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Studies on the Metabolism of D- and L-Isomers of 3,4-Dihydroxyphenylalanine (DOPA). IV.[1] Urinary and Tissue Metabolites of D- and L-DOPA-^{14}C after Intravenous and Oral Administration to Rats[2]

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The radioactive metabolites of D- and L-isomers of 3,4-dihydroxyphenylalanine-(DOPA)-2-^{14}C in the urine and the main tissues were comparatively investigated after intravenous and oral administration to rats. After administration of L-DOPA, eighteen metabolites were detected in the urine and dopamine conjugate, dopamine, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxy-4-hydroxyphenyl ethanol (MHPE) conjugate were found to be the main metabolites, while excretion as DOPA was only less than 1%. After administration of D-DOPA, an appreciable amount (20%) was excreted as unchanged DOPA, but dopamine, mostly in the free form, was found to be the main metabolite. After administration of L-DOPA, dopamine and its metabolites were the main component in the tissues including the brain and skeletal muscle, while after that of D-DOPA, 3-O-methyl-DOPA was the only metabolite, except the kidney where dopamine and its metabolites were found to be formed. It was demonstrated that approximately 67 and 53% of the brain radioactivity was dopamine and its metabolites at 10 and 60 min after intravenous and oral administration of L-DOPA-^{14}C, respectively. The main metabolite in the pancreas and intestine from L-DOPA was DOPAC and dopamine conjugate, respectively, while unchanged DOPA from D-DOPA. In the liver, where D-DOPA dose not accumulate, dopamine conjugate was the main metabolite from L-DOPA, while no dopamine from D-DOPA. From these results, D-DOPA was considered to be metabolized to dopamine only in the kidney, most of which is excreted directly into the urine.

In the previous papers,[1,4,5] the absorption, distribution and excretion of radioactivity were compared between ^{14}C-labeled D- and L-isomers of 3,4-dihydroxyphenylalanine (DOPA) following intravenous and oral administration to rats. The results revealed that there are marked differences in the fates of the two isomers, which could be interpreted as being due to specificity in the transport and metabolic systems with respect to the optical isomers. The present investigations were performed to establish further the relation between the distribution of radioactivity and the metabolic fates of the two isomers. The radioactive metabolites of D- and L-DOPA-^{14}C in the urine and the main tissues including the brain were comparatively investigated following the intravenous and oral administration to rats.

Material and Method

Materials——D- and L-DOPA-2-^{14}C were prepared from DL-DOPA-2-^{14}C which was purchased from the Radiochemical Center, Amersham, England, as described in the previous paper.[4] The specific activity

1) Part III: H. Shindo, E. Nakajima, K. Kawai, N. Miyakoshi, and K. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **21**, 817 (1973).
2) This work was presented at the 3rd Symposium on Drug Metabolism and Action, Fukuoka, November 1971.
3) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*
4) H. Shindo, N. Miyakoshi, and I. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **19**, 2490 (1971).
5) H. Shindo, N. Miyakoshi, and E. Nakajima, *Chem. Pharm. Bull.* (Tokyo), **20**, 966 (1972).

was 24.9 and 26.4 μCi/mg for D- and L-DOPA-^{14}C, respectively. The radiochemical purity as radioactive D- and L-DOPA was over 97% for the both preparations. 3,4-Dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA), dopamine (DA), adrenaline and noradrenaline used as authentic samples are the commertial products. D- and L-DOPA, L-3-O-methyl-DOPA, 3-O-methyldopamine, 3,4-dihydroxyphenylethanol (DHPE), 3-methoxy-4-hydroxyphenylethanol (MHPE) and 3,4-dihydroxyphenylpyruvic acid were prepared in the Research and Development Department of Sankyo Chemical Industries, Ltd.

Administration of Labeled Drugs——Male rats of Wistar-Imamichi strain weighing about 150 g were used without fasting. For intravenous administration, D- and L-DOPA-^{14}C were dissolved in physiological saline containing 0.001N HCl to the concentration of 3.3 mg/ml and the solution was injected from the tail vein in the dose of 10 mg/kg. For oral administration, D- and L-DOPA-^{14}C were diluted with non-radioactive D- and L-DOPA, respectively, to one sixth of the original specific activity and after dissolving in the physiological saline the solution, usually 0.5 ml, was administered orally with stomach tube in the dose of 60 mg/kg. For the period of 12 hr after the administration only water was given *ad lib.*

Collection of Urine——In order to avoid any further decomposition of the metabolites, the urine was collected into 5 ml of 0.2N HCl directly from an animal placed in an individual metabolic cage. After a given time, the cage was rinsed with 10 ml of 0.02N HCl and the washings were combined with the collected urine. The samples were stored under refrigeration to be supplied for chromatographic separation. A part of the urine was hydrolized with glusulase (Endo Laboratories Inc.) in acetate buffer of pH 5.5 at 37° overnight.

