Studies on the Metabolism of D- and L-Isomers of 3,4-Dihydroxyphenylalanine (DOPA). VI.¹⁾ Metabolism of D-DOPA in Rat Kidney

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The metabolism of the D-isomer of 3,4-dihydroxyphenylalanine (D-DOPA) in rat kidney was investigated. After incubation of $9.05 \times 10^{-3} \text{ M}$ D-DOPA with rat kidney homogenate, 3,4-dihydroxyphenylpyruvate (DHPP) was detected as the main metabolite, whereas at a lower concentration of D-DOPA ($5.22 \times 10^{-4} \text{ M}$), dopamine was the dominant product. It was shown *in vitro* that D-DOPA is changed to L-DOPA through two steps and decarboxylated immediately to dopamine in rat kidney. The first step is an oxidative deamination of D-DOPA to DHPP by D-amino acid oxidase. This step was inhibited by D-alanine and benzoic acid wherein these substances act as a substrate and inhibitor, respectively. L-Alanine had almost no effect. The K_m value of D-DOPA for purified hog kidney D-amino acid oxidase was 24 mM. The second step is conversion of DHPP to L-DOPA by transaminase. This step was accelerated by aspartate, glutamate, tyrosine, tryptophan and phenylalanine. The latter two were particularly effective as amino donor. L-DOPA was identified by thin-layer chromatography, paper electrophoresis and reverse isotope dilution method. The transaminase activity was observed both in the 9000 g supernatant and precipitate fractions from rat kidney. The rat kidney slice had greater activity than the liver slice in metabolizing D-DOPA to dopamine. This difference is attributable to the content of D-amino acid oxidase in these organs and may give an explanation of the fact that D-DOPA is metabolized *in vivo* almost exclusively in the kidney.

In the previous paper,³⁾ the metabolic differences of D- and L-isomers of 3,4-dihydroxyphenylalanine (DOPA) were investigated and it was shown that D-DOPA is also metabolized *in vivo* to a considerable extent to dopamine though the rate is much lower than that of L-DOPA. It was further shown that dopamine and its metabolites were not detected in any other tissues than the kidney after administration of D-DOPA-2-¹⁴C to rat, suggesting that the metabolism of D-DOPA occurs in the kidney rather than in the liver. Several D-isomers of essential amino acids are known to compensate their L-isomers in animals.⁴⁾ As a possible mechanism for such a conversion, it is believed that the corresponding α -ketoacid formed from the D-isomer by D-amino acid oxidase is transaminated to the L-isomer. As for D-DOPA, the same transformation mechanism to L-DOPA followed by an immediate decarboxylation by aromatic L-amino acid decarboxylase has been supposed by Sourkes, *et al.*⁵⁾ The details are not known however. In the present paper, the metabolism of D-DOPA in rat kidney was investigated *in vitro* and the following two steps were ascertained individually: D-DOPA $\rightarrow$ 3,4-Dihydroxyphenylpyruvic acid (DHPP), and DHPP $\rightarrow$ L-DOPA. Effective amino donors to DHPP were also examined.

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Material and Method

Materials—L-DOPA-2-¹⁴C was purchased from the Radiochemical Center, Amersham, England. The specific activity was 49.2 μ Ci/mg. D-DOPA-2-¹⁴C was prepared by the optical resolution of DL-DOPA-2-¹⁴C which was purchased from the Radiochemical Center, Amersham, England, as described in the previous paper.⁶⁾ The specific activity was 24.9 μ Ci/mg. DHPP-2-¹⁴C was prepared from D-DOPA-2-¹⁴C by purified hog kidney D-amino acid oxidase. A solution of 0.1 mCi D-DOPA-2-¹⁴C was incubated at 37° for 1 hr with 250 μ g of D-amino acid oxidase and 3 mg of catalase in 1 ml of potassium phosphate buffer at pH 7.0 with constant shaking. The reaction mixture was separated by paper chromatography with the solvent system, *n*-butanol-methanol-1*N* formic acid (6:2:1). The part of the chromatogram corresponding to DHPP was cut off and DHPP-¹⁴C was extracted with 100 ml of acetone. 3,4-Dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxyphenylacetic acid (HVA), dopamine (DA), adrenaline and noradrenaline used as authentic samples are the commercial products. D- and L-DOPA, DHPP, L-3-O-methyl-DOPA, 3-O-methyl-dopamine were prepared in the Research and Development Department of Sankyo Chemical Industries, Ltd. 4-Bromo-3-hydroxybenzylamine hydrochloride (NSD 1055), used as DOPA decarboxylase-inhibitor, was prepared in this laboratories. D-Amino acid oxidase and catalase were purchased from Sigma Chemical Co., Ltd.

