ENZYMATIC DECARBOXYLATION OF L-THREO-3,4-DIHYDROXYPHENYLSERINE IN RAT HEART

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Abstract—Decarboxylation of L-threo-3,4-dihydroxyphenylserine (L-threo-DOPS) by the higher speed supernatant of the rat heart homogenate and the regional distribution of L-threo-DOPS decarboxylase activity were examined. Decarboxylation was demonstrated to occur specifically with L-isomer but not with D-isomer. Addition of pyrogallol was necessary for maximal recovery of norepinephrine. The optimal condition for decarboxylation of L-threo-DOPS by the rat heart enzyme was similar to conditions required with the enzymes from brain and kidney. Under the optimal conditions, K_m and V_{max} for L-threo-DOPS were 2.1 mM and 6.4 nmoles/mg protein/15 min, respectively. Decarboxylation of L-threo-DOPS was markedly inhibited by D-threo-DOPS and D-DOPA. The L-aromatic amino acid decarboxylase activity was highest in the right auricle followed by the atrial body, the left auricle, the right ventricle and the left ventricle.

Studies *in vivo* have demonstrated the decarboxylation of the 3,4-dihydroxyphenylserine (DOPS) to norepinephrine in mammalian tissues including the heart (1-9). The norepinephrine formed from L-threo-DOPS was found to be natural norepinephrine, while L-erythro-DOPS yielded unnatural norepinephrine (10). We have reported the enzymic decarboxylation of the isomers of DOPS using rat kidney and brain (10, 11) and hog kidney (12).

To elucidate the basis of the effect of L-threo-DOPS on the heart, we examined the optimal conditions for decarboxylation of threo-DOPS and the regional enzyme activity required to form norepinephrine from threo-DOPS in the rat heart.

MATERIALS AND METHODS

Enzyme preparation: Male Wistar rats, weighing 200 to 250 g, were decapitated and the hearts removed, weighed and homogenized in 5 volumes of distilled water. For the assay of L-threo-DOPS decarboxylase activity in various regions, the heart was separated into 5, i.e., left auricle, right auricle, atrial body, left ventricle and right ventricle. The right auricle was cut from the atrium on the right side along the inferior and superior vena cava, and the left auricle on the left side along the pulmonary veins from atrium. The homogenates were centrifuged at $8,000 \times g$ for 10 min at 4°C. The supernatant fractions were used as L-aromatic amino acid decarboxylase. Protein concentration of the enzyme was determined by the method of Lowry et al. (13).

Decarboxylation of L-threo-DOPS: Decarboxylation was carried out by the method of Lovenberg et al. (14). The reaction was started by addition of the substrate and stopped by the addition of 4 ml of ice cold 0.4 N perchloric acid with 10 mg of sodium metabisulfite and 200 mg of ethylenediaminetetraacetic acid disodium salt.

Determination of reaction product, norepinephrine: After decarboxylation, the mixture was centrifuged at $5,000\times g$ for 10 min at 4°C . The amount of norepinephrine in the mixture was determined by the method of Bertler et al. (15).

Reagents: Optical isomers of threo-DOPS, L-threo- $([\alpha]_D^{20} = -42.6$ ($c=1$, N-HCl, purity: 99.5%) and D-threo- $([\alpha]_D^{20} = +43.2$ ($c=1$, N-HCl), purity: 99.8%) (Kyowa Hakko Kogyo Co. Ltd., Japan). D-DOPA (Sigma), pargyline (Abbott) and pyridoxal phosphate (Sigma) were used. Other chemicals used were of reagent grade and were not purified further.

RESULTS

Effect of pyrogallol on the recovery of reaction product, norepinephrine

To prevent the decomposition of reaction product by catechol-*o*-methyltransferase, pyrogallol was added to the incubation medium in concentrations ranging from 10^{-9} to 10^{-6} M. The recovery of the reaction product was increased by the addition of pyrogallol and the maximum recovery was observed at 10^{-6} M (Fig. 1).

Time course

When 10^{-3} M L-threo-DOPS was incubated at 37°C in medium containing 125 mM

FIG. 1. Effect of concentration of pyrogallol on decarboxylation of L-threo-DOPS by supernatant fraction of rat heart homogenate. The reaction was carried out at 37°C for 30 min in the presence of 125 mM tris-HCl (pH 8.6), 0.1 mM pyridoxal phosphate, 0.2 mM pargyline, 1 mM L-threo-DOPS, 1 to 3 mg enzyme protein and various concentrations of pyrogallol. Each point represents the mean of two determinations.

FIG. 2. Time course of decarboxylation of L- and D-threo-DOPS by supernatant fraction of rat heart homogenate. Reaction was carried out in the medium containing 125 mM tris-HCl (pH 8.6), 0.1 mM pyridoxal phosphate, 0.2 mM pargyline, 1 μM pyrogallol, 1 mM L- or D-threo-DOPS and 1 to 3 mg enzyme protein at 37°C . Each point represents the mean of two determinations.

tris-HCl buffer (pH 8.6), 0.2 mM pargyline, 0.1 mM pyridoxal phosphate, 1 μ M pyrogallol and 1 to 3 mg of protein of supernatant fraction, the reaction product, norepinephrine increased almost linearly for 45 min. Norepinephrine production was not observed in the incubation medium without the enzyme, even after 30 min of incubation (Fig. 2). On the other hand, norepinephrine was not produced from D-threo-DOPS (Fig. 2).

