The Enzymatic Decarboxylation of DOPA in Human Liver Homogenates[1,2] (34816)

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(Introduced by J. M. Coon)

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The enzymatic decarboxylation of dihydroxyphenylalanine (DOPA) and other aromatic amino acids has been demonstrated in many animal tissues including the brain (1–3); and, the enzyme aromatic amino acid decarboxylase has been isolated and purified from mammalian liver and kidney (4–6). In contrast, a study of this enzyme in various human tissues revealed activities which were low when compared to activities found in most laboratory animals (7). Studies of the enzymatic decarboxylation in human brain, except for a single report (8) to the contrary, have indicated that the activity of this enzyme is either absent or present at only extremely low levels in this tissue (7, 10–12). Thus, the enzymatic decarboxylation in human tissues seems to differ either qualitatively or quantitatively from that in animal tissues.

Since this enzyme is considered to play an important role in the biosynthesis of various biogenic amines such as dopamine, norepinephrine, and serotonin, we have studied the decarboxylation of DOPA in human liver homogenates, and also the effects of various drugs and metabolites on the enzyme activity. Liver was selected for these studies because we had previously found demonstrable enzyme activities in this tissue, whereas, the enzymatic activity, in human brain homogenates was not sufficient for quantitative investigations (7).

Materials and Methods. Human liver samples were obtained between 6 and 11 hr after death from males and females, 39–65 years of age, who had died of coronary occlusion. The tissue samples were frozen and stored at —73° for periods of 1–7 weeks. The use of autopsy material seems justified since previous studies indicated that the animal enzyme was quite stable postmortem (7, 9).

The radioactive substrate, DL-DOPA-1-^{14}C, was obtained from New England Nuclear Corp., Boston, Mass. Paper chromatography of the substrate using butanol:acetic acid: water 25:4:10 indicated only a single radioactive component corresponding to DOPA.

EDTA, *p*-chloromercuribenzoate, DOPA, α-methyl-DOPA, norepinephrine, 5-hydroxytryptophan, 5-hydroxytryptamine, phenylalanine, phenylpyruvic acid and pyridoxal-5′-phosphate were obtained from Sigma Chemical Co., St. Louis, Mo. Chlorpromazine was obtained through the courtesy of Smith, Kline and French, Inc., Philadelphia, Pa., and α-methyl tyrosine was purchased from Regis Chemical Co., Chicago, Ill.

Decarboxylation of DOPA was determined by measuring the formation of $^{14}CO_2$ (7). A typical enzyme assay contained 25 mg of liver, 5×10^{-5} *M* D,L-DOPA-1-^{14}C (2.4×10^6 dpm/μmole), 5×10^{-5} *M* pyridoxal-5′-phosphate (B_6), 0.1 *M* phosphate pH 7.5 buffer, and additions, if specified, in a total volume of 1.7 ml. Liberated $^{14}CO_2$ was collected in 5 *N* NaOH, and an aliquot of this solution was counted in a Packard liquid-scintillation counter. Activities of rat liver homogenates determined by this method were similar to values reported for this tissue in the literature (10). Enzyme activities were calculated by difference between "fresh" homogenates and aliquots which had been quickly brought to a boil and then allowed to cool. Since most data in the literature are based on wet weight, values in this study are

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[2] A preliminary report of this study has been published in Fed. Proc. 28, 544 (1969).

TABLE I. Effect of Various Substances on Human Liver Aromatic Amino Acid Decarboxylase Activity.[a]

Addition	Molar concentration resulting in 50% inhibition of enzyme activity
p-Chloromercuribenzoate	3×10^{-5}
EDTA[b]	$>1 \times 10^{-3}$
Dopamine	7×10^{-5}
5-Hydroxytryptamine	1×10^{-4}
Norepinephrine	2×10^{-3}
5-Hydroxytryptophan	4×10^{-4}
Phenylalanine	8×10^{-4}
Phenylpyruvic acid[c]	5×10^{-6}
Phenylpyruvic acid[c]	2×10^{-4}
α-Methyl tyrosine	3×10^{-3}
α-Methyl DOPA[b]	$>1 \times 10^{-3}$
Chlorpromazine[b]	$>1 \times 10^{-3}$

[a] All experiments were done in duplicate on liver samples obtained from at least two different individuals. Activities of the uninhibited reaction ranged between 250 and 400 mμmoles/g/hr.

[b] No inhibition was seen at the concentration of 1×10^{-3} *M* although higher concentrations were slightly inhibitory.

[c] Liver homogenates obtained from two individuals showed high sensitivity to phenylpyruvic acid; homogenates from the livers of two other individuals were much less sensitive.

reported on the same basis for purposes of control and comparison.