Separation of Metabolites—Thin-layer chromatography (TLC) was performed on cellulose plates (Merck, F₂₅₄, 0.1 mm thickness) with the solvent system, *n*-butanol-methanol-1*N* formic acid (6:2:1). Paper electrophoresis (PEP) was performed in 0.01*M* potassium phosphate buffer at pH 6.0 for 1.5 hr applying 500 V. The spots were detected by spraying the equivolume mixture of 1% ferric chloride and 1% potassium ferricyanide solution. The metabolites containing phenolic hydroxyl group were recognized as blue spots.

Metabolism of D-DOPA-2-¹⁴C by Rat Kidney and Liver Homogenates and Slices—Male rats of Wistar-Imamichi strain reared for 7–8 weeks were exsanguinated to death. The kidney and the liver were removed and homogenized by Polytron (Kinematica Co., Ltd. Switzerland) in 4–5 fold volume of 0.05*M* potassium phosphate buffer (pH 7.0). The protein concentration was determined by Lowry's method.⁷⁾ The kidney and the liver slices of 0.3–0.5 mm thickness were prepared manually by the hand slicer (Natume Co., Ltd.).

The homogenates (about 10 ml) were preincubated at 37° for 5 min under constant shaking. The reaction was started by the addition of D-DOPA-2-¹⁴C solution. One ml of the incubation mixture was pipetted out every 10 min and heated for 2 min at 100°. After centrifugation at 2000 rpm, 10 μ l of the supernatant was spotted on cellulose thin layer plates and developed.

The reaction using the slices were performed in Krebs-Henselite bicarbonate buffer at pH 7.4 in an atmosphere of 95% O₂–5% CO₂ gas. The slices (0.5–1 g) were floated in about 4 ml of the buffer. Ten μ l of the floating medium was taken by microsyringe periodically and spotted on the thin layer plate directly. The radioactive metabolites were detected by autoradiography after exposing the chromatogram onto Industrial X-ray film (Sakura Type N). The radioactive spots were scraped into the counting vials. One ml of 70% ethanol and 10 ml of liquid scintillator (8 g PPO, 200 mg dimethyl-POPOP, 200 ml of toluene and 800 ml of dioxane) were added successively. The radioactivity was measured in a Packard Model 3380 liquid scintillation counter.

Reverse Isotope Dilution Method for L-DOPA Identification—DHPP-2-¹⁴C (8.65 μ Ci) was incubated with 5 ml of the rat kidney homogenates and 10 mg of sodium glutamate at 37° for 5 hr in an atmosphere of nitrogen gas and the reaction was terminated by heating at 100°. The supernatant after centrifugation at 2000 rpm for 10 min was passed through an anion exchange column (Dowex 1, Cl form, 200–400 mesh) and the elution was secured by 5 ml of 1*M* potassium chloride. The eluate was concentrated to dryness by rotary evaporator and dissolved in 20 ml of distilled water. In 10 ml of the solution, 250 mg of non-radioactive L-DOPA was added and dissolved by heating. The recrystallization was then repeated until a constant specific radioactivity was obtained. When a constant value was obtained, the content of radioactive L-DOPA (W_x mg) and the percentage ($X\%$) in the original extract (R dpm) were calculated from the mean specific activity of the crystals (S dpm/mg), the amount of nonradioactive L-DOPA added (W mg) and the specific activity of the original DHPP-2-¹⁴C (S_0 dpm/mg) according to the following equations.

$$W_x = WS/(S_0 - S)$$

$$X = 100S(W + W_x)/R$$

Assay of D-Amino Acid Oxidase Activity—The rat kidney and liver homogenates were centrifuged at 9000 *g* and the precipitate fractions were resuspended with potassium phosphate buffer to the original volume. The activity of D-amino acid oxidase was observed only in the 9000 *g* precipitate fractions. One ml of 9000 *g* precipitate fraction from the rat kidney and liver homogenates were preincubated at 37° for 5 min under constant shaking. The reaction was started by the addition of 0.5 ml of D-DOPA solution (4 mg of D-DOPA dissolved in 1 ml of 0.05*M* potassium phosphate buffer at pH 7.0). One ml of 10% trichloro-

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acetic acid was added for termination. Aliquots of the supernatant after the centrifugation at 2000 rpm were analyzed for DHPP by the Brigg reaction.⁸⁾ Four ml of the diluted sample was added with 1 ml of phosphomolybdate reagent prepared by mixing equal volume of 1% potassium phosphate in water and 3% solution of ammonium molybdate in 5N hydrochloric acid. After 2hr, OD at 830 m μ was measured.

