Brit. J. Pharmacol. (1962), **18**, 175–182.

OBSERVATIONS ON THE SUBSTRATE SPECIFICITY OF DOPA DECARBOXYLASE FROM OX ADRENAL MEDULLA, HUMAN PHAEOCHROMOCYTOMA AND HUMAN ARGENTAFFINOMA

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(*Received December* 8, 1961)

The substrate specificity of the dopa decarboxylases of ox adrenal medulla, human phaeochromocytoma and human argentaffinoma have been studied. The enzymes from all three tissues decarboxylated dopa, metatyrosine, orthotyrosine and 5-hydroxytryptophan. Dopa was decarboxylated most rapidly and 5-hydroxytryptophan least rapidly by the enzyme from all three tissues. Competition experiments indicate that all four substrates are decarboxylated by the one enzyme. Attempts to demonstrate decarboxylation of [C^{14}]-tyrosine, [C^{14}]-tryptophan or [C^{14}]-histidine by these enzyme preparations were unsuccessful.

The enzyme, dopa decarboxylase, was discovered in kidney tissue by Holtz, Heise & Lüdtke in 1938. Subsequently some of the properties of dopa decarboxylases from different sources were investigated and some general principles regarding the substrate specificity of the enzymes were formulated (Blaschko, 1950). The requirements for activity of the mammalian enzyme were that the amino acid should be of the L configuration, the amino group should be unsubstituted and that there should be a hydroxyl group either in the 2 or 3 position on the ring. The absence of activity towards tyrosine was established by Blaschko, Holton & Sloane Stanley (1949) and by Blaschko & Holton (1950). The importance of the ring *m*-hydroxyl group in the enzymic reaction was further demonstrated by Sourkes, Heneage & Trano (1952), who reported that if the hydroxyl group of metatyrosine were blocked by methyl substitution the resulting compound was not a substrate.

Already, in 1939, Blaschko had proposed a physiological role for dopa decarboxylase as the catalyst of one of the steps in the conversion of tyrosine to adrenaline. This suggestion gained support from Langemann's demonstration (1951) of dopa decarboxylase in adrenal medulla. Subsequently this enzyme was shown to be localized in the non-particulate cytoplasm of adrenal medulla tissue (Blaschko, Hagen & Welch, 1955) and its role in noradrenaline biosynthesis substantiated with the demonstration of the conversion of dopa and of dopamine to noradrenaline (Demis, Blaschko & Welch, 1956 ; Hagen, 1956).

In 1953 Udenfriend, Clark & Titus reported an enzyme in kidney preparations which decarboxylated 5-hydroxytryptophan but not tryptophan. That both

5-hydroxytryptophan decarboxylase and dopa decarboxylase might be one and the same enzyme was suggested by Langemann (1958) when he found both activities together in a human argentaffin cell tumour. Mouse mastocytoma also contains dopa, 5-hydroxytryptophan and histidine decarboxylase activities (Hagen, Weiner, Ono & Lee, 1960). More recently Udenfriend, Lovenberg & Weissbach (1960) have postulated that there is only a single aromatic L-amino acid decarboxylase which acts on 5-hydroxytryptophan, dopa, tryptophan, phenylalanine, tyrosine and histidine.

This paper reports studies on the substrate specificity of dopa decarboxylase (5-hydroxytryptophan decarboxylase) prepared from ox adrenal medulla, human phaeochromocytoma and human argentaffinoma. In these studies DL-orthotyrosine (*o*-hydroxyphenyl-DL-alanine), DL-metatyrosine (*m*-hydroxyphenyl-DL-alanine), L-tyrosine, L-dopa, L-tryptophan, DL-5-hydroxytryptophan, L-histidine, and L-tyrosine were the aromatic amino acids investigated in manometric studies of the enzyme activity. In addition, C^{14}-labelled L-tryptophan, L-histidine, DL-5-hydroxytryptophan and L-tyrosine were also incubated with the enzyme and the corresponding amines sought by isotope dilution and autoradiography. These three tissues produce and store pharmacologically active amines, and the decarboxylases are known to catalyse an important step in amine formation. Because the amount of the enzyme present in homogenates of adrenal medulla, phaeochromocytoma and argentaffinoma was insufficient for these manometric studies, it was necessary to partially purify and concentrate the enzyme from a large amount of the tissue cytoplasm in order to detect activity towards some of the substrates.

In the course of this work two other accounts of the purification of dopa decarboxylase have appeared, one from kidney (Werle & Aures, 1959) and one from adrenal medulla (Fellman, 1959).

