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AN ABNORMAL REACTION OCCURRING IN THE PRESENCE OF L-AROMATIC AMINOACID DECARBOXYLASE (o)

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SUMMARY. The reaction of L-aromatic aminoacid decarboxylase (EC 4.1.1.28) with α-methyl-L-DOPA or 5-hydroxy-L-tryptophan leads to the formation of dihydroxyphenylacetone or, respectively, 5-hydroxyindolacetaldeyde. These are produced in amounts far exceeding, on molar basis, that of the coenzyme, pyridoxal-5'-phosphate. The reaction cannot therefore be simply a decarboxylation-dependent transamination, using the coenzyme as an amino group acceptor. Evidence is presented which rules out the possibility that this phenomenon is due to an oxidative deamination.

L-aromatic amino acid decarboxylase (EC 4.1.1.28), commonly known as DOPA decarboxylase, has been purified in several laboratories either from pig (1-3) or guinea pig kidney (4).

It has been noticed that some substrates slowly inactivate the enzyme, with a concomitant shift of the enzymic absorption spectrum, the formation of the amino form of the coenzyme, and the deamination of a fraction of the decarboxylation product. This reaction, which has been described for α-methyl-L-DOPA (5), L-DOPA (6), and 5-hydroxy-L-tryptophan (7), has been attributed to a transamination between the amine, produced by decarboxylation, and the enzyme-bound pyridoxal-P. The same reaction has been described for other amino acid decarboxylases (8,9) and might be of importance for the modulation of the activity of this class of

(o) This paper is dedicated to Prof. A. Rossi-Fanelli in occasion of his 75th birthday.

Abbreviations; DPA = 3,4 dihydroxyphenylacetone; HIA = 5-hydroxyindolacetaldeyde; pyridoxal-P = pyridoxal-5'-phosphate; L-DOPA = L-3-(3,4 dihydroxyphenyl)-alanine.

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enzymes (10). However, when we investigated in more detail the reaction of DOPA decarboxylase with α-methyl-DOPA or with 5-hydroxy-L-tryptophan we found that the reaction cannot be explained by the simple model which has been proposed. We report here the results of this investigation.

MATERIALS AND METHODS.

DOPA, 5-hydroxytryptamine, pyridoxal-P, and iproniazid were obtained from Sigma, α-methyl-L-DOPA from Fluka. Horse liver NADH was purchased from Boehringer. DPA was synthesized as described by Slates et al. (11). α-methyldopamine was a kind gift from Prof. I.J. Kopin (Laboratory of Clinical Science, National Institute of Mental Health, Bethesda - Md., U.S.A.) [^{3}H] α-methyl-L-DOPA with a specific activity of 1.25 Ci/mmole was purchased from Amersham. The enzyme was prepared from pig kidney (3) and was essentially homogeneous on polyacrylamide gel electrophoresis and analytical centrifugation. Apoenzyme was prepared according to Borri Voltattorni et al. (5).

Enzymic activity was measured according to Charteris and John (12). Reaction between α-methyl-L-DOPA and the enzyme was carried out at 25°C in 0.01 M phosphate buffer, pH 6.8. The products of the reaction, i.e. α-methyldopamine and DPA, were determined by high performance liquid chromatography (13). Alternatively, DPA was measured by the following method: the reaction was started with [^{3}H] α-methyl-L-DOPA (7 mM, 0.07 μC/ml), then stopped by heating at 100°C. The reaction mixture was extracted several times with ethyl ether, the extract was taken to dryness, redissolved in water and counted, using the scintillation cocktail of Patterson and Greene (14). The ethereal extract contained essentially only DPA, as judged by thin layer chromatography in formic acid/tert-butanol/water (15/70/15) (v/v/v) followed by visualization with 2,6-dichloroquinone-4-chloroimide.

Control experiments were performed without enzyme or in the presence of 10^{-4} M iproniazid as a monoamine oxidase inhibitor.

Concerning the reaction between 5-hydroxy-L-tryptophan and the enzyme, it was noticed that it leads to a rapid decarboxylation of the substrate, followed by a slower deamination of 5-hydroxytryptamine; therefore the latter was used directly to investigate the deamination reaction, which was performed in 0.1 M triethanolamine-HCl buffer, at pH 8.4. The product (i.e. HIA) was measured by its reaction with 0.25 mM NADH, in the presence of alcohol dehydrogenase from horse liver (15), after stopping the reaction catalysed by the decarboxylase by heating at 100°C.

Pyridoxal-P-content of the enzyme was measured either according to Wada and Snell (16), or according to O'Leary and Baughn (6) or from the optical density at 390 nm of the enzyme dissolved in 0.1 M NaOH, after removal of the substrate and products by means of gel filtration on a Sephadex G 25 column (cm 0.5x15).