Determination of K_m Value of D-DOPA for D-Amino Acid Oxidase—Purified hog kidney D-amino acid oxidase was used for the determination of K_m value. Twenty μ g of D-amino acid oxidase in potassium phosphate buffer at pH 7.0 was preincubated at 37° for 5 min under constant shaking. D-DOPA-2-¹⁴C at various concentrations was added. The reaction was stopped after 10 min by adding 6N hydrochloric acid. Ten μ l of the solution was spotted on cellulose plate and developed. The spots corresponding to DHPP and DOPAC were scraped into the counting vials and the radioactivity was measured. The reaction was evaluated by the sum of these two products. The amount gave a linear plot against the reaction time at least for 40 min. The K_m value was determined from Lineweaver-Burk reciprocal plots.

Assay of Transaminase Activity—Two ml of the kidney and liver homogenates were preincubated with 0.1 ml of pyridoxal-5'-phosphate (10^{-4} M), 0.1 ml of dithiothreitol (10^{-3} M) and 0.1 ml of NSD 1055, DOPA decarboxylase inhibitor,⁹⁾ (10^{-4} M) at 37°. One ml of DHPP (3.63×10^{-3} M) and 0.5 ml of glutamate or phenylalanine (10^{-2} M) in 0.05M potassium phosphate buffer at pH 7.0 were added. The tubes were filled with nitrogen gas and then plugged tightly with rubber stoppers. The reaction was terminated by heating the tubes in boiling water for 2 min. The supernatant after centrifugation at 2000 rpm was passed through an anion exchange resin column (Dowex 1, Cl Form, 200—400 mesh, 0.7×2 cm). L-DOPA was eluted completely by 2 ml of 1M potassium chloride. The elution with distilled water was continued further until the volume of eluate reached 10 ml. L-DOPA was determined by Nair's method.¹⁰⁾ Two ml of the eluate was added with 1 ml of 10% sodium tungstate, 1 ml of 5% sodium nitrite in water and 0.5 ml of 10% trichloroacetic acid in 0.5N hydrochloric acid, successively. After 5 min, 1 ml of 3N sodium hydroxide was added and then OD at 510 m μ was measured after 15 min. In the experiments to see the effect of pyridoxal-5'-phosphate and dithiothreitol on the transaminase activity, the homogenate were first dialyzed against 0.05M potassium phosphate buffer (pH 7.0) containing 10^{-2} M hydroxylamine for 24 hr and then against the same buffer without hydroxylamine for 48 hr.

Examination of Amino Donors to DHPP—A partially purified transaminase preparation was used. The kidneys from 10 rats were homogenized by Polytron in 4—5 fold volume of 0.05M potassium phosphate buffer (pH 7.0) and then centrifuged at 9000 *g*. The supernatant was fractionated by ammonium sulphate and the precipitate at 40—60% saturation was redissolved in 50 ml of the buffer and then dialyzed against 5 liter of the same buffer for 48 hr. The transaminase activity was measured in the same way as described above. Two ml of this enzyme preparation was preincubated with dithiothreitol, pyridoxal-5'-phosphate and NSD 1055 for 5 min and then incubated with DHPP and various amino acids for 1 hr.

Result

D-DOPA Metabolism with Rat Kidney Homogenates and Slices

The time course of metabolite formation after incubation of 9.05×10^{-3} M D-DOPA-2-¹⁴C with rat kidney homogenates is shown in Fig. 1. DHPP was found to be the main metabolite and dopamine, DOPAC and HVA were also detected on the TLC, demonstrating that D-DOPA is metabolized to a considerable extent in the kidney and that the transformation of D-DOPA to DHPP is involved in the D-DOPA metabolism. The fact that dopamine and its metabolites appeared from D-DOPA in spite of a high specificity¹¹⁾ of the aromatic amino acid decarboxylase for the L-antipode suggests the formation of L-DOPA from D-DOPA.