Separation of Urinary Metabolites——Two dimentional chromatography combined of paper partition chromatography and paper electrophoresis[6] was used in order to separate and follow all of the radioactive metabolites quantitatively. About 20 μl of the urine sample was spotted on the central line at about 15 cm above the lower end of a paper, with a mixture of an appropriate amount of eight nonradioactive standards as carriers. Development was performed with solvent system, *n*-butanol: acetic acid: water (4: 1: 1) for 14 to 16 hr at the room temperature. After drying under a cold air, the paper chromatogram was again developed in the transverse direction by electrophoresis in 0.025M phosphate buffer of pH 6.5, applying 400 V for 1.5 hr. The radioactive metabolites were detected by autoradiography after exposing the chromatogram onto Industrial X-ray film. The identification of the radioactive spots with those of the authentic samples were easily done by comparison with colored (amines and amino acids) or decolored (acids) spots of the standard after Ninhydrine reaction on the paper.

Extraction and Separation of Tissue Metabolites——Ten minutes after intravenous and 60 min after oral administration of radioactive D- and L-DOPA, the rats were sacrificed by bleeding from the carotid artery. The brain, liver, kidney, pancreas, heart, small intestine, skin (back) and skeletal muscle (femoral) were quickly removed by dissection. The tissues were, after weighing, homogenized in 6 ml of ice-cold 4% $HClO_4$ with Potter glass homogenizer and centrifuged (8000 rpm $\times$ 10 min). The precipitates were again extracted with 10 ml of 4% $HClO_4$ and the combined extracts were followed by the chromatographic separation.

The metabolites in the kidney, heart, intestine, skeletal muscle and skin could be separated well by applying directly with two-dimentional paper partition–paper electrophoretic chromatography (PPC–PEP). The extracts were adjusted to pH 5.0 with 2.0N KOH solution and after allowing to stand at 0° overnight the precipitates removed by filtration. The solution was concentrated to dryness under a reduced pressure and the residue dissolved in 1 ml of 50% 10^{-3}N HCl–ethanol. The chromatographic separation was performed in the same way as for the urinary metabolites except that usually 50μl of the solution was spotted.

The metabolites in the brain and pancreas could be separated by PPC-PEP after removing the lipids by Sephadex column chromatography. The extracts were concentrated *in vacuo* to about 4 ml, charged on Sephadex G-25 column (1.7 cm $\times$ 17 cm) and eluted with 80 ml of 0.004% $HClO_4$ under a constant rate of 0.4 ml/min. The radioactivity was recovered in the fractions (each 4 ml) from No. 5 to 13, while the lipid components were separated in those before No. 4 as white turbid eluates. The combined eluates from No. 5 to 15 were desalted with addition of 2.0N KOH, evaporated to dryness, dissolved in 1 ml of 50% 10^{-3} N HCl–ethanol and spotted to be separated by PPC–PEP chromatography.

For the separation of metabolites in the liver, the extracts were at first separated by Dowex 50 column into three fractions[7] and each of them was separated by thin–layer chromatography. Dowex-50 (H^+ form, 200—400 mesh) was washed and transformed to K^+ form according to the method of Hirs, *et al.*[8] The extracts were concentrated *in vacuo* to about 5 ml, desalted by adjusting the pH to 2.0 with 2N KOH and charged on the column (0.8 cm $\times$ 6 cm). On washing the column with 50 ml of O_2-free distilled water, the effluent contained phenolcarboxylic acids. Amino acids were eluted with 150 ml of 1N HCl and finally amines with 70 ml of 2.5N HCl. Dopamine glucuronide which was the main metabolite of L-DOPA-^{14}C was found to be

6) B. Duhm, W. Maul, H. Medenwald, K. Patzschke, and L. A. Wegner, *Z. Naturforschg.*, **20b**, 434 (1965).
7) K.F. Gey and A. Pletscher, *Biochem. J.*, **92**, 300 (1964).
8) C.H.W. Hirs, S. Moore, and W.H. Stein, *J. Biol. Chem.*, **200**, 493 (1953).

eluted in both of the latter two fractions. The total recovery of radioactivity by this procedures was over 95%. Each fraction was evaporated to dryness *in vacuo* and dissolved in 1 ml of 50% 10^{-3}N HCl–ethanol. Thin-layer chromatography (TLC) was performed on Cellulose F_{254} plate (Merck, 0.1 mm thickness) with solvent system, *n*-butanol: methanol: 1N formic acid (3: 1: 1). The radioactive spots were detected and identified with the authentic samples in the same way as that described for the paper chromatography.

Radioactive Measurements——The radioactive spots detected on the paper chromatograms were cut out and transferred into the counting vials, after cutting into smaller sections. After shaking in 0.5 or 1.0 ml of methanol, 15 ml of liquid scintillator (8 g PPO, 200 mg dimethyl-POPOP, 200 ml toluene and 800 ml dioxane) was added. The radioactivity was counted in the Beckman LS-250 Liquid Scintillation Spectrometer. For samples of low cpm values, the counting time was increased to 30 min. The radioactive spots on the TLC plates were also quantitatively transferred into the counting vials scraping carefully with a spatula and the radioactivity was counted after shaking in 0.5 ml of methanol and adding with 15 ml of the liquid scintillator.

