites were separated by cellulose thin layer chromatography (E. Merck, F_{254}, 0.1 mm thickness) using a solvent system of *n*-butanol: acetic acid: water (12: 3: 5, v/v), after adding appropriate amounts of the authentic standards to the extracts. The standards used and their *Rf* values were: L-5-HTP (0.20), D-5-HTP (0.20), DL-5-methoxytryptophan (0.42), 5-HT (0.47), 5-methoxytryptophan (0.66), 5-hydroxyindole 3-acetic acid (5-HIAA) (0.80), 5-methoxyindole-3-acetic acid (0.94) and N-acetylserotonine (0.86). The radioactive spots were detected by autoradiography and quantitatively transferred into the counting vials by scraping carefully with a spatula and their radioactivities were counted after shaking in 15 ml of liquid scintillator.

***In Vitro* Experiments on Uptake by Intestinal Tissue Segments and Brain Slices**——The rats were sacrificed by bleeding from the carotid artery and the upper small intestine and the brain were removed and immediately placed in ice-cold physiological saline. The intestine was everted with a stainless steel rod and cut into rings of about 3 mm in length. The brain slices of 0.3 to 0.5 mm thickness were prepared from the cerebral cortex using a Stadie-Riggs slicer. About 500 mg of the intestinal segments or the brain slices were selected at random and placed in a 100 ml Erlenmeyer flask with 15 ml Krebs-Henseleit bicarbonate buffer (pH 7.1) containing 0.3% glucose and, in some cases, an appropriate amount of inhibitor. After preincubation for 10 min at 37° under gassing with 95% O_2—5% CO_2, L-5-HTP-^{14}C or other test compound was added and the incubation was continued over a certain period. The segments or slices were then removed from the medium, washed four times each with 20 ml ice-cold saline, blotted on a filter paper, weighed and frozen in a mixture of acetone and dry ice. The radioactive substances were extracted in the same way as described above and

8) H. Shindo and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **24**, 3158 (1976).

assayed for radioactivity. The extracellular and intracellular spaces were determined in the control experiments using inulin-^{14}C in the same way as described previously,[9] and the tissue accumulation was expressed as an intracellular to extracellular concentration ratio of radioactivity, C_{ICF}/C_{ECF}. The radioactive metabolites were then separated by cellulose thin layer chromatography by treating the extracts in the same way as described above.

Determination of Decarboxylase Activity of Rat Tissues——Male rats of Wistar-Imamichi strain weighing about 200 g were used after fasting for 16 hr. After the rats were sacrificed by bleeding, the liver, upper small intestine (jejunum) and brain were removed. The tissues were homogenized in ten (liver) or five (brain and intestine) volumes of cold 0.02M phosphate buffer of pH 7.0 for 2 min with Potter-Ervehjem homogenizer and centrifuged at 9000×*g* or 20 min at 0° and the supernatants were used as the enzyme solution. After preincubation of 1 ml enzyme solution treated with 0.3 ml pyridoxal phosphate solution (final: 0.05 mM) and 0 2 ml iproniazide (final: 1 mM) for 10 min, 0.5 ml solution of L-DOPA-^{14}C or L-5-HTP-^{14}C (final: 1 mM) was added and the incubation was continued under a nitrogen atmosphere. After 10 and 30 min, 1 ml of the reaction mixture was pipetted out and the reaction was stopped by heating at 100° for 2 min. After deproteinization, the reaction products were separated by cellulose thin layer chromatography and the formed dopamine or 5-HT was assayed for radioactivity.

Result

Excretion of D- and L-5-HTP-^{14}C after Oral Administration to Rats

The urinary and fecal excretion of radioactivity following oral administration of 5 mg/kg ^{14}C-labeled D- and L-5-HTP and 5-HT are shown in Table I. As can be seen from the table, L-5-HTP showed a significantly higher urinary excretion and correspondingly a lower fecal excretion than those of D-5-HTP, suggesting a much higher absorbability of the L-isomer from the intestine than the D-isomer. 5-HT was found to be also well absorbed from the intestine, but much more slowly than L-5-HTP.