When a lower concentration of D-DOPA-2-¹⁴C (5.22×10^{-4} M) was incubated with rat kidney homogenates, however, dopamine became a major metabolite rather than DHPP as shown in Fig. 2. Incubation of 5.22×10^{-4} M D-DOPA-2-¹⁴C with rat kidney slices also gave a similar result (Fig. 3).

The fact that DHPP was detected dominantly from a high concentration of D-DOPA may be explained by the fact that DHPP has an inhibitory effect on aromatic amino acid decarboxylase¹²⁾ and/or by a saturation mechanism for the transamination reaction.

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Fig. 1. Time Course of Metabolite Formation from D-DOPA-2- ^{14}C ($9.05 \times 10^{-3}\text{M}$) with Rat Kidney Homogenate

specific activity of D-DOPA-2- ^{14}C : $2.08 \mu\text{Ci/mg.}$

- : total counts of metabolites
- : DHPP
- : Dopamine
- : DOPAC
- ▲—: HVA
- △—: unknown metabolite (electrically neutral)

Fig. 2. Time Course of Metabolite Formation from D-DOPA-2- ^{14}C ($5.22 \times 10^{-4}\text{M}$) with Rat Kidney Homogenate

Specific activity of D-DOPA-2- ^{14}C : $24.9 \mu\text{Ci/mg.}$

- : total counts of metabolites
- : DHPP
- : dopamine
- : DOPAC
- ▲—: HVA
- △—: unknown metabolite (electrically neutral)

Participation of D-Amino Acid Oxidase in the Transformation of D-DOPA to DHPP

Examination for whether the enzyme responsible for the deamination of L-DOPA to DHPP is D-amino acid oxidase or not was conducted. After the incubation of D- and L-DOPA with 9000 *g* precipitate fraction from rat kidney homogenate, DHPP was found to be formed only from D-DOPA as shown in Fig. 4. The supernatant fraction was almost devoid of activity.

The effect of D-alanine which is known to be a good substrate for D-amino acid oxidase was then examined. As shown in Fig. 5, the equimolar addition of D-alanine inhibited considerably the formation of DHPP from D-DOPA and the ten fold molar addition of D-alanine inhibited the formation of DHPP almost completely. On the other hand, the ten fold molar addition of L-alanine exhibited almost no inhibitory effect. The effect of benzoic acid which is a well-known inhibitor of this enzyme¹³⁾ was also examined. As shown in Fig. 6, benzoic acid exhibited a strong inhibition on the formation of DHPP from D-DOPA and in proportion to the concentration, the inhibitory effect increased.

It was examined with the purified enzyme system whether D-DOPA is a substrate of D-amino acid oxidase or not. D-DOPA-2- ^{14}C was incubated with purified hog kidney D-amino

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Fig. 3. Time Course of Metabolite Formation from D-DOPA-2- ^{14}C ($5.22 \times 10^{-4}\text{M}$) with Rat Kidney Slice

specific activity of D-DOPA-2- ^{14}C : $24.9 \mu\text{Ci/mg}$

- : total counts of metabolites
- : DHPP
- : dopamine
- : DOPAC
- ▲: HVA
- △: unknown metabolite (electrically neutral)

Fig. 5. Inhibitory Effect of D-Alanine on DHPP Formation from D-DOPA

- : control. D-DOPA concentration: $4.83 \times 10^{-3}\text{M}$
- : with equimolar D-alanine
- : with ten fold molar D-alanine
- : with ten fold molar L-alanine

Fig. 4. DHPP Formation from D- and L-DOPA

Fig. 6. Inhibitory Effect of Benzoic Acid on DHPP Formation from D-DOPA

- I: control. D-DOPA concentration: $5.08 \times 10^{-3}\text{M}$
- II: with $5 \times 10^{-6}\text{M}$ benzoate
- III: with $15 \times 10^{-6}\text{M}$ benzoate
- IV: with $25 \times 10^{-6}\text{M}$ benzoate
- V: with $35 \times 10^{-6}\text{M}$ benzoate