Result

Urinary Metabolites after Oral Administration of D- and L-DOPA-^{14}C to Rats

The autoradiogram obtained after two-dimentional PPC–PEP separation of 24 hr urine after oral administration of L-DOPA-^{14}C (60 mg/kg) is shown in Fig. 1. Eighteen radioactive metabolites were detected and the following seven spots could be identified from the comparison with the mobilities of authentic standards: dopamine, DOPA, 3-O-methyldopamine, 3-O-methyl-DOPA, HVA, DOPAC and noradrenaline. None of the remaining spots corresponded to any of the authentic standards available and they were referred as A-1 to A-8. On hydrolysis of the urine with glusulase, however, all of these eight spots disappeared or decreased in the concentration markedly, while the concentration in the spots corresponding to dopamine, 3-O-methyldopamine, DOPAC and noradrenaline showed a significant increase and, in addition, four radioactive new spots referred as N-1 to N-4 were detected, as illustrated in Fig. 1-B. Therefore, all of the eight unknown spots can be attributed to the glucuronide or sulfate conjugates of the metabolites.

The distribution of radioactivity in each spot in 24 hr urine was tabulated in Table I, in comparison with that after glusulase hydrolysis. As can be seen from the table, it is evident that the spot A-5 which has the largest amount in the urinary metabolites corresponds to dopamine conjugate. The spots N-1 and N-2 could be identified to be 3-methoxy-4-hydroxyphenylethanol (MHPE) and 3,4-dihydroxyphenylethanol (DHPE), respectively, from the

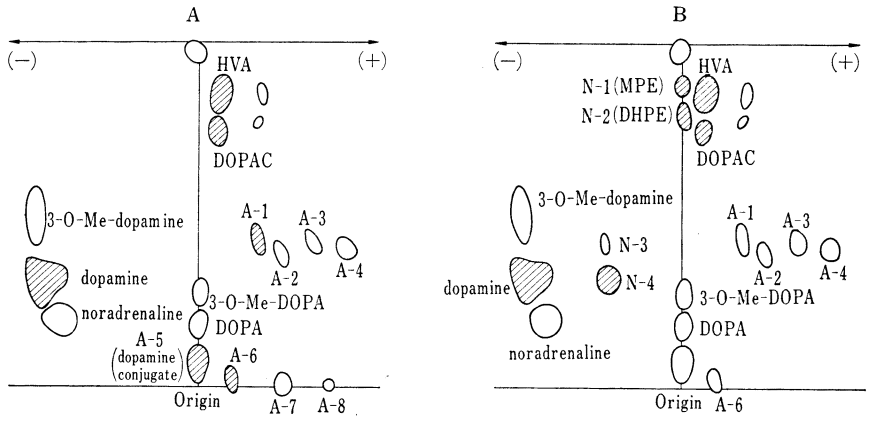

Fig. 1. Two-dimentional Autoradiogram of Urinary Metabolites 24 hr after Oral Administration of L-DOPA-^{14}C to Rats (60 mg/kg)

A: untreated urine; B: urine treated with glusulase
Separated by paper chromatography in the longitudinal direction (*n*-butanol: acetic acid: water= 4: 1: 1) and paper electrophoresis in the transverse direction (0.1M phosphate buffer, pH 6.5).
The shadowed spots represent the main metabolites.

comparison with mobilities of the authentic standards. The spot A-1 appears to be attributed to a conjugate of MHPE. Excretion of unchanged L-DOPA was only slight, less than 1% of the urinary radioactivity, indicating an almost complete metabolic change of L-DOPA in the body. Dopamine conjugate, probably the glucuronide, and HVA were excreted in the largest amounts, being about 34 and 19% of the total urinary metabolites, respectively, followed by MHPE-conjugate, free dopamine and DOPAC. Thus, it was indicated that, from the result after glusulase hydrolysis, L-DOPA is transformed mainly to dopamine, HVA, MHPE and DOPAC in an approximate ratio of 4: 2: 1: 1.

TABLE I. Metabolites of D- and L-DOPA-^{14}C in 24 hr Urine after Oral Administration (60 mg/kg) to Rats

Metabolites	% to total radioactivity			
	L-DOPA			D-DOPA
	Urine	+Glusulase		Urine
Dopamine-conjugate (A-5)	34.29	6.15	−29.14	5.28
Dopamine	6.62	32.49	+25.87	42.75
DOPA	0.73	0.56		16.86
3-O-Methyl-DOPA	0.23	0.64		4.32
3-O-Methyldopamine	1.21	3.62	+ 2.41	3.44
Noradrenaline	0.02	0.68	+ 0.66	0.42
HVA	18.68	18.28		7.09
DOPAC	6.30	9.14	+ 2.84	2.84
MHPE (N-1)	—	10.27	+10.27	—
DHPE (N-2)	—	5.00	+ 5.00	—
A-1	9.76	0.23	− 9.53	1.06
A-2	2.35	0.08	− 2.27	1.88
A-3	3.90	1.03	− 2.87	1.95
A-4	4.20	1.40	− 2.80	2.50
A-6	6.10	0.19	− 5.19	—
A-7	1.38	—	− 1.38	—
A-8	0.50	—	− 0.50	—
N-3	—	4.13	+ 4.13	—
N-4	—	1.16	+ 1.16	—

TABLE II. Urinary Excretion of L-DOPA Metabolites after Oral Administration (60 mg/kg) to Rats