Table I. Urinary and Fecal Excretion of D- and L-5-HTP-^{14}C and 5-HT-^{14}C after Oral Administration to Rats (5 mg/kg)

Compound	% to dose ±S.E. (n=3)				
	Urine			Feces	
	6 hr	24 hr	48 hr	24 hr	48 hr
L-5-HTP	68.08±4.46	74.79±2.28	75.46±2.13	1.37±0.34	2.47±0.14
D-5-HTP	44.25[a]	54.31±4.91	55.68±4.50	13.58±2.24	18.14±4.13
5-HT[b]	41.81±3.47	58.05±2.82	60.10±2.99	8.96±3.06	14.00±2.08

a) mean from two experiments
b) 9.5 mg/kg 5-HT creatinine sulfate

Absorption of D- and L-5-HTP from *in Situ* Rat Intestine

The time course of the absorption of radioactive D- and L-5-HTP from an acute loop of rat small intestine are shown in Table II. The results revealed that the L-isomer disappeared from the lumen much more rapidly than the D-isomer. After 1 hr, about 90% of L-5-HTP-^{14}C administered was shown to be absorbed into the blood stream, while only about 25% of the D-isomer. It was noted that D-5-HTP showed a relatively high retention in the intestinal wall and after 60 min about 40% of the administered D-5-HTP remained in the intestianl tissue as compared to about 5% of the L-isomer. Since D- and L-5-HTP are expected to have a very close similarity in their physico-chemical nature, the above result suggests that there is involved some specific transport mechanism in the absorption of the L-isomer rather than a simple diffusion process. The absorption of different doses of L-5-HTP from the intestinal loop were thus compared and it was found that the absorption

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Table II. Absorption of D- and L-5-HTP-^{14}C from Ligated Loop of Rat Small Intestine (Dose: 0.55 mg/loop)

Compound	min after administration	% to dose ± S.E.		
		Residual amount in lumen	Retained in tissue	Absorption into blood
L-5-HTP	5	74.14±4.26	17.64±2.18	8.23±2.58
	10	44.61±4.61	27.98±0.57	27.41±5.17
	30	23.97±1.01	19.95±0.77	56.08±0.81
	60	3.39±0.59	4.85±0.34	91.76±0.82
D-5-HTP	5	85.33±3.71	13.74±3.51	0.93±0.37
	10	74.39±1.82	22.21±1.32	3.40±0.71
	30	38.75±1.63	41.62±2.04	19.63±3.67
	60	33.66±4.12	40.56±0.57	25.78±3.58

Each value represents the mean from 3 to 4 experiments.

rate was decreased markedly with increasing the amount of dose. As shown in Fig. 1, the Lineweaver-Burk plots of the amount absorbed during the first 10 min indicated a typical saturation kinetics, one of the characteristics for an active transport mechanism, which gave V_{max}=1.82 μmoles/min and $Kt=9.1\times10^{-2}$ M.

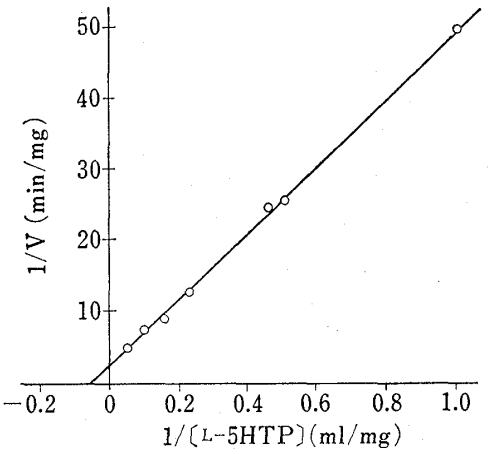

Fig. 1. Lineweaver-Burk Plots of the Absorption of Different Amount of L-5-HTP

Solution (0.5 ml) of different amount of L-5-HTP-^{14}C was injected into ligated loop of rat small intestine and the amount absorbed was determined from the residual activity in the lumen after 10 min.
Each value represents the mean from three experiments.