acid oxidase and the products were analyzed by TLC. It was shown that D-DOPA is a good substrate of D-amino acid oxidase. When D-DOPA was oxidized by the purified enzyme, however, DOPAC was detected as a main product on TLC, whereas DHPP was present in only small amount as shown in Table I. It was considered that DHPP formed from D-DOPA undergoes immediate oxidative decarboxylation to DOPAC by hydrogen peroxide which is produced as by-product in D-DOPA oxidation by D-amino acid oxidase. In fact, DHPP was detected as a main product when catalase was added to the incubation mixture (Table I). It was also verified in a separate experiment that DHPP is oxidized nonenzymatically to DOPAC easily. The spot of DOPAC was detected on TLC with simultaneous disappearance of DHPP after 30 min oxidation by 3% hydrogen peroxide at room temperature. As will be described later, the rat kidney homogenates contain enough catalase to destroy hydrogen peroxide and oxidative decarboxylation of DHPP to DOPAC by hydrogen peroxide might be prevented.

TABLE I. Incubation Products of D-DOPA-2-¹⁴C with Hog Kidney D-Amino Acid Oxidase with or without Catalase

	DOPAC (%)	DHPP (%)
With catalase	8.73	91.27
Without catalase	75.95	24.05

D-amino acid oxidase: 25 μ g catalase: 500 μ g
 D-DOPA concentration: 1.27×10^{-2} M
 incubation period: 1 hr

Fig. 7. Lineweaver-Burk Plot of D-DOPA Oxidation with Hog Kidney D-Amino Acid Oxidase

A Lineweaver-Burk plot of D-DOPA oxidation by purified D-amino acid oxidase from hog kidney is shown in Fig. 7, and K_m value was obtained as 24 mM.

From these results, it was concluded that the enzyme responsible for the transformation of D-DOPA to DHPP is D-amino acid oxidase.

Transamination of DHPP to L-DOPA by Transaminase

L-DOPA formation from DHPP was subsequently proved. DHPP (1.52×10^{-2} M) was incubated with rat kidney homogenates for 3 hr and the metabolites were separated by TLC and PEP. The main spot was recognized as corresponding to DOPA after color development (Fig. 8).

Because it is impossible to separate D- and L-isomers of DOPA by chromatographic techniques,¹⁴⁾ the identification of the L-isomer was achieved by the reverse isotope dilution method. DHPP-2-¹⁴C was incubated with rat kidney homogenates in the presence of glutamate for 5 hr in an atmosphere of nitrogen gas as described in the experimental section.

After the separation of DOPA fraction by ion exchange chromatography, nonradioactive L-DOPA was added in excess and the recrystallization was repeated. A constant specific

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Fig. 8. Incubation Products of DHPP with Rat Kidney Homogenate

radioactivity of L-DOPA was obtained as shown in Table II. DOPA formed from DHPP was shown to be the L-isomer since there was a good coincidence between the amount of L-DOPA calculated from this method and that of DOPA fraction from TLC method. It was ascertained in the control experiment that radioactive D-DOPA is eliminated completely from the solution in the course of 3 to 4 times recrystallization with an excess of nonradioactive L-DOPA.

TABLE II. Reverse Isotope Dilution Method for L-DOPA Formation from DHPP-2-¹⁴C

Specific radioactivity (dpm/mg) of the crystals				Content of L-DOPA (%)	
1st	2nd	3rd	4th	This method	TLC method
3012	1741	1697	1616	9.68	8.68

Transaminase and Amino Donors to DHPP

The transaminase activity was observed both in the 9000 *g* supernatant and precipitate fractions of the rat kidney homogenates. After the partial purification of supernatant transaminase by ammonium sulphate fractionation, the highest activity was observed in the precipitate at 40–60% saturation (Fig. 9). The transaminase activity in the 9000 *g* precipitate fraction might be located in mitochondria like other transaminases.^{15–17} This is also supported by Gey's work on L-DOPA transaminase in rat brain.¹⁸ The effect of pyridoxal-5'-phosphate (PLP) and dithiothreitol (DTT) on the transamination reaction was examined. As shown in Fig. 10, absence of each component resulted in an appreciable decrease in the enzyme activity. The heat treated homogenate was devoid of the activity.