Metabolites	% to urinary radioactivity			
	1 hr	3 hr	6 hr	24 hr
Dopamine-conjugate	18.51	34.53	35.23	34.29
Dopamine	23.55	14.59	10.07	6.62
DOPA	0.78	1.03	0.93	0.73
3-O-Methyl-DOPA	0.69	0.30	0.47	0.23
3-O-Methyldopamine	2.90	1.59	1.71	1.21
Noradrenaline	0.51	0.30	0.26	0.02
HVA	23.65	16.37	15.21	18.68
DOPAC	14.66	8.72	6.02	6.30
A-1 (MHPE-conjugate)	3.05	4.45	9.77	9.76
A-2	1.41	1.79	3.44	2.35
A-3	1.51	—	0.37	3.90
A-4	3.56	0.88	0.60	4.20
A-6	1.10	4.17	5.15	6.10
A-7	—	0.53	0.94	1.38
A-8	—	—	0.16	0.50
Urinary excretion (% to Dose)	20.23	54.26	71.96	87.32

All of these metabolites were detected in the urine samples collected in the first 1, 3 and 6 hr after administration as well as in the 24 hr urine. The relative amount of each metabolite was, however, significantly different depending upon the time of collection, as shown in Table II. In the urine collected in the earliest period after administration, free dopamine and HVA were the major metabolites, both being about 24% of the urinary radioactivity, with a smaller amount of dopamine conjugate (about 19%). With increasing the time, however, the excretion of free dopamine was decreased, while that of its conjugate increased. As shown in Fig. 2, the same trend was observed for A-1 and A-6 as for dopamine conjugate, showing a gradual increase in the excretion after a certain lag time in the earliest period after administration. Since no such a phenomenon could be seen in other metabolites as dopamine, HVA and DOPAC, this is considered to be a characteristic for the excretion of the conjugate form. It was also noted that DOPA and 3-O-methyl-DOPA appear to be excreted only in the earliest period as the free form, while dopamine, DOPAC, 3-O-methyldopamine and noradrenaline as the free form in the earliest period and as the conjugate form in the later period. HVA appears to be excreted as the free form for the whole period.

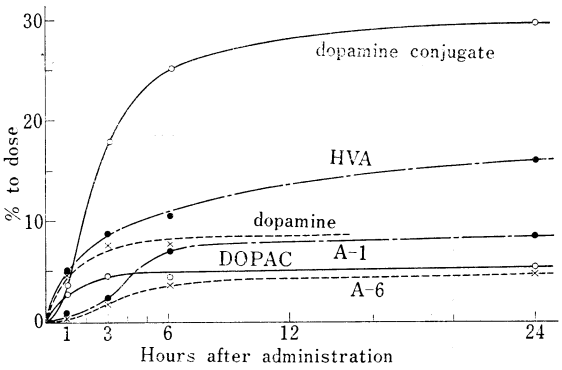

Fig. 2. Time Course in the Urinary Excretion of Metabolites after Oral Administration of L-DOPA-^{14}C(60 mg/kg) to Rats

After oral administration of D-DOPA-^{14}C, on the other hand, the two-dimentional autoradiogram of the 24 hr urine indicated that all radioactive metabolites detected correspond to those found for L-DOPA-^{14}C and it was found that free dopamine was the main metabolite and unchanged DOPA was excreted in an appreciable amount, while dopamine conjugate and other conjugated metabolites only in a small amount. The distribution of radioactivity in these metabolites was compared in Table I. The fact that unchanged DOPA was excreted in about 17% of the total urinary radioactivity as well as the fact that the excretion of HVA and DOPAC was considerably lower than that after L-DOPA administration indicates that D-DOPA is not so easily metabolized in the body as the L-isomer. However, the finding that the main metabolite was free dopamine, being about 43% of the total urinary radioactivity, appears to indicate that the D-isomer is also decarboxylated to a considerable extent in the body, in spite of the fact that[9] DOPA-decarboxylase is highly specific for the L-isomer.

The total amount of excretion of dopamine as free and conjugate forms was, therefore, the same extent for D- and L-DOPA, being approximately 40 to 50% of the total urinary radioactivity. A marked difference between the isomers was that after administration of D-DOPA the most of dopamine was excreted in the free form, while after that of L-DOPA mostly in the conjugated form. The site and a possible mechanism of D-DOPA metabolism will be discussed later.

Urinary Metabolites after Intravenous Administration of D- and L-DOPA-^{14}C to Rats

In 24 hr urine after intravenous administration of D- and L-DOPA-^{14}C to rats, the same radioactive metabolites were detected as those found in the urine after oral administration. The distribution of radioactivity in the metabolites was shown in Table III. From a comparison with Table I, it was noted that after intravenous administration of L-DOPA-^{14}C the relative percentages of each metabolite are considerably different from those after oral admini-

9) W. Lovenberg, H. Weissbach, and S. Udenfriend, *J. Biol. Chem.*, **237**, 89 (1962).

stration. The excretion of unchanged DOPA was also very slight, but the amount of dopamine conjugate was decreased with an increased amount of free dopamine, as compared to those after oral administration. After intravenous administration of D-DOPA-^{14}C, on the contrary, the relative percentages of each metabolite coincided in values very well with those found after oral administration. Free dopamine and unchanged DOPA were excreted as the main metabolites, being about 44 and 19% of the total urinary metabolites, respectively. This difference can be interpreted as being due to that between the site of metabolism of the D- and L-isomers, as will be discussed later.