Effect of Metabolic Inhibitors on L-5-HTP Uptake by Intestine

The intestinal tissue segments were incubated with L-5-HTP-^{14}C *in vitro* with or without a metabolic inhibitor or an amino acid and the uptake was compared in terms of the intracellular concentration of radioactivity after 30 min incubation. As shown in Table III, both 2,4-dinitrophenol (10^{-4} M) and sodium azide (5×10^{-4} M) showed a significant inhibition on the uptake of L-5-HTP-^{14}C and anerobic conditions also inhibited the uptake appreciably. The uptake was also inhibited by non-radioactive L-5-HTP and L-tryptophan, while no inhibition was shown by D-5-HTP. These results are again consistent with the assumption that L-5-HTP is transported into the intestinal epithelial cells by an active transport mechanism, as in the case of L-DOPA.[6]

Metabolism of D- and L-5-HTP in the Intestinal Tissue

In order to see the possible metabolic change of D- and L-5-HTP in the intestinal tissue during the absorption, the radioactive substances were extracted from the intestinal tissue and the lumen at various times after administration of D- and L-5-HTP-^{14}C into the ligated intestinal loop and were separated by thin-layer chromatography. The autoradiograms revealed that in the lumen only one radioactive spot corresponding to 5-HTP was detected, indicating that both D- and L-5-HTP is stable in the intestinal lumen. In the intestinal tissue after administration of L-5-HTP-^{14}C, on the contrary, radioactive spots corresponding to 5-HT and 5-HIAA and, in addition, a spot at *Rf* 1.2 were detected as well as that of unchanged 5-HTP.

Table III. Effect of Metabolic Inhibitors and Amino Acids on L-5-HTP Uptake by Rat Small Intestinal Segments

Inhibitor or amino acid	Concentration (M)	Uptake[a] (mμmoles/ml ICF)	%-inhibition
None	—	18.69±1.41	—
2,4-Dinitrophenol	10^{-4}	13.39±1.51	28.35
Sodium azide	5×10^{-4}	14.60±1.29	21.88
95% N_2–5% CO_2	—	14.14±0.96	24.34
None	—	13.26±1.72	—
L-5-HTP	5×10^{-4}	7.77±2.29	41.40
D-5-HTP	5×10^{-4}	13.66±2.37	−3.01
L-Tryptophan	5×10^{-4}	11.02±1.99	16.89

a) Uptake was determined after 30 min incubation in the medium containing 5×10^{-5}M L-5-HTP-^{14}C. Extracellular space of the intestinal segments was determined to be 21.59% with inulin-^{14}C.
Each value represents the mean ±S.E. from five experiments.

The relative amounts of 5-HTP and its metabolites thus observed in the tissue and lumen are shown in Table IV. The results indicated that a part of L-5-HTP is metabolized to some extent in the intestinal tissue by suffering decarboxylation, while D-5-HTP is not metabolized to any appreciable extent. When the extent of the metabolism was compared between L-5-HTP and L-DOPA,[6] however, L-5-HTP was shown to be metabolized in the intestinal tissue to a much lesser extent than L-DOPA. Five minutes after the administration, about 80% of L-DOPA-^{14}C in the intestinal tissue was found to be metabolized to dopamine and its further metabolites and only about 15% remained as unchanged DOPA.[6] However, over 50% of the total radioactivity in the tissue was found to remain in the unchanged form at a comparable time after administration of L-5-HTP-^{14}C.