METHODS

Source of tissue. Adrenal glands were removed from oxen as soon as possible after death and placed on ice for transport to the laboratory, where the medulla was immediately dissected free of cortex. The phaeochromocytoma and the argentaffinoma were surgically removed from human patients and immediately placed on ice for transport to the laboratory.

Preparation of the enzyme. All manipulations were carried out in a cold room at a temperature of between 1° and 3° C. The tissue was finely cut up and then homogenized in 0.4 M sucrose containing 0.02 M sodium versenate. The volume of the sucrose solution added was adjusted to make a one-in-five homogenate. Centrifugation at 100,000×*g* for 1 hr yielded a dense sediment and a clear supernatant with a thin layer of fat on top. The sediment was set aside for other experiments. Measured volumes of a neutral saturated solution of ammonium sulphate were added to the supernatant to give a 10%, 20%, 30%, 40%, 45%, 50% and 55% saturation. After each addition of ammonium sulphate the precipitate was removed by centrifugation before the addition of further ammonium sulphate solution. In this way seven precipitates were obtained. Each precipitate was resuspended in 0.02 M sodium phosphate buffer of *p*H 6.5 containing 1.0 M mercaptoethanol, and the volume made up to 10 ml. with this buffer. These resuspended precipitates will be referred to as the 10%, 20%, 30%, 40%, 45%, 50% and 55% ammonium sulphate fractions.

The enzymic activities were measured manometrically. Either 0.5 or 1.0 ml. of enzyme fraction was placed in the main compartment of the manometer flask together with 1 ml. of 0.2 M phosphate buffer of *p*H 6.5 and 100 μg pyridoxal-5-phosphate. Ten mg of amino acid was placed in the side arm. The flasks were gassed with nitrogen and incubated at

37° C. Manometer readings were taken before tipping and then at frequent intervals over a 20-min period. In preliminary experiments the reaction was stopped by tipping in perchloric acid from a second side arm and the further changes in manometric reading due to the additional carbon dioxide output were recorded. However, such an insignificant evolution of carbon dioxide occurred on adding acid that this step was dispensed with in the experiments reported in this paper.

Firstly, the dopa decarboxylase activities of the different ammonium sulphate fractions were determined and the most active fraction, either the 45%, 50% or 55%, used for the substrate specificity studies. As different amounts of tissue were used in different experiments, the enzyme activities are reported as μl. carbon dioxide formed/10 g tissue/hr. Thus, if 100 g tissue was used, and if the 45% fraction was the most active, and if 1 ml. of that fraction (total volume of each fraction was 10 ml.) liberated 50 μl. in 30 min, then the activity of that fraction would be expressed as 100 μl./10 g/hr.

Detection of tyrosine, tryptophan and 5-hydroxytryptophan decarboxylase activities was also attempted in a 1 hr incubation of these amino acids labelled with C^{14} with enzyme fractions that were highly active in decarboxylating dopa, 5-hydroxytryptophan, metatyrosine and orthotyrosine. The incubation was carried out in Warburg flasks as described above and was terminated by the addition of 5N perchloric acid. 0.4 μM of the non-radioactive amine, tyramine, tryptamine or histamine, according to the [C^{14}] amino acid used in the incubation, was added to each flask, and the protein removed by centrifugation. The supernatant was neutralized with potassium hydroxide and the potassium perchlorate removed by centrifugation. The supernatant solution was then passed through a column of Amberlite XE 64 carboxylic ion exchange resin in the ammonium form. The radioactive amino acids passed through the column, which was then washed with water. The amines were eluted with N acetic acid. The eluate was evaporated to a small volume (about 0.2 ml.) and a portion of it used for paper chromatography.

Ascending paper chromatography was carried out with Whatman no. 1 filter paper and either butanol acetic acid or isopropanol ammonia solvent. When the solvent had travelled about 20 cm the paper was removed from the tank and dried for 24 hr. The amines were located with ninhydrin and the ninhydrin spots fixed with cupric nitrate. For autoradiography each sheet of paper was marked in two places with radioactive ink, placed against *x*-ray film and left in darkness. After a month the *x*-ray film was developed. Black marks on the film indicated the location of radioactivity on the paper chromatogram.