TABLE III. Metabolites of D- and L-DOPA-^{14}C in 24 hr Urine after Intravenous Administration (10 mg/kg) to Rats

Metabolite	% to total radioactivity	
	L-DOPA	D-DOPA
Dopamine Conjugate	14.36	2.46
Dopamine	23.50	48.36
DOPA	1.01	15.96
3-O-Methyl-DOPA	1.74	4.04
3-O-Methyldopamine	2.57	2.14
HVA	28.09	5.74
DOPAC	6.43	2.13
A-1	5.60	1.38
A-2	2.57	1.58
A-3	2.02	0.98
A-4	5.10	3.57
A-6	0.52	—
A-7	1.00	—

Metabolites in Rat Tissues after Intravenous and Oral Administration of D- and L-DOPA-^{14}C

The radioactive metabolites in the main tissues including the brain were analysed 10 min after intravenous (10 mg/kg) and 1 hr after oral (60 mg/kg) administrations of D- and L-DOPA-^{14}C to rats and the results on the main metabolites are shown in Tables IV and V. It can be seen generally that after administration of L-DOPA the metabolites including dopamine and its metabolites are the main components in most of the tissues, while after that of the D-isomer unchanged DOPA and its 3-O-methyl derivative are the main components in most of the tissues with an apparent exception of the kidney.

In the brain, a formation of dopamine was demonstrated both after intravenous and oral administration of L-DOPA-^{14}C. Ten minutes after the intravenous administration, about 15% of the brain radioactivity was found to be dopamine and about 50% to be HVA and DOPAC, the dopamine metabolites. Since dopamine cannot pass through the blood-brain barrier,[4] the result indicates a rapid metabolism of L-DOPA to dopamine and its metabolites in the brain. One hour after oral administration, when the maximum uptake of radioactivity was observed, about 5 and 50% of the brain radioactivity was found to be dopamine and its metabolites, respectively. It was noted that the amount of 3-O-methyl-DOPA was higher than that after intravenous administration. After administration of D-DOPA-^{14}C, on the other hand, no dopamine and its metabolites could be detected, while the most of radioactivity was found to be unchanged DOPA and 3-O-methyl-DOPA, indicating that D-DOPA cannot be decarboxylated in the brain to form dopamine.

The metabolites in the skeletal muscle were found to be quite similar to those in the brain. Ten minutes after intravenous administration of L-DOPA-^{14}C, about 15% of the total radioactivity was detected as dopamine. The only metabolite detected after administration of D-DOPA-^{14}C was 3-O-methyl-DOPA.

In the pancreas, where a rapid and high accumulation of radioactivity occurrs in both D- and L-DOPA-^{14}C,[1,4] DOPAC was found to be the main metabolite from L-DOPA, while unchanged DOPA from D-DOPA. In the intestine, where a high accumulation of radioactivity also occurrs in both isomers,[4] dopamine conjugate was found to be the main metabolite from L-DOPA, while unchanged DOPA from D-DOPA.

In the skin, unchanged DOPA was found to be present in the highest percentage among the tissues investigated after administration of L-DOPA-^{14}C. After administration of D-DOPA-^{14}C, the most of radioactivity was unchanged DOPA. From the autoradiographic studies,[4] it was shown that the radioactive uptake by the skin is mostly concentrated in the hair follicles and the above results suggest a concentrative uptake of DOPA by the hair.

In the liver, dopamine conjugate was the main metabolite after administration of L-DOPA-^{14}C, being over 70% of the total radioactivity. After administration of D-DOPA-^{14}C, on the contrary, almost no metabolite other than 3-O-methyl-DOPA could be detected. This result indicates that D-DOPA is not decarboxylated in the liver to any appreciable extent and might give an explanation for the fact[1,4] that D-DOPA-^{14}C does not accumulate in the liver in contrast to a high accumulation of L-DOPA-^{14}C.

In the kidney, on the other hand, the formation of dopamine and its metabolites could be demonstrated from both D- and L-DOPA. After intravenous and oral administration of L-DOPA-^{14}C, free and conjugated dopamine was the main metabolite, respectively, being about 30% of the total radioactivity, followed by HVA (about 20%). After intravenous administration of D-DOPA-^{14}C, free and conjugated dopamine were the main metabolites, being about 30 and 18% of the total radioactivity, respectively, and HVA and DOPAC were also detected in appreciable amounts.

TABLE IV. Main Metabolites in Rat Tissues 10 min after Intravenous Administration of D- and L-DOPA-^{14}C (10 mg/kg)

Metabolite	% to total radioactivity							
	Brain		Skel. Muscle		Pancreas		Intestine	
	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA
DOPA	12.2	50.6	30.2	90.0	3.4	52.6	5.1	88.7
3-O-Methyl-DOPA	6.4	49.4	31.8	4.0	1.0	+[a]	1.0	7.2
Dopamine	15.6	—[b]	14.6	—	6.5	—	1.9	+
3-O-Methyldopamine	—	—	5.7	—	1.3	—	+	+
HVA	26.8	—	4.5	—	8.5	+	3.5	—
DOPAC	24.3	—	3.6	—	69.5	+	6.9	—
Dopamine Conjugate	—	—	—	—	4.3	—	78.5	—

Metabolite	% to total radioactivity					
	Skin		Liver		Kidney	
	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA
DOPA	47.2	91.0	+	38.6	2.6	19.3
3-O-Methyl-DOPA	14.5	—	2.3	25.9	1.5	3.3
Dopamine	6.3	—	+	+	30.0	29.3
3-O-Methyldopamine	3.8	—	1.0	+	7.4	3.3
HVA	6.7	—	—	4.0	21.9	1.4
DOPAC	5.4	—	2.6		9.6	2.1
Dopamine Conjugate	7.6	—	72.4	+	8.4	18.2

a) A trace of radioactivity was detected.
b) No radioactivity was detected.