Table IV. Metabolism of D- and L-5-HTP-^{14}C in the Intestinal Tissue during the Absorption

Metabolite	% to total radioactivity								
	Tissue					Lumen			
	L-5-HTP			D-5-HTP		L-5-HTP		D-5-HTP	
	5 min	10 min	30 min	10 min	30 min	10 min	30 min	10 min	30 min
5-HTP	50.20	60.43	29.91	94.01	86.17	89.31	86.21	92.10	89.09
5-HT	9.74	9.40	18.85	—[a]	—	—	—	—	—
5-HIAA	1.10	3.26	5.26	—	—	—	—	—	—
5-HT glucuronide	31.46	21.89	39.72	—	—	—	—	—	—

0.5 mg D- and L-5-HTP-^{14}C was injected into the loop of rat small intestine and the radioactive substances in the tissue and lumen were extracted and separated by cellulose thin-layer chromatography. Each value represents the mean from two experiments.
a) No radioactivity was detected.

Comparison of Tissue Decarboxylase Activity on L-5-HTP and L-DOPA

Decarboxylase activity of 9000×*g* supernatants from rat liver, small intestine and brain homogenates were compared between L-DOPA and L-5-HTP as the substrate under the same conditions. As shown in Table V, the decarboxylation activity was found to be much higher for L-DOPA than for L-5-HTP in all the tissues tested. In the small intestine and liver, L-DOPA was decarboxylated approximately four and seven times more rapidly than L-5-HTP, respectively, indicating that L-5-HTP is significantly more stable in the peripheral organs than L-DOPA.

TABLE V. *In Vitro* Decarboxylation of L-5-HTP and L-DOPA in Rat Liver, Small Intestine and Brain Homogenates

Tissue	Substrate	Formation of amine (μmole/g tissue)		%-decarboxylation		Activity ratio[a] L-DOPA/L-5-HTP
		10 min	30 min	10 min	30 min	
Liver	L-DOPA	9.10	16.7	46.86	85.97	6.9
	L-5-HTP	1.32	3.73	6.52	18.40	
Small intestine	L-DOPA	1.17	3.22	4.24	11.66	3.4
	L-5-HTP	0.34	0.88	1.19	3.06	
Brain	L-DOPA	0.47	1.27	4.73	12.86	7.8
	L-5-HTP	0.06	0.16	0.62	1.57	

After 10 or 30 min incubation of ^{14}C-labeled L-DOPA or L-5-HTP in 9000×*g* supernatant of homogenates, formed dopamine or 5-HT was assayed after separation by cellulose thin-layer chromatography. Each value represents the mean from three experiments.
a) calculated from the results of 10 min incubation.

Uptake of L-5-HTP and L-DOPA by Brain Slices

In vitro uptake of radioactivity by the rat brain cortex slices when incubated with 0.05 mM D- and L-5-HTP-^{14}C is shown in Table VI. L-5-HTP was found to be taken up rapidly and accumulated in a high concentration in the brain tissue and after 30 min incubation the intracellular to extracellular concentration ratio of radioactivity reached a value as high as 10: 1. That this accumulation was not caused from a metabolic change of L-5-HTP in the brain was proved by the extraction and separation of the brain radioactivity. After 30 min incubation, about 84% of the total radioactivity in the brain tissue was found to be unchanged 5-HTP, concomitant with 4.8, 2.1 and 3.0% of 5-HT, 5-HIAA and 5-methoxytryptophan (5-MTP), respectively. When the concentration ratio was calculated based on the concentration of unchanged 5-HTP in the tissue and medium, the value of 7.8: 1 was obtained, indicating that L-5-HTP was transported into the brain tissue against the concentration gradient. On the contrary, D-5-HTP did not accumulate in the brain tissue and the intra- to extracellular concentration ratio did not exceed the unity. These results suggest that a transport mechanism which is specific for the L-antipode is involved. Furthermore, the uptake of L-5-HTP was inhibited by the presence of metabolic inhibitors such as 2,4-dinitrophenol (10^{-4} M) and anerobic conditions and by that of non-radioactive L-5-HTP and L-tryptophan significantly, while it was not inhibited at all by that of D-5-HTP, as shown in Table VII. From these results, it must be concluded that L-5-HTP, but not D-5-HTP, is transported into the brain tissue by an active transport mechanism.