RESULTS

Table 1 shows the rates of decarboxylation of dopa and 5-hydroxytryptophan by different fractions prepared by ammonium sulphate treatment of the 100,000 × *g*

TABLE 1

RATES OF DECARBOXYLATION OF DOPA AND 5-HYDROXYTRYPTOPHAN BY FRACTIONS PREPARED BY TREATING THE PARTICLE-FREE SUPERNATANT OF AN ADRENAL MEDULLA HOMOGENATE WITH DIFFERENT CONCENTRATIONS OF AMMONIUM SULPHATE

The values are μl. carbon dioxide evolved/ml. of fraction hr (measured over a 15-min period). The protein content is in mg/ml. of each fraction. The percentage values in brackets indicate relative rate of decarboxylation of 5-hydroxytryptophan compared with the rate of decarboxylation of dopa (100%)

Fraction	Dopa	5-Hydroxy-tryptophan	Protein content mg/ml.
40%	396	64 (16%)	101
45%	848	140 (17·5%)	33
50%	448	88 (20%)	30
55%	40	8 (20%)	—

supernatant of an adrenal medulla homogenate. The rate of decarboxylation of 5-hydroxytryptophan is between 16% and 20% of the rate of decarboxylation of dopa in all four fractions.

Table 2 shows the rates at which dopa, 5-hydroxytryptophan, metatyrosine, and orthotyrosine are decarboxylated by dopa decarboxylase prepared from bovine

TABLE 2

THE ACTIVITIES OF THE MOST ACTIVE AMMONIUM SULPHATE FRACTIONS PREPARED FROM HOMOGENATES OF FOUR BOVINE ADRENAL MEDULLAE, OF A PHAEOCHROMOCYTOMA, AND AN ARGENTAFFINOMA

The values are a μl. carbon dioxide/hr/10 g tissue from which the fraction was derived. The percentage values in brackets indicate the relative rate of decarboxylation of the different amino acids compared with the rate of decarboxylation of dopa (100%)

Fraction	Dopa	Meta-tyrosine	Ortho-tyrosine	5-Hydroxy-tryptophan
		Ox adrenal medulla		
55%	1,000	840 (84%)	465 (47%)	240 (24%)
55%	980	750 (77%)	420 (43%)	180 (19%)
45%	780	570 (73%)	390 (50%)	105 (13%)
50%	600	510 (84%)	270 (45%)	90 (15%)
		Human phaeochromocytoma		
55%	736	457 (62%)	265 (36%)	132 (18%)
		Human argentoffinoma		
55%	1,782	590 (33%)	382 (21%)	212 (12%)

adrenal medulla, human phaeochromocytoma and human argentaffinoma. Of these four amino acids, dopa was decarboxylated most rapidly, then followed in order: metatyrosine, orthotyrosine, and 5-hydroxytryptophan. The relative rates of decarboxylation of these amino acids were fairly consistent from one preparation to another of the ox adrenal medulla enzyme. Although the human phaeochromocytoma enzyme decarboxylated the dopa, metatyrosine, orthotyrosine, and 5-hydroxytryptophan at rates comparable to those of ox adrenal medulla, the argentaffinoma decarboxylated dopa relatively much more rapidly than it decarboxylated the other three amino acids.

The results of competition experiments are shown in Figs. 1, 2, 3 and 4. These indicate that with the enzyme from all three tissues 5-hydroxytryptophan competes with dopa and 5-hydroxytryptophan also competes with metatyrosine. The reduction in the rate of decarboxylation of dopa caused by the 5-hydroxytryptophan was less with the human argentaffinoma preparation than with the ox adrenal medulla or with the human phaeochromocytoma preparations.

Similarly, in an experiment with bovine adrenal medulla homogenate the rate of carbon dioxide formation with dopa and orthotyrosine was competitive and not additive.

No carbon dioxide evolution could be detected when either tyrosine, tryptophan or histidine was incubated with the enzyme preparations, and no reduction in the rates of decarboxylation of dopa or 5-hydroxytryptophan could be observed by the addition of tyrosine, tryptophan or histidine to the incubation medium.