Results. The addition of B_6 to human liver homogenates was necessary for optimal enzyme activity. Omission of B_6 decreased the activity by approximately 60%. In rat liver homogenates, the corresponding decrease was approximately 25%.

Under the conditions of the enzyme assay, the rate of DOPA decarboxylation was constant for 90 min. Enzymatic DOPA decarboxylation showed a linear relationship to DOPA concentrations over the range from 1.5×10^{-5} to 7.5×10^{-5} *M*. The pH optimum of the reaction was found to be between 7 and 8. Below pH 6 and above pH 9 the rate of decarboxylation decreased markedly.

The effects of various chemicals, metabolites, and drugs on the enzymatic decarboxylation of DOPA are shown in Table I. The sulfhydryl group inhibitor *p*-chloromercuribenzoate inhibited the reaction, whereas, the chelating agent EDTA was without effect. Of the amines tested, dopamine, the decarboxylation product of DOPA, and 5-hydroxytryptamine (serotonin) inhibited the reaction at much lower concentrations than did norepinephrine. In animals, DOPA and 5-hydroxytryptophan (5-HTP) are thought to be decarboxylated by the same enzyme (3). 5-HTP also inhibited the enzymatic decarboxylation of DOPA in the human liver preparations, and a Lineweaver-Burk type analysis indicated that the inhibition was competitive. Phenylpyruvic acid proved to be a powerful inhibitor when added to livers from two of the autopsies, but a weak inhibitor in the case of the livers from two other autopsies. Chlorpromazine, phenylalanine, α-methyl-DOPA, and α-methyl-tyrosine produced either no inhibition or inhibited the reaction only at high concentrations.

Table II shows the decarboxylation of DOPA as a function of various liver homogenate concentrations. A reduction of the "fresh" tissue concentration from 100 to 2.5 mg/vessel produced a linear decrease in the decarboxylation of DOPA as would be expected of an enzymatic reaction. However, a further reduction of tissue below 2.5 mg/vessel resulted in increased DOPA decarboxylation and the highest value was obtained when the tissue was completely omitted from the incubation mixture. When boiled homogenate was provided in place of the fresh homo-

TABLE II. Decarboxylation of DOPA as Function of the Liver Concentration in the Incubation Vessel.

Tissue concentration (mg/vessel)	CO_2 formation (mμmoles/vessel/hr)[a]	
	Fresh	Boiled
100	8.8	0
50	4.4	0
25	2.4	0
2.5	0.2	—
0.25	0.8	3.5
0.125	2.1	—
0.025	—	11.0
0	15.2	15.2

[a] Values represent the means of duplicates of liver samples obtained from two autopsies.

TABLE III. The Effects of Dialysis on the Decarboxylation of DOPA.

Conditions[a]	$^{14}CO_2$ formed[b] (mμmoles/vessel/hr)
Homogenate before dialysis	0.8
Homogenate after dialysis	3.2
Boiled homogenate before dialysis	0.0
Boiled homogenate after dialysis	1.2

[a] One-half the homogenate was kept in the refrigerator overnight while the second half was dialyzed against 20 vol of phosphate buffer.

[b] Values represent the means of duplicates of liver samples obtained from two autopsies.

genate, no decarboxylation of DOPA was observed at concentrations of boiled homogenate above 2.5 mg/vessel. However, a decrease in the amount of boiled homogenate below 2.5 mg/vessel again resulted in increased DOPA decarboxylation, and the reaction rate was highest in the absence of boiled tissue. These results show that DOPA decarboxylation can occur by both a nonenzymatic and an enzymatic reaction and that the nonenzymatic reaction is inhibited in the presence of fresh or boiled homogenate.

The effects of dialysis on the decarboxylation of DOPA in fresh and boiled homogenates is shown in Table III. In these experiments, one-half of each of the homogenates was stored in the refrigerator overnight while the second half was placed in a dialysis tube and dialyzed against 20 vol of 0.1 *M* phosphate buffer, pH 7.4, for the same period of time. In both cases, dialysis increased the CO_2 formation from DOPA.

The buffer outside the dialysis tube (diffusate) was concentrated 10-fold after dialysis and 1 ml was added to vessels containing DOPA but no tissue. The addition of this diffusate reduced the nonenzymatic decarboxylation of the amino acid to 50% of the value obtained with similarly concentrated buffer which had not been involved in the dialysis. In contrast, DOPA decarboxylation in "fresh" tissue homogenates was not affected by the presence of the same amount of diffusate or by the addition of boiled homogenate (100 mg). These results suggest that a small molecule is present in fresh and boiled homogenates which interferes with the nonenzymatic decarboxylation of DOPA.