Almost the same results were obtained on the uptake of L-DOPA-^{14}C by the brain slices; the data is in Table VI and VII. In order to minimize the metabolic change of L-DOPA

TABLE VI. *In Vitro* Uptake of D- and L-5-HTP-^{14}C and D- and L-DOPA-^{14}C by Rat Brain Cortex Slices

Substrate	Uptake[a] (mμmole/ml ICF ±S.E.)	C_{ICF}/C_{ECF}[b]
L-5-HTP	329.3±32.0	9.41
D-5-HTP	33.4± 1.9	0.74
L-DOPA	394.5± 6.93	10.70
D-DOPA	106.0± 3.68	2.40

a) The brain slices were incubated with 5×10^{-5} M substrate at 37° for 30 min.
b) Extracellular space was obtained to be 27.52% by inulin-^{14}C.
Each value represents the mean ± standard error from four experiments.

TABLE VII. Effect of Metabolic Inhibitors and Amino Acids on Uptake of L-5-HTP and L-DOPA by Rat Brain Cortex Slices

Inhibitor	Conc. (M)	L-5-HTP		L-DOPA	
		Uptake[a] (mμmole/ml ICF)	%-inhibition	Uptake[a] (mμmole/ml ICF)	%-inhibition
None	—	390.7±25	—	394.5± 6.9	—
2,4-Dinitrophenol	10^{-4}	295.5± 3	24.36	254.9± 8.3	36.07
95% N_2– 5% CO_2		216.1± 3	44.69	143.3± 8.6	63.69
L-5-HTP or L-DOPA	5×10^{-4}	292.5±31	25.13	266.7± 7.8	32.40
D-5-HTP or D-DOPA	5×10^{-4}	418.2±21	−7.04	399.2±17.7	−1.02

a) The brain slices were incubated at 37° for 30 min with 5×10^{-5} M ^{14}C-labeled substrate. Values represent the mean ± standard error from four experiments.

in the brain tissue, 100 mg/kg of NSD-1055, a central dopa-decarboxylase inhibitor,[10] was administered intraperitoneally to rats 1 hr before the brain was removed. After 30 min incubation of the brain slices with 0.05 mM L-DOPA-^{14}C, it was found that the intracellular to extracellular concentration ratio of radioactivity reached a value as high as 10.3: 1 and that about 97% of the brain radioactivity remained as unchanged DOPA. The uptake was inhibited significantly by 2,4-dinitrophenol and anerobic conditions and by the presence of non-radioactive L-DOPA and L-phenylalanine, while not by D-DOPA. All these results indicate that L-DOPA, as well as L-5-HTP, is transported into the brain tissue by an active transport mechanism. In the case of D-DOPA-^{14}C, the concentration ratio of radioactivity also exceeded unity and reached a value of 2.4: 1 after 30 min. This suggest the possibility that the D-isomer also shares the same transport systems to some extent.

Discussion

Following the oral administration of L-DOPA and L-5-HTP to animal organisms as a precursor of the corresponding brain amine, they must encounter metabolic and transport barriers as illustrated in Chart 1 before reaching in the central nervous system. In the case of L-DOPA, it was found by rat whole-body autoradiography that[7] the distribution pattern of radioactivity changes depending on the amount of oral dose and the brain uptake was detected when the dose was increased to a level higher than 50 mg/kg. Such a dose is in accord with the effective dose level of L-DOPA clinically applied in the treatment of Parkinsonism. This was considered to be due to extensive peripheral decarboxylation of L-DOPA, resulting in a low L-DOPA concentration in the circulating blood. Our autoradiographic work on L-5-HTP-^{14}C,[8] on the contrary, revealed that the distribution pattern does not change appreciably on varying the amount of the oral dose and an appreciable uptake of radioactivity by the brain was observed even with a dose as low as 2 and 10 mg/kg. In order to see the cause of this difference, therefore, possible barriers in the metabolic and transport processes were comparatively investigated between L-DOPA and L-5-HTP.