Several amino acids were examined for ability as an amino donor for DHPP using supernatant transaminase. As shown in Fig. 11-A and 11-B, aspartate, glutamate, tyrosine, tryptophan and phenylalanine were found to be effective amino donors. Phenylalanine and tryptophan (10⁻²M) were particularly effective. Because of a very low solubility of tyrosine, comparison of the ability as an amino donor between glutamate, tyrosine and phenylalanine

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Fig. 9. Partial Purification of Transaminase by Ammonium Sulphate Fractionation

kidneys from four rats were used in the experiment. incubation period: 3 hr

was achieved at a low concentration of $1.11 \times 10^{-3} \text{M}$. As shown in Fig. 11-B, tyrosine was proved to be also an effective amino donor, but not so active as phenylalanine. For the 9000 g precipitate fraction, effective amino donors were almost the same as those for the supernatant transaminase. It was noted, however, that the control experiment showed a relatively high value without addition of any amino acid. This might be caused from free amino acids in mitochondria which can not be eliminated easily by dialysis.

Fig. 10. Effect of PLP and DTT on Transamination of DHPP

—○—: control. glutamate: 10^{-2}M , PLP: 10^{-4}M , DTT: 10^{-3}M , NSD 1055: 10^{-4}M , DHPP: $3.63 \times 10^{-3} \text{M}$
 —△—: -PLP
 —●—: -DTT
 —▲—: Heat treated homogenate was incubated.

Fig. 11-A. Amino Donors to DHPP for Supernatant Transaminase

amino acid concentration: 10^{-2}M

Fig. 11-B. Amino Donors to DHPP for Supernatant Transaminase

amino acid concentration: $1.11 \times 10^{-3} \text{M}$

Comparison of D-DOPA Metabolism between Kidney and Liver

The ability of D-DOPA metabolism was compared between the kidney and the liver. D- and L-DOPA-2- ^{14}C was incubated with rat kidney and liver slices in an atmosphere of 95% O_2 —5% CO_2 gas for 60 min and metabolites were separated by TLC and determined as described in the experimental section. As shown in Table III, no significant differences in the formation of metabolites from L-DOPA was recognized between the kidney and the liver

and both of these organs metabolized L-DOPA rapidly. In both organs, the main metabolites were dopamine, DOPAC and HVA. As to D-DOPA metabolism, on the other hand, a remarkable difference was proved in the metabolic rate of D-DOPA between these organs and it was revealed that the kidney has an extremely high ability to metabolize D-DOPA. The main metabolite was dopamine in both organs, but total amount of the metabolites formed from D-DOPA with kidney slices was about ten fold larger than that with liver slices as shown in Table III.

D-Amino acid oxidase and transaminase were thus compared for their activity between the kidney and the liver homogenates. While the content of D-amino acid oxidase was shown to be much higher in the kidney than that in the liver, there was no significant difference in the activity of transaminase between these two organs as shown in Fig. 12 and 13.

TABLE III. Metabolites from D- and L-DOPA-2-¹⁴C with Rat Kidney and Liver Slices

Metabolites	% of total radioactivity			
	L-DOPA		D-DOPA	
	Kidney	Liver	Kidney	Liver
DOPA	8.73	18.76	66.05	96.70
Dopamine	36.49	23.63	18.04	1.69
3-O-Methyldopamine	3.19	16.93	—	—
DHPP	4.26	2.52	2.75	—
DOPAC	35.94	31.29	9.71	1.61
HVA	11.39	6.87	3.45	—
Sum of the metabolites	91.27	81.24	33.95	3.3

incubation period: 1 hr D-DOPA: $3.69 \times 10^{-4}M$ L-DOPA: $2.52 \times 10^{-4}M$
Organs slices (1 g) were floated in 4 ml of the buffer.

Fig. 12. Content of D-Amino Acid Oxidase in the Liver and Kidney

incubation period: 20 min

Fig. 13. Content of Transaminase in the Liver and Kidney

incubation period: 30 min
amino donor ($10^{-2}M$)

□: phenylalanine
■: glutamate

Thus D-amino acid oxidase must be a key enzyme in D-DOPA metabolism.

Discussions

The following results were obtained in the present study: 1) after the incubation of D-DOPA-2-¹⁴C with rat kidney homogenates at high concentration, DHPP was detected as the main metabolite and dopamine was also recognized as a minor component, indicating that the transformation of D-DOPA to DHPP is involved in the D-DOPA metabolism; 2) both benzoic acid and D-alanine inhibited the oxidation of D-DOPA to DHPP and thus the

participation of D-amino acid oxidase in this transformation was demonstrated; 3) the formation of L-DOPA from DHPP was demonstrated by TLC and PEP and identified by reverse isotope dilution method after the incubation of DHPP with rat kidney homogenates; 4) aspartate, glutamate, tyrosine, tryptophan and phenylalanine were proved to be effective amino donors to DHPP in transamination reaction and 5) the rat kidney slices showed a much higher activity than the liver slices in metabolizing D-DOPA.