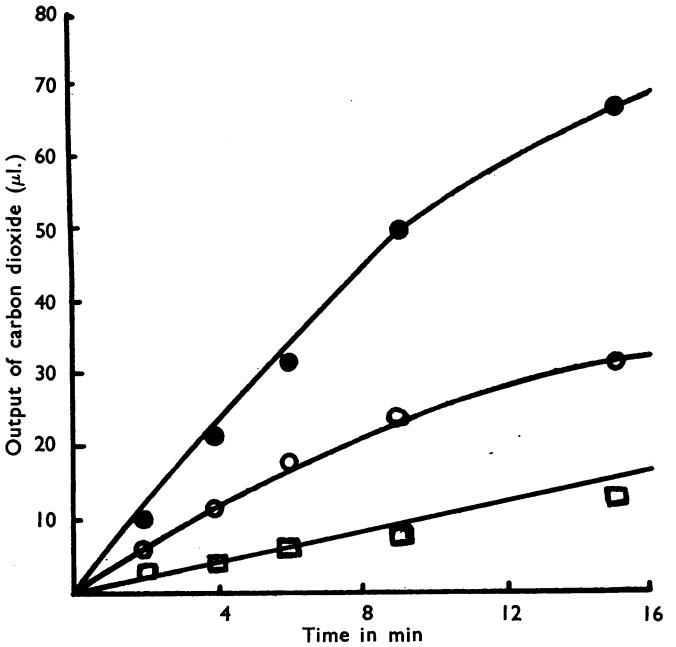

Fig. 1. The rates of decarboxylation of dopa (●), of 5-hydroxytryptophan (□), and of both amino acids together (○) by bovine adrenal medulla decarboxylase.

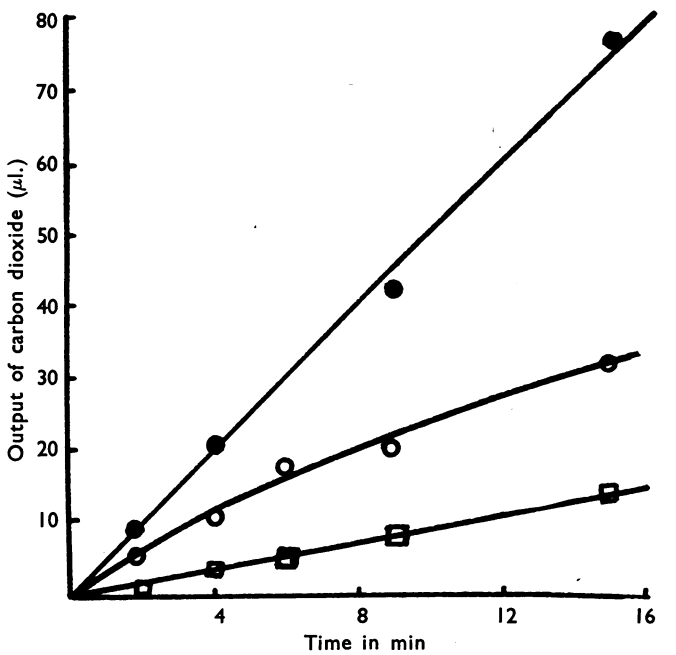

Fig. 2. The rates of decarboxylation of metatyrosine (●), of 5-hydroxytryptophan (□), and of metatyrosine and 5-hydroxytryptophan together (○) by bovine adrenal medulla decarboxylase.

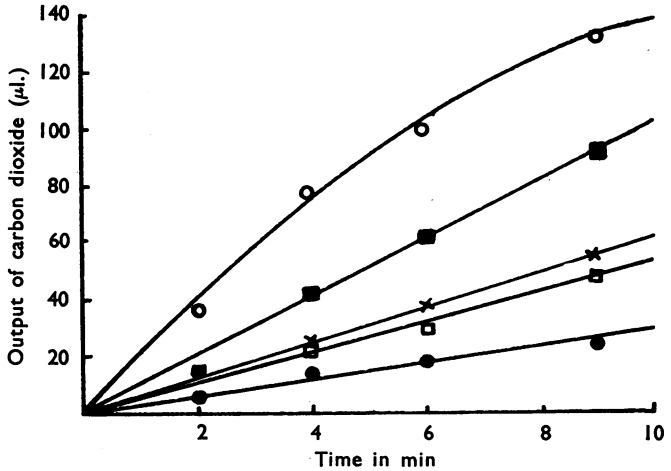

Fig. 3. The rates of decarboxylation of dopa (○), of metatyrosine (■), of orthotyrosine (X), of metatyrosine and 5-hydroxytryptophan (□), and of 5-hydroxytryptophan (●) by human phaeochromocytoma decarboxylase.

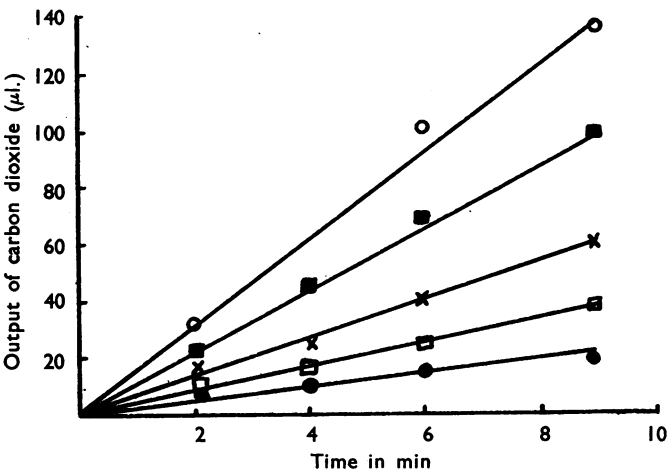

Fig. 4. The rate of decarboxylation of dopa (○), dopa and 5-hydroxytryptophan (■), metatyrosine (X), metatyrosine and 5-hydroxytryptophan (□), and 5-hydroxytryptophan (●) by human argentaffinoma decarboxylase.