In the first barrier, intestinal absorption, L-5-HTP was proved to be transported by an active transport mechanism as well as L-DOPA.[6] The maximum velocity of the transport (V_{max}) and the transport constrant (K_t) were found to be 1.82 μmoles/min and 9.1×10^{-2} M, respectively, which are almost comparable to the corresponding values found for L-DOPA:[6] 0.91 μmoles/min and 2.0×10^{-2} M, respectively. An appreciably larger value of V_{max} for L-5-HTP suggests that the rate of absorption is even higher in L-5-HTP than L-DOPA in large doses.

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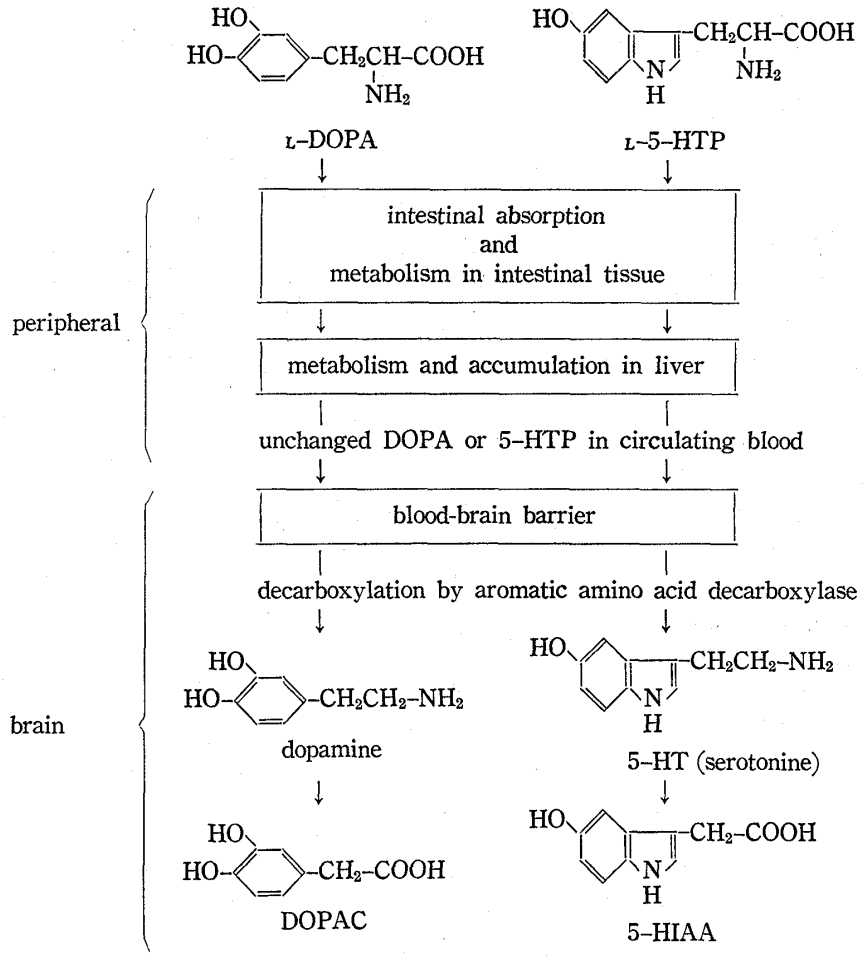

Chart 1. Metabolic Changes and Possible Barriers for the Brain Uptake of L-DOPA and L-5-HTP after Oral Administration