Chart 1. Main Metabolic Pathways of D-DOPA in Rat Kidney

It was concluded from these results that D-DOPA is transformed to L-DOPA *via* two steps in the kidney as shown in Chart 1. The first step is the oxidative deamination of D-DOPA to DHPP by D-amino acid oxidase and the second step is the conversion of DHPP to L-DOPA by transaminase. It has been shown in the previous paper³⁾ that dopamine and its metabolites were not detected in any other tissues than the kidney after the administration of D-DOPA-2-¹⁴C to rats. The much higher activity of the rat kidney slices than the liver slices in metabolizing D-DOPA in the present study demonstrated that the metabolites found in the urine and the kidney after administration of D-DOPA-2-¹⁴C to rats are derived from the metabolism in the kidney. The fact that dopamine was the main metabolite and a little DHPP was detected in rat urine after the administration of D-DOPA-2-¹⁴C to rats in the

previous paper agrees well with the present result that DHPP is a minor metabolite while dopamine is the major one when a low concentration of D-DOPA-2-¹⁴C was incubated with rat kidney homogenates or slices.

The result that DHPP was detected as the main metabolite from a high concentration of D-DOPA-2-¹⁴C ($9.05 \times 10^{-3}\text{M}$) is considered to be resulted from the inhibitory effect of DHPP on aromatic amino acid decarboxylase and/or the saturation of transaminase with DHPP formed from D-DOPA. The fact that dopamine was not detected when DHPP of a comparable concentration ($1.52 \times 10^{-2}\text{M}$) was incubated with rat kidney homogenates might support the above consideration because the concentration of DHPP fairly exceeds the K_i value of DHPP on aromatic amino acid decarboxylase ($2.5 \times 10^{-3}\text{M}$).¹²⁾

The following two alternate mechanisms for the formation of L-DOPA from D-DOPA might be improbable; one is the direct racemization of D-DOPA to L-DOPA by racemase and another is the transamination of D-DOPA to DHPP by D-specific transaminase and subsequent retransamination of DHPP to L-DOPA by L-specific enzyme. Both racemase and D-specific transaminase are known to be present in microorganisms but they are not known in animals.¹⁹⁻²⁵⁾ Furthermore, the fact that D-DOPA metabolism was inhibited by benzoic acid is considered to be sufficient to eliminate these possibilities.

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The mechanism of D-DOPA metabolism in the kidney *in vivo* is presumed to proceed in the following way.

- 1) Uptake of D-DOPA into the kidney cells from the blood flows.
- 2) Oxidation of D-DOPA to DHPP by D-amino acid oxidase in peroxisome. The localization of D-amino acid oxidase in peroxisome has been revealed by Duve, *et al.* both in the kidney and the liver. They have also shown that the particle contains catalase.²⁶⁾ Thus, the oxidative decarboxylation of DHPP to DOPAC by hydrogen peroxide might be prevented. In fact, Krebs demonstrated that D-amino acid is transformed to corresponding α -ketoacid by the crude kidney extracts and oxidative decarboxylation of α -ketoacid does not proceed.²⁷⁾
- 3) Transamination of DHPP to L-DOPA by transaminase both in supernatant and mitochondria.
- 4) Decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase in the supernatant. It is known that the enzyme is located in the cytoplasm.²⁸⁾
- 5) Active excretion of dopamine from the renal tubule cells. The active excretion of dopamine was verified in chicken by Sanner.²⁹⁾

It is known that L-DOPA is transaminated by tyrosine aminotransferase (TAT) which occurs in the liver and kidney, and by aromatic amino acid transaminase in the brain.³⁰⁻³²⁾ It can not be deduced whether the L-DOPA formation from DHPP is attributed to the L-DOPA specific enzyme or not because the transaminase was not purified in the present study. Because DHPP is convertible to L-DOPA easily by a transamination reaction and transaminase is distributed widely in many organs, the possibility of DHPP as another precursor of dopamine from the view point of the Parkinsonism therapy is now under investigation in this laboratory.

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