Thus, after administration of D-DOPA, dopamine and its metabolites could be detected in an appreciable amount only in the kidney, suggesting that the formation of dopamine from D-DOPA occurrs only in the kidney.

TABLE V. Main Metabolites in Rat Tissues 1 hr after Oral Administration of D- and L-DOPA-^{14}C (60 mg/kg)

Metabolite	% to total radioactivity							
	Brain		Skel. Muscle		Pancreas		Intestine	
	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA
DOPA	10.8	37.5	23.6	54.5	10.7	54.8	3.7	82.9
3-O-Methyl-DOPA	21.8	52.1	14.5	34.0	20.9	30.1	1.0	9.0
Dopamine	5.2	—[a]	2.9	+[b]	—	—	19.5	—
3-O-Methyldopamine	—	—	3.0	+	—	—	+	—
HVA	31.8	—	9.0	—	13.7	+	2.9	—
DOPAC	16.0	—	4.0	—	34.3	+	12.4	—
Dopamine Conjugate	—	—	27.7	—	—	—	37.6	—

Metabolite	% to total radioactivity					
	Skin		Liver		Kidney	
	L-DOPA	D-DOPA	L-DOPA	D-DOPA	L-DOPA	D-DOPA
DOPA	53.8	78.1	—	15.3	1.6	53.7
3-O-Methyl-DOPA	14.6	—	—	40.9	1.6	4.9
Dopamine	1.9	—	0.5	—	3.8	4.8
3-O-Methyldopamine	—	—	1.2	—	4.6	2.3
HVA	—	—	—	—	24.0	9.5
DOPAC	—	—	—	—	2.8	—
Dopamine Conjugate	+	—	76.0	—	31.7	19.1

a) No radioactivity was detected.
b) A trace of radioactivity was detected.

Discussion

After intravenous and oral administration of L-DOPA-^{14}C to rats, dopamine and its conjugate, probably the glucuronide, HVA and DOPAC were found to be excreted as the main metabolites in the urine. MHPE conjugate was identified as another major metabolite and DHPE conjugate, 3-O-methyl-DOPA, 3-O-methyldopamine and noradrenaline were identified as minor metabolites. The excretion of unchanged DOPA was only slight, being less than 1% of the total urinary radioactivity. The results indicate that L-DOPA administered exogeneously in a high dose is metabolized almost exclusively by the pathway through dopamine to give finally HVA, DOPAC and MHPE by the action of monoamine oxidase (MAO) and/or catechol O-methyltransferase (COMT), as shown in Chart 1, in accordance with the previous studies.[10,11]

After intravenous and oral administration of D-DOPA-^{14}C, on the other hand, unchanged DOPA was excreted in about 20% of the total urinary radioactivity during 24 hr, indicating a considerably slower rate of metabolism of the D-isomer than the L-isomer. This might be due to the fact that DOPA-decarboxylase which is the first step of DOPA metabolism is highly specific for the L-isomer.[9] The main metabolite in the urine was, however, found to be also dopamine and its conjugate, being about 50% of the total radioactivity in 24 hr urine,

10) M. Sandler and C.R.J. Ruthven, *Progress in Medicinal Chem.*, **6**, 200 (1969).
11) D.B. Calne, F. Karoum, C.R.J. Ruthven, and M. Sandler, *Br. J. Pharmacol.*, **37**, 57 (1969).