The second possible barrier is metabolism in the intestinal tissue during the absorption and the third is that in the liver before being transferred into the circulating blood. It has been established[11] that a single enzyme, aromatic amino acid decarboxylase, participates in the decarboxylation of both L-DOPA and L-5-HTP in animal organisms. Thus the extent of decarboxylation was compared between the two substrates under the same conditions. Five minutes after administration of L-DOPA-^{14}C into *in situ* rat intestinal loop, only about 15% of the total radioactivity in the intestinal tissue was unchanged DOPA, while under the same conditions over 50% L-5-HTP-^{14}C administered was found to be in the unchanged form. It might be said, therefore, that although L-5-HTP is decarboxylated in the intestinal tissue to some extent during the absorption, the extent is much less than that of L-DOPA. Furthermore, a comparison of the decarboxylase activity against L-DOPA and L-5-HTP in the intestinal and liver homogenates revealed that L-DOPA is decarboxylated four and seven times more rapidly than L-5-HTP, respectively. The fact that L-5-HTP is decarboxylated to a much lesser extent in the liver is consistent with our previous autoradiographic observation[8] that L-5-HTP-^{14}C did not accumulate in the liver appreciably whereas L-DOPA-^{14}C accumulate in a high concentration, because the latter accumulation has been clarified as being due to the accumulation of dopamine glucuronide.[12] All these results indicate

11) J.G. Christensen, W. Dairman, and S. Udenfriend, *Proc. Nat. Acad. Sci.*, **69**, 343 (1972).
12) H. Shindo, T. Komai, K. Tanaka, E. Nakajima, and N. Miyakoshi, *Chem. Pharm. Bull.* (Tokyo), **21**, 826 (1973).

that generally L-5-HTP can be much more easily transferred into the circulating blood without suffering decarboxylation in the peripheral organs than L-DOPA, after oral administration.

As to the final barrier, the blood-brain barrier, comparative studies using rat brain cortex slices revealed that, i) both L-5-HTP and L-DOPA, but not the D-isomers, are transported into the brain tissues against the concentration gradient, reaching the intracellular to extracellular concentration ratio of over 10: 1, ii) the transport is inhibited significantly by metabolic inhibitors such as 2,4-dinitrophenol and anerobic conditions, indicating that the process requires metabolic energy, and iii) the transport is inhibited significantly by L-amino acids, but not by D-amino acids. All these results indicate that both L-5-HTP and L-DOPA, but not their D-isomers, penetrate into the brain tissue by an active transport mechanism and that there is no substantial difference in the uptake between L-5-HTP and L-DOPA. Participation of an active transport in the brain uptake of L-DOPA has already been reported,[13] by the same method but using L- and DL-DOPA-^{14}C. In the present investigations, D-5-HTP-^{14}C was proved not to accumulate in the brain tissue, while D-DOPA-^{14}C was shown to more easily penetrate into the brain tissue and the intracellular to extracellular concentration ratio of radioactivity exceeded unity, reaching a value of 2.4: 1. The latter finding may be possibly due to that D-DOPA shares the active transport system to some extent or is more easily penetrated by simple diffusion mechanism than D-5-HTP followed by its metabolism, *e.g.* O-methylation by COMT, or binding to some brain tissue. This is consistent with our autoradiographic finding that an appreciable radioactive uptake by the brain was observed after administration of D-DOPA-^{14}C,[7] while no uptake was detected in the brain after that of D-5-HTP-^{14}C.[8] A detailed distribution and metabolism of L-5-HTP-^{14}C in the cat brain have been investigated in this laboratories, which will be published elsewhere.[14]

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13) H. Yoshida, J. Nambu, K. Kaniike, and R. Imaizumi, *Jap. J. Pharmacol.*, 13, 1 (1963).
14) N. Miyakoshi, Y. Nishijima, and H. Shindo, *Jap. J. Pharmacol.*, to be published.