Enzyme concentrations

Decarboxylation of 10^{-3} M L-threo-DOPS was examined at the concentrations of enzyme protein ranging 0 to 7.8 mg. Norepinephrine production increased linearly in proportion to the enzyme concentration in the incubation medium (Fig. 3).

Temperature dependency

Incubation was performed at 0, 20, 30, 37 and 45°C. The maximum rate of decarboxylation was found at 37°C (Fig. 4-A).

FIG. 3. Effect of concentration of enzyme protein on decarboxylation of L-threo-DOPS by supernatant fraction of rat heart homogenate. The reaction was carried out at 37°C for 30 min in the presence of 125 mM tris-HCl (pH 8.6), 0.1 mM pyridoxal phosphate, 0.2 mM pargyline, 1 μ M pyrogallol, 1 mM L-threo-DOPS and various concentrations of enzyme protein. Each point represents the mean of two determinations.

FIG. 4. Effects of temperature and pH on decarboxylation of L-threo-DOPS by supernatant fraction of rat heart homogenate. A: The reaction was carried out for 30 min in the presence of 125 mM tris-HCl (pH 8.6), 0.1 mM pyridoxal phosphate, 0.2 mM pargyline, 1 μ M pyrogallol, 1 mM L-threo-DOPS and 1 to 3 mg enzyme protein at temperature of 0 to 45°C. Each point represents the mean of two determinations. B: The reaction was carried out at 37°C for 30 min in the presence of 0.1 mM pyridoxal phosphate, 0.2 mM pargyline, 1 μ M pyrogallol, 1 mM L-threo-DOPS, 1 to 3 mg enzyme protein and 125 mM buffer solution of the pH ranging 7.0 to 9.4. pH of incubation medium was adjusted by phosphate buffer (pH 7.0, 7.8 and 8.2) (○ ○ ○) and tris-HCl buffer (pH 8.2, 8.6, 9.0 and 9.4) (● ● ●). Each point represents the mean of two determinations.

pH dependency

pH in the incubation medium was altered using phosphate buffer (pH 7.0, 7.8 and 8.2) and tris-HCl buffer (pH 8.2, 8.6, 9.0 and 9.4). The maximum rate of decarboxylation of 10^{-3} M L-threo-DOPS was observed at pH 8.6 (Fig. 4-B).

Pyridoxal phosphate requirement

Pyridoxal phosphate (PALP) was required as a cofactor of the decarboxylation of L-threo-DOPS. The rate of decarboxylation without PALP was increased by addition of PALP. The maximum rate of decarboxylation was observed at 10^{-4} M PALP (Fig. 5). At the higher concentration of PALP, the rate of decarboxylation was decreased.

K_m and V_{max} for L-threo-DOPS

Under the optimal condition, Michaelis constant (K_m) and maximum velocity (V_{max}) were obtained from Lineweaver-Burk plots of the initial velocity of the decarboxylation of L-threo-DOPS. K_m was 2.1×10^{-3} M and V_{max} was 6.4 nmoles norepinephrine/mg protein/15 min (Fig. 6).

Effect of D-threo-DOPS and D-DOPA on the decarboxylation of L-threo-DOPS

When 10^{-4} , 5×10^{-4} and 10^{-3} M of D-isomer of threo-DOPS were added to the incubation medium, the rate of the L-threo-DOPS decarboxylation was inhibited in a concentration-dependent manner (Fig. 7). D-DOPA at the same concentration also markedly inhibited the rate of L-threo-DOPS decarboxylation (Fig. 7).

FIG. 5. Effect of concentration of pyridoxal phosphate on decarboxylation of L-threo-DOPS by supernatant fraction of rat heart homogenate. The reaction was carried out at 37°C for 30 min in the presence of 125 mM tris-HCl (pH 8.6), 0.2 mM pargyline, 1 μ M pyrogallol, 1 mM L-threo-DOPS, 1 to 3 mg enzyme protein and various concentrations of pyridoxal phosphate. Each point represents the mean of two determinations.

FIG. 6. Lineweaver-Burk plots of the initial rate of L-threo-DOPS decarboxylation by supernatant fraction of rat heart homogenate at 37°C and pH 8.6.

FIG. 7. Inhibitory effect of D-threo-DOPS or D-DOPA on decarboxylation of L-threo-DOPS by supernatant fraction of rat heart homogenate. The reaction was carried out at 37°C for 30 min in the presence of 125 mM tris-HCl (pH 8.6), 0.1 mM pyridoxal phosphate 0.2 mM pargyline, 1 μ M pyrogallol, 1 mM L-threo-DOPS, 1 to 3 mg enzyme protein and various concentrations of D-threo-DOPS or D-DOPA. Each point represents the mean of two determinations.