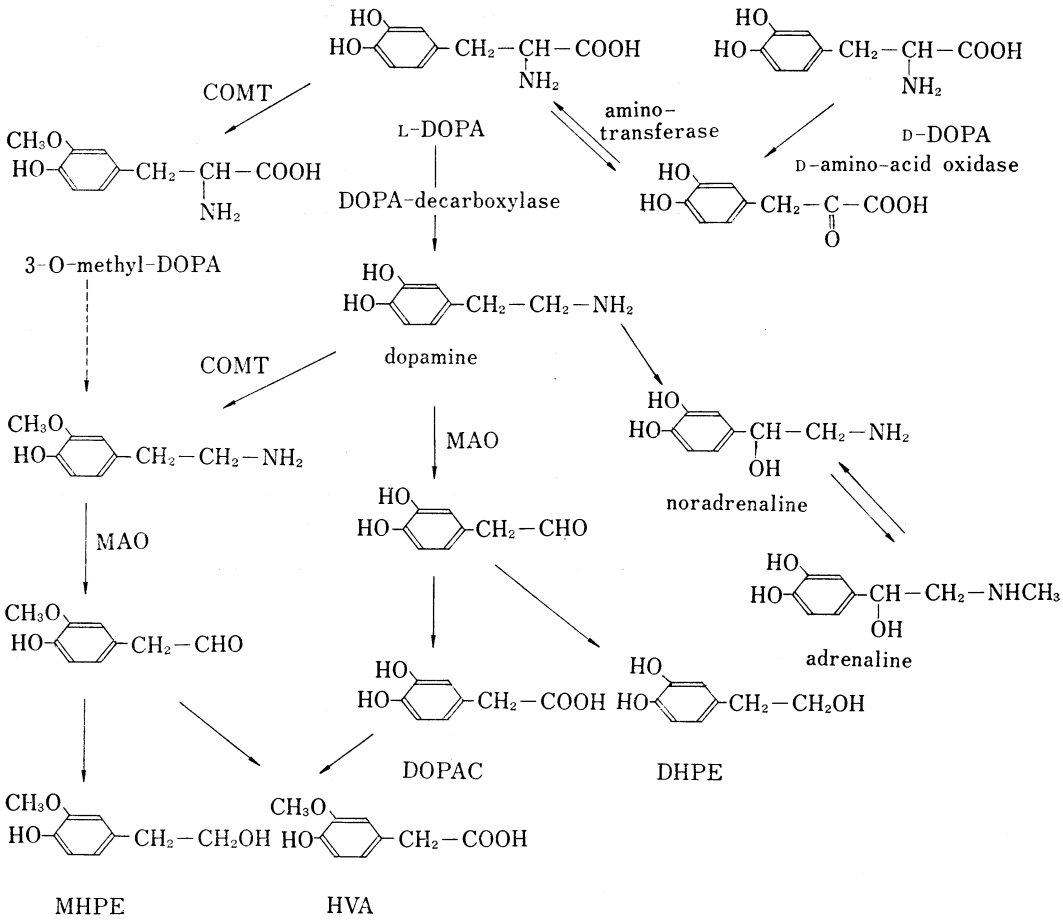

Chart 1. Main Metabolic Pathways of D- and L-DOPA

which is the same extent as that excreted from L-DOPA-^{14}C. A marked difference between the two isomers was the finding that in the case of D-DOPA the most part of dopamine was excreted as the free form in contrast to the case of L-DOPA where the most part as the conjugated form. This is considered to indicate that dopamine formed from D-DOPA was excreted into the urine without passing through the liver to any appreciable extent. In fact, dopamine conjugate was found to be the main metabolite accumulated in the liver after administration of L-DOPA, while after that of D-DOPA no appreciable amount of dopamine and its metabolites was detected. This is in accord with the above consideration and might give an explanation for the observation that no appreciable accumulation of radioactivity occurrs in the liver after administration of D-DOPA-^{14}C in contrast to a high accumulation of L-DOPA-^{14}C.[1,4] On the other hand, the finding that a prominent formation of dopamine and its metabolites from D-DOPA was detected only in the kidney suggests that the formation of dopamine from D-DOPA is proceeded mostly in the kidney, followed by its direct excretion into the urine.

As to a possible metabolic pathway of D-DOPA, Sourkes, *et al.*[12] supposed that D-DOPA is at first oxidized with D-amino acid oxidase to give 3,4-dihydroxyphenylpyruvic acid, which

12) T.L. Sourkes, M.H. Wiseman-Distler, J.F. Moran, G.F. Murphy, and S.S. Cyr, *Biochem. J.*, **93**, 469 (1964).

is then transformed specifically to L-DOPA with transaminase and thus formed L-DOPA is decarboxylated to dopamine, as shown in Chart 1. It is well known that D-amino acid oxidase is localized in the kidney[13] and that both transaminase[14] and DOPA-decarboxylase is abundant in the kidney. Furthermore, an active secretion of dopamine from the renal tubule has been reported in chicken.[15]

Thus, considering from all these results, it is quite plausible that D-DOPA administered is, after circulation in the blood, metabolized mostly at the kidney to dopamine, which is then excreted directly into the urine without being reabsorbed back into the blood circulation to any appreciable extent. A more detailed mechanism of D-DOPA metabolism in the kidney is now under investigation in this laboratory.

Another present finding that the urinary metabolites from L-DOPA changed considerably depending upon the route of administration, while those from D-DOPA did not can be interpreted as being due to the difference in the site of metabolism. After oral administration of L-DOPA, a contribution of the metabolism at the peripheral tissues as the gastric and intestinal mucosa and liver may result in the significant differences in the relative amount of urinary metabolites from those after intravenous administration, such as an increased excretion of dopamine conjugate. For D-DOPA, on the other hand, the kidney is only the site of metabolism and, therefore, the urinary metabolites may not depend upon the route of administration.