Discussion. Under the conditions employed DOPA can undergo an enzymatic and a nonenzymatic decarboxylation reaction. The nonenzymatic reaction proceeds in the absence of liver homogenate. In the presence of tissue an enzyme-mediated reaction takes place, while the nonenzymatic reaction is inhibited by an endogenous, heat-stable, diffusable molecule.

Based on these observations the nonenzymatic reaction has been studied in some detail, and the results have been published separately (13). Although it is known that amino acids decarboxylate nonenzymatically, these reactions usually require B_6, certain metal ions, and/or elevated temperatures (14). In contrast, DOPA decarboxylation was found to proceed in the absence of B_6 and at room temperature. The pH optimum of the reaction was between 7 and 8. The reaction rate was decreased by the addition of 5-HTP and increased in the presence of dopamine, norepinephrine, and certain metal ions (13).

The enzymatic decarboxylation of DOPA and the effects of various metabolites and drugs on this reaction have been further investigated in this study. Enzymatic DOPA decarboxylation in human liver homogenates was found to be similar to that observed with the purified rat liver and guinea pig kidney enzymes (4–6). All three enzymes exhibit similar pH maxima, require B_6, are inhibited by low concentrations of *p*-chloromercuribenzoate, and are not affected by EDTA.

Both DOPA and 5-HTP are decarboxylated by the same enzyme in animal tissues (3). The competitive inhibition of DOPA decarboxylation by 5-HTP in human liver homogenates suggests that humans, like animals, may utilize a single enzyme for the decarboxylation of both of these aromatic amino acids. The inhibition of DOPA decarboxylation by dopamine and serotonin, the products of the enzymatic decarboxylation of DOPA and 5-HTP, might be indicative of a regulation of the human enzyme by its products. α-Methyl DOPA, which inhibits the pig kidney enzyme but not the bacterial en-

zyme (15), had no effect on the human enzyme.

The reduction of DOPA decarboxylation in human liver homogenates by phenylpyruvic acid is of interest since the inhibition of aromatic amino acid decarboxylase by phenylpyruvic acid has been implicated in the pathogenesis of phenylketonuria (PKU) (16). This hypothesis was subsequently rejected because phenylalanine derivatives are inhibitory to the animal enzyme at concentrations of approximately 2×10^{-2} *M* whereas the concentration encountered in phenylketonuria is approximately 2×10^{-3} *M* (17). Our results obtained with the human enzyme show that concentrations encountered *in vivo* in phenylketonuria could interfere with the decarboxylation of DOPA *in vitro,* and thus could indeed implicate decarboxylase inhibition in the pathogenesis of phenylketonuria. Furthermore, the enzymes in human livers from different individuals did not exhibit the same sensitivity to phenylpyruvic acid. DOPA decarboxylation in homogenates obtained from two different livers was inhibited 50% by 2×10^{-4} *M*; whereas, the same inhibition was observed by 5×10^{-6} *M* in homogenates obtained from two other human livers. Although many factors, such as diseases or drug intake, could have been responsible for this finding, the possibility that there are at least two types of human aromatic amino acid decarboxylase has to be considered. Pseudocholinesterase is an example of an enzyme with multiple forms (18). The enzymatic transformation of succinylcholine is inhibited by low concentrations of dibucaine in some individuals; whereas, higher concentrations of the chemical are needed to produce the same inhibition in other individuals. The possibility that aromatic amino acid decarboxylase exists in two forms with different sensitivities to phenylpyruvic acid could then explain the observation that not all individuals with high phenylalanine or phenylpyruvic acid levels show the classical PKU syndrome. If decarboxylase inhibition plays a major role in PKU, only individuals with the sensitive enzyme would be expected to suffer from a reduction in DOPA decarboxylation; whereas, individuals with the "insensitive" enzyme might be unaffected by the increased levels of phenylpyruvic acid.

Summary. DOPA decarboxylation occurred enzymatically and nonenzymatically. In the absence of tissue the reaction proceeded nonenzymatically. In the presence of liver tissue an enzymatic reaction took place while the nonenzymatic reaction appeared to be inhibited by a dialyzable compound in the liver homogenate. The enzymatic decarboxylation of DOPA was extremely slow when compared with similar preparations from animal tissues. The pH optimum was between 7 and 8. The reaction was inhibited by *p*-chloromercuribenzoate, dopamine, 5-hydroxytryptamine, and competitively by 5-hydroxytryptophan. Phenylpyruvic acid strongly inhibited the reaction in homogenates of livers obtained at two autopsies but proved to be a weaker inhibitor in the case of homogenates of liver obtained at two other autopsies. Norepinephrine, EDTA, chlorpromazine, α-methyl DOPA and phenylalanine did not markedly influence the enzymatic DOPA decarboxylation.

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