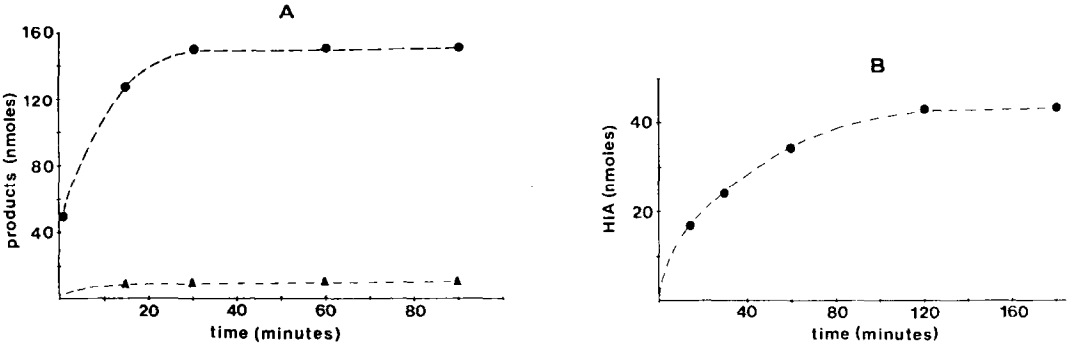

Fig. 1. Product formation from 2.8 mM α-methyl-L-DOPA (A) and 1.3 mM 5-hydroxytryptamine (B).
A: -•- DPA; -▲- α-methyldopamine. Total amount of enzyme: 12 nmoles; pH 6.8.
B: -•- HIA; total amount of enzyme: 4.8 nmoles; pH 8.4.

Oxygen was measured with a GME oxygraph Model ICM equipped with a Clark electrode at 25°C. Ammonia was measured either with a specific ammonia electrode (Model 95-10, Orion Res.), essentially by the procedure of Attili et al. (17), or by the reaction of glutamic dehydrogenase (18).

RESULTS AND DISCUSSION.

The reaction of α-methyl-L-DOPA with L-aromatic amino acid decarboxylase leads to the production of a small amount of α-methyldopamine, which is the expected decarboxylation product, and of a larger amount of DPA, which is the expected product of a decarboxylation-dependent transamination (Figure 1). However, DPA is formed in molar amounts significantly exceeding those of pyridoxal-P, one mole of which is present per mole of enzyme (1-3). In fact the molar ratio DPA/pyridoxal-P ranges from 8 to 15,depending on the enzyme preparation.

These results, obtained by determinations based on HPLC, were confirmed by independent measurements obtained by the use of [^{3}H] α-methyl-L-DOPA and extraction of DPA formed in diethyl ether. Again amounts of DPA equal to 15 times that of pyridoxal-P were found.

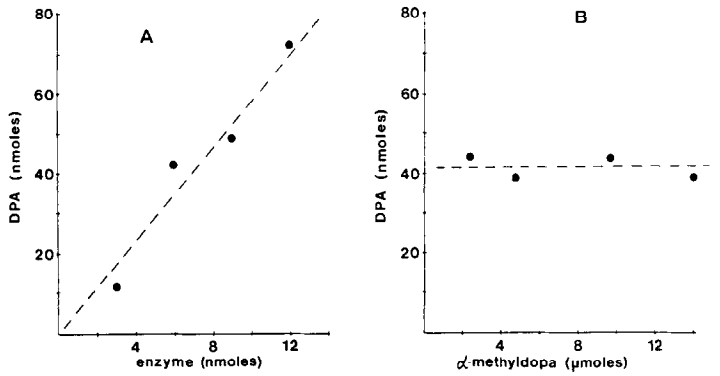

Fig. 2. Maximum amount of DPA produced after reaction of α-methyl-L-DOPA as a function of enzyme concentration (A) or substrate concentration (B).
A: 2.8 mM methyl DOPA.
B: 5 μM enzyme.

The reaction of α-methyl-L-DOPA was followed at different levels of substrate (Figure 2A) and enzyme (Figure 2B). The maximum amount of ketone produced was independent of the substrate concentration, but directly dependent on enzyme concentration. DPA formation was not accompanied by significant ammonia production nor by oxygen consumption, as shown in Table I. DPA was produced also when the reaction was performed under a nitrogen atmosphere.

Ketone formation was observed also by direct reaction of α-methyldopamine with the enzyme. In this case, however, the reaction was so slow that it did not go to completion after several hours. This can be attributed to the low affinity of the amines for the enzyme at pH 6.8; the use of higher pH values is precluded by the rapid oxidation of the cathecholamine. The higher rate of formation of DPA starting the reaction with α-methyl-L-DOPA can be explained by assuming that α-methyldopamine, formed as an intermediate, is deaminated before leaving the active site.