In the present studies, a prominent formation of dopamine was demonstrated in the brain after administration of L-DOPA. Ten and 60 min after intravenous and oral administration of L-DOPA-^{14}C, respectively, when the maximum concentration of radioactivity was reached, the most part of the brain radioactivity was detected as dopamine and its metabolites. Since dopamine itself cannot pass through the blood-brain barrier,[4] dopamine detected in the brain must be formed from L-DOPA in the brain tissues. From the facts that the radioactivity is accumulated in the caudate nucleus of the brain, as was demonstrated by autoradiographic technique,[4] and that DOPA-decarboxylase activity in the brain is also localized in the striatum,[16] the metabolites thus found in the whole brain might be regarded as representing mainly the metabolism in the caudate nucleus, the site of dopamine depletion in Parkinsonism.[17] The result that the percentage of dopamine was appreciably higher after intravenous than oral administration may indicate a more efficient increase in the brain dopamine level by the intravenous administration, as well as a much more efficient uptake of radioactivity by the brain after intravenous than oral administration of L-DOPA-^{14}C.[1]

On the other hand, after administration of D-DOPA only unchanged DOPA and its 3-O-methylated derivative were detected in the brain, indicating that D-DOPA is not decarboxylated in the brain tissues and thus might have no therapeutic effect on Parkinsonism. A high accumulation and a long retention of radioactivity in the whole brain observed[1,5] after oral administration of D-DOPA-^{14}C is, therefore, considered to be due to a retention of unchanged DOPA and, more probably, 3-O-methyl-DOPA for a long period without being metabolized further.

As already discussed in the preceding paper,[1] the distribution and behavior of radioactive D- and L-DOPA in the skeletal muscle are quite similar to those in the brain and an active transport mechanism is expected to be involved in their uptake in a similar way to that in the brain.[18] In the present results, the metabolites were also found to be similar to those in the brain. After administration of D-DOPA-^{14}C only unchanged DOPA and 3-O-methyl-

13) L. Birkofer and R. Wetzel, *Z. Physiol. Chem.*, **264**, 31 (1940).
14) E.C.C. Lin and W.E. Knox, *J. Biol. Chem.*, 233, 1186 (1958).
15) E. Sanner, *Acta Pharmacol. et Toxicol.*, **20**, 375 (1963).
16) A. Bertler and E. Rosengren, *Acta Physiol. scand.*, **47**, 350 (1959).
17) O. Hornykiewicz, *Pharmacol. Revs.*, **18**, 925 (1966).
18) H. Yoshida, J. Namba, K. Kaniike, and R. Imaizumi, *Jap. J. Pharmacol.*, **13**, 1 (1963).

DOPA could be detected as the metabolites. A high accumulation and a long retention of radioactivity in the skeletal muscle after oral administration of D-DOPA-^{14}C might be, therefore, interpreted in the same way as that in the brain.

The fact that a prominent formation of 3-O-methyl-DOPA was observed both in the brain and skeletal muscle from D-DOPA indicates that COMT can act on the D-isomer to the same extent as that on the L-isomer. It has been shown that[19] after administration of L-DOPA-^{14}C to rats the percentage of 3-O-methyl-DOPA in the brain increases with time after 30 min and that 3-O-methyl-DOPA is a poor substrate for DOPA-decarboxylase[20] and, when administered to rats, is not easily metabolized and remains for a long period in the body.[21] Since 3-O-methyl-DOPA is only an important metabolite in the tissues after administration of D-DOPA, it is most probable that a long retention of radioactivity observed after administration of D-DOPA-^{14}C is mainly due to the retention of 3-O-methyl-DOPA in the tissues. Whole-body autoradiographic study using L-3-O-methyl-DOPA-^{14}C is now under investigation.

In the previous papers,[1,4] a rapid and high accumulation of radioactivity was observed in the pancreas and intestine after administration of both D- and L-DOPA-^{14}C and was interpreted as an uptake of amino acid by the sites of a rapid protein synthesis through some mechanism which is not specific with respect to optical isomers. In the present results, however, the main metabolite in the pancreas and intestine was found to be DOPAC and dopamine conjugate, respectively, as early as 10 min after intravenous injection of L-DOPA-^{14}C. After administration of D-DOPA-^{14}C, on the other hand, unchanged DOPA was the main component of radioactivity in both organs. Since dopamine does not accumulate in the pancreas to any appreciable extent,[4] it is suggested that L-DOPA once accumulated in the pancreas was rapidly metabolized to DOPAC through dopamine. In the intestinal mucosa, on the other hand, dopamine also accumulates as well as DOPA and a higher accumulation of L-DOPA in the intestine than the D-isomer[1] is suggested to be caused from its decarboxylation followed by the conjugate formation. Tjälve reported that[22] both DOPA-2-^{14}C and -1-^{14}C showed a strong accumulation of radioactivity in the mouse pancreas 1 and 30 min after intravenous injection, but in his study the DL-racemate has been used rather than the L-isomer. In our unpublished results from wholebody autoradiography using L-DOPA-1-^{14}C, it has been observed that the concentration of radioactivity 10 min after intravenous injection in the pancreas is very low, while increases gradually with time and remains for a long period. This result agrees well with the present finding of a rapid decarboxylation of L-DOPA in the pancreas and might be explained by assuming that 3-O-methyl-DOPA is gradually accumulated in the pancreas, probably through uptake from the blood circulation.

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20) R. Ferrini and A. Glässer, *Biochem. Pharmacol.*, **13**, 798 (1964).
21) G. Bartholini, I. Kuruma, and A. Pletscher, *Br. J. Pharmacol.*, **40**, 461 (1970).
22) H. Tjälve, *Acta Physiol. scand.*, Suppl. **1971**, 360.