In the experiments in which the enzyme from all three tissues was incubated with [C^{14}]-5-hydroxytryptophan, tyrosine, tryptophan or histidine, and non-radioactive 5-hydroxytryptamine, tyramine, tryptamine or histamine added to the acidified incubation medium after the incubation, clear ninhydrin staining spots due to the amines could be observed on the paper chromatograms. However, radio-activity, as indicated by blackening of the *x*-ray film, was observed only in association with the 5-hydroxytryptamine spot. No trace of radioactivity was observed in association with the histamine, tyramine or tryptamine spots. Some unidentified

areas of radioactivity were present on the papers, but these did not correspond to identifiable amines or amino acids. Thus, no decarboxylation of tyrosine, tryptophan or histidine could be detected.

DISCUSSION

These experiments indicate that the three amine-producing and -storing tissues, ox adrenal medulla, human phaeochromocytoma and human argentaffinoma, contain an amino acid decarboxylase, which can decarboxylate dopa, metatyrosine, orthotyrosine and 5-hydroxytryptophan, but which is inactive towards tyrosine, tryptophan and histidine. The rates of decarboxylation of the four different amino acids are of the same relative order in all three tissues. Dopa is decarboxylated most rapidly, 5-hydroxytryptophan least rapidly, metatyrosine is decarboxylated more rapidly than orthotyrosine. It is noteworthy that the relative rates of decarboxylation of dopa and 5-hydroxytryptophan are different from those reported for the decarboxylase of mouse mastocytoma. In preparations of the latter tissue the rate of decarboxylation of 5-hydroxytryptophan was between 25% and 50% of the rate of decarboxylation of dopa (Hagen *et al.*, 1960), whereas in the preparations reported in this paper 5-hydroxytryptophan was decarboxylated at between 10% and 25% of the rate for dopa.

Further support for the idea that the same enzyme is involved in the decarboxylation of dopa and 5-hydroxytryptophan is indicated by the fairly constant ratios of the rates of carbon dioxide evolution from these two amino acids by the different ammonium sulphate fractions in a single experiment. Additional evidence that one enzyme is involved is provided by the competition experiments, in which the rate of carbon dioxide evolution from dopa was reduced by the addition of 5-hydroxytryptophan. That 5-hydroxytryptophan was less effective in reducing the decarboxylation of dopa by the argentaffinoma enzyme suggests that the argentaffinoma enzyme has a lower affinity for 5-hydroxytryptophan than the enzyme derived from bovine adrenal medulla or from human phaeochromocytoma tissue. Whether a low affinity for 5-hydroxytryptophan is a characteristic of dopa decarboxylases of argentaffin tissue in general, or just of this particular tumour, can only be determined by examining the enzymes from more argentaffinomas as they become available.

The inability of this enzyme to decarboxylate L-histidine, L-tyrosine or L-tryptophan is at variance with the claim (Udenfriend *et al.*, 1960) that a single aromatic L-amino acid decarboxylase can act on dopa, 5-hydroxytryptophan, tryptophan, tyrosine and histidine, as is also the report of an enzyme in human urticaria pigmentosa tissue which decarboxylates histidine but is unable to decarboxylate 5-hydroxytryptophan (Birt, Hagen & Zebrowski, 1961). The divergent findings cannot be attributed to insensitivity of the technique, as the method used, paper chromatography and autoradioaugraphy of the amine produced, is probably as sensitive as any available. Thus the difference is probably a reflection of the different properties of the decarboxylase of different tissues. Whereas Udenfriend and his group used kidney as the source of the enzyme, the enzyme in the present study was derived from tissues the sole function of which appears to be the synthesis.

storage and secretion of amines. Direct comparisons of the amino acid decarboxylase of kidney with that of these tissues might throw some light on this interesting difference.

This work has been carried out with the support of a grant (B 2282) from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, and of a grant from the National Research Council of Canada. I am grateful to Mr William Black for skilful technical assistance.

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