TABLE 1. Formation of norepinephrine from L-threo-DOPS by L-aromatic amino acid decarboxylase in various regions of rat heart

Regions	Amount of Norepinephrine (nmoles/mg protein/30 min)
Right auricle	0.582 $\pm$ 0.093
Left auricle	0.358 $\pm$ 0.008
Atrial body	0.469 $\pm$ 0.067
Right ventricle	0.374 $\pm$ 0.057
Left ventricle	0.226 $\pm$ 0.098

Each value represents the mean $\pm$ S.E. of 3-4 determinations. All regions from 10 animals were used for each determination. The heart was separated into 5 regions, i.e., left auricle, right auricle, atrial body, left ventricle and right ventricle. The reaction was carried out at 37°C in 2 ml of medium consisting of 125 mM tris-HCl (pH 8.6), 10⁻⁴ M pyridoxal phosphate, 10⁻⁶ M pyrogallol, 2 $\times$ 10⁻⁴ M pargyline, 1 to 3 mg of protein of the supernatant fraction and in the presence or absence of 10⁻³ M L-threo-DOPS.

The formation of norepinephrine from L-threo-DOPS by L-aromatic amino acid decarboxylase in various regions of rat heart

The heart was separated into five regions, i.e., left auricle, right auricle, atrial body, left ventricle and right ventricle. Norepinephrine formation from L-threo-DOPS was more extensive in the atrium than in the ventricle and the formation in both the atrium and the ventricle was higher in the right than in the left side (Table 1).

DISCUSSION

The formation of norepinephrine occurred when rat heart enzyme was incubated with L-threo-DOPS, but was not observed after the incubation with D-threo-DOPS. The optimal conditions for the L-threo-DOPS decarboxylation were examined.

The heart enzyme activity for the L-threo-DOPS decarboxylation was assayed using the

incubation medium with monoamine oxidase inhibitor as described previously (10, 12), however, the lowest recovery of reaction product, norepinephrine was obtained in the heart preparation as compared with kidney and brain preparations. The addition of pyrogallol, catechol-*o*-methyltransferase (COMT) inhibitor, resulted in an increase in the recovery of norepinephrine formed from L-threo-DOPS, suggesting the presence of COMT intermingled in the $8,000 \times g$ supernatant of heart homogenate which was used as the crude decarboxylase preparation.

The maximal rate of the L-threo-DOPS decarboxylation was obtained at pH 8.6 and 37°C. The optimal pH and temperature obtained here were similar to those in our previous studies on rat kidney and brain (10, 11) and hog kidney (12). A longer incubation time and larger amount of enzyme protein were required for the assay of L-threo-DOPS decarboxylation by heart enzyme as compared with the kidney and brain enzymes (10, 11) because of the lower activity of L-aromatic amino acid decarboxylase in the heart.

The maximum formation of norepinephrine from L-threo-DOPS by the rat heart decarboxylase was obtained by adding 10^{-4} M PALP. The increased concentration of PALP, however, inhibited the norepinephrine formation. This inhibition is presumably due to the formation of complex of PALP with the substrate as described in the previous reports (11, 12).

Decarboxylation of L-threo-DOPS by the rat heart enzyme was inhibited in a concentration-dependent manner by D-DOPA and D-threo-DOPS. This inhibition of L-threo-DOPS decarboxylation by D-amino acid is attributed to the interaction with the enzyme as described previously (10–12).

K_m of the heart enzyme for L-threo-DOPS was 2.1 mM which is quite similar to K_m of the brain enzyme (1.43 mM) and K_m of the kidney enzyme (1.3 mM) (10, 11). K_m values of L-aromatic amino acid decarboxylase for various substrates have been reported in the guinea pig (14). K_m of the heart enzyme for L-threo-DOPS in our study herein was of the same order as that for tryptophan (3 mM) and larger than those for DOPA (0.4 mM) and 5-hydroxytryptophan (0.02 mM). On the other hand, the maximum velocity of decarboxylation of L-threo-DOPS by the heart enzyme was smaller than that by the rat kidney enzyme (10). These results suggest that the affinity of L-threo-DOPS for the heart enzyme is of the same grade but the rate of L-threo-DOPS decarboxylation is lower than in the case of rat kidney and brain (10, 11).

The formation of norepinephrine from L-threo-DOPS with the enzyme from the atria was more extensive than that with the enzyme from the ventricle and formations in both atria and ventricle were greater on the right side than on the left. These regional distributions of L-threo-DOPS decarboxylase activity are closely correlated to those of the heart norepinephrine contents in the rat, guinea pig, rabbit, cat (16) and dog (17), and to the adrenergic innervation in the guinea pig and rabbit (18).

We reported elsewhere that L-threo-DOPS produced an increase in atrial rate, and that the positive chronotropic effect of L-threo-DOPS was inhibited in the atria from rats treated with benserazide (19, 20). Our results obtained herein may provide a biochemical basis for

the effect of L-threo-DOPS on the cardiac functions.

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