Substrate deamination was also observed with 5-hydroxytryptamine. In this case a higher pH for the reaction could be used, and 8 moles of HIA were formed per mole of coenzyme present (Fi-

TABLE I

Effects of reaction of α-methyl-L-DOPA or 5-hydroxytryptamine with aromatic amino acid decarboxylase.

Substrate	Measured effect	Moles per mole of enzyme
α-methyl-L-DOPA	Oxygen consumed	0.2
α-methyl-L-DOPA	Ammonia produced	0.5[a], 1.0 ± 0.3[b]
α-methyl-L-DOPA	Residual pyridoxal-P	0.6[c], 0.75[d]
5-hydroxytryptamine	Residual pyridoxal-P	0.15[c], 0.25[e]

a: determined by ammonia electrode.
b: determined with glutamic dehydrogenase. This value represents the average of three determinations; it is smaller than the value of the reagent blank, and is equal to one-tenth of the DPA formed in the same experiment.
c: determined spectrophotometrically in 0.1 M NaOH.
d: determined with phenylhydrazine.
e: determined by reactivation of apo-tyrosine-decarboxylase.

gure 1B). There is, therefore, a satisfactory agreement with what is found with α-methyl-L-DOPA.

The presence of pyridoxal-P is indispensable for the deamination step, since no HIA was formed from 5-hydroxytryptamine by the apoenzyme, while an excess of HIA was again formed when just one equivalent of the coenzyme was added to the apoenzyme (Table II).

TABLE II

Coenzyme dependence of the production of HIA from 5-hydroxytryptamine.

Enzyme	Coenzyme added (moles/moles of enzyme)	Carbidopa added (moles/moles of enzyme)	HIA formed (moles/moles of enzyme)
Apoenzyme	-	-	0.2
Apoenzyme	1	-	6.6
Apoenzyme	2	-	5.6
Holoenzyme	-	1	0

The reaction was carried out in triethanolamine-HCl buffer, pH 8.4, 0.1 M, with 7.5 nanomoles of enzyme and 1 micromole of 5-hydroxytryptamine.

The requirement of pyridoxal-P is also shown by the inhibitory effect of carbidopa (which forms a stoichiometric complex with pyridoxal-P (19)) on the holoenzyme.

Determination of residual coenzyme showed that after reaction with α-methyl-L-DOPA or with 5-hydroxytryptamine an appreciable fraction of the coenzyme remained in its original aldehydic form (Table I); however the value of this fraction was different with the two substrates.

Measurements of pyridoxamine phosphate were not satisfactory since we experienced the same difficulties reported by O'Leary and Baughn (16) for the enzymatic determination of this coenzyme.

These data rule out the possibility that the formation of DPA or HIA is due to a decarboxylation-dependent transamination, in which pyridoxal-P acts as the amino group acceptor.

An oxidative deamination, due either to a secondary catalytic activity of the enzyme itself, or to a contaminating amine oxidase can also be excluded on the basis of the following considerations: a) there is neither a significant ammonia production nor oxygen consumption (Table I); b) α-methyldopamine is a poorer substrate for DPA formation than α-methyl-L-DOPA; this is the opposite of what is expected for the presence of an amine oxidase; c) one equivalent of pyridoxal-P (with respect to the enzyme) is necessary for the deamination step; d) the carbonyl compounds are produced up to a maximum value which is related to the enzyme content and not to the substrate content.

The last observation might fit the idea that a transamination does indeed take place, and that some unknown amino group acceptors are located on (or strongly bound to) the enzyme itself.

This hypothesis is compatible with the results of preliminary experiments in which the decarboxylase was treated with $[^3H]$ sodium borohydride; tritium incorporation in the protein exceeded by a factor of about ten the value expected for the presence of pyridoxal phosphate. This might indicate the presence of reducible groups (possibly carbonyl groups) on the enzyme, capable of

accepting the amino groups, in an amount sufficient to explain the production of DPA and HIA.

The role of the coenzyme is obscure although it is established from the data of Table II that the deamination process has an absolute requirement for pyridoxal-P.

It should be noticed that reaction with α-methyl-L-DOPA leads to the formation of quite small amounts of α-methyldopamine, usually not higher than enzyme on a molar basis (Figure 1). This is not compatible with the idea that the hypotensive effect of α-methyl-L-DOPA is due to α-methylnorepinephrine, derived from α-methyldopamine (20).

Finally, considering the amounts of HIA produced from 5-hydroxytryptamine, the possibility that HIA has a physiological importance requires investigation.

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