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A POSSIBLE FEED-BACK MECHANISM CONTROLLING THE BIOSYNTHESIS OF 5-HYDROXYTRYPTAMINE

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Abstract—The effect of 5-hydroxytryptamine on the decarboxylation of 5-hydroxytryptophan by partially purified guinea pig L-aromatic amino acid decarboxylase was studied. 5-Hydroxytryptamine was found to inhibit the enzyme. The degree of inhibition was related to the time of preincubation of enzyme and 5-hydroxytryptamine and to the concentration of added pyridoxal phosphate, the co-factor for decarboxylation of 5-hydroxytryptophan. The possibility of a feed-back mechanism regulating the metabolism of 5-hydroxytryptamine is considered.

THE DISCOVERY of an enzyme capable of decarboxylating 5-hydroxytryptophan (5HTP) to 5-hydroxytryptamine (5HT) was first made in 1953.[1] It was purified from guinea pig kidneys,[2] and the evidence available now indicates that it is identical with the enzyme dihydroxyphenylalanine (DOPA) decarboxylase first isolated from guinea pig kidney in 1938.[3] The enzyme acts on the naturally occurring L-isomers of 5HTP and DOPA,[4–7] and its action on other aromatic L-amino acids has led to its being designated aromatic L-amino acid decarboxylase.[6]

Pyridoxal phosphate (PP) added to dialysed DOPA decarboxylase increases the activity of this enzyme[8] and it has been established as the coenzyme necessary for the enzymic decarboxylation of both DOPA and 5HTP.[9–11]

Extensive studies have been made on the inhibition of this enzyme by a large number of compounds.[12] The present study is concerned with the inhibition of a partially purified preparation of guinea pig kidney aromatic L-amino acid decarboxylase (5HTPD) by 5HT the product of 5HTP decarboxylation.

MATERIALS AND METHODS

DL—(1 — ^{14}C) 5HTP was obtained from Baird Atomic Co.; DL—5HTP and pyridoxal phosphate from L. Light & Co.; 5HT creatinine sulphate from Roche Products Ltd.

Enzyme preparation

The 5HTPD preparation used in these experiments was obtained from an aqueous homogenate of guinea pig kidney; this was partially purified by the method of Clark, Weissbach and Udenfriend as far as Stage III.[2] This fraction, i.e. the solution obtained after dialysis of the redissolved protein precipitated between 37 and 55% $(NH_4)_2SO_4$ saturation, was stored at −20°. The thawed enzyme was diluted with distilled water (1 : 10, v/v) just before use.

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Enzyme assay

Decarboxylase activity was determined by trapping the $^{14}CO_2$ released from L(1-^{14}C) 5HTP in hyamine.[13] The incubation of the enzyme was carried out in 25 ml conical flasks with one side arm closed by a gassing stopper. The main flask was sealed by a rubber serum stopper from which a 1 ml glass tube was suspended by means of a stainless steel wire inserted through the stopper. The main flask contained 1·0 ml 0·25M phosphate buffer pH 8·0, 0·1 ml dilute enzyme solution and 0·1 ml aqueous pyridoxal phosphate of the appropriate concentration as necessary. 0·1 ml of 5HT in 0·01 N HCl at the indicated concentration was added to the main flask in experiments where 5HT was preincubated with the enzyme, and to the side arm when 5HT was added simultaneously with the substrate; 0·1 ml of 0·01 N HCl was added to the control flasks. Water was added to make up the final incubation volume to 2 ml. The 5HTP substrate (0·1 ml) of sp. act. 4·4 μc/m-mole was added to the side arm of the flask. The enzyme solution and 5HT were always added last. The flasks were flushed through with N_2 for 5 min, sealed, placed in a water bath at 37° and preincubated (with shaking) for the stated time. The reaction was started by tipping the contents of the side arm into the main flask. Incubation was continued for the appropriate time, then 0·3 ml hyamine hydroxide in methanol was injected through the serum stopper into the suspended tubes and 0·3 ml 25% H_2SO_4 injected into the flask to terminate the reaction and release dissolved CO_2. The flasks were shaken for a further hour at 37° to allow all the evolved CO_2 to diffuse into the hyamine; after this time the tube containing the hyamine and dissolved $^{14}CO_2$ was dropped into a vial containing 15 ml of 0·4% PPO (2,5-diphenyloxazole) and 0·01% POPOP 1,4*bis* 2-(5-phenyloxazolyl) benzene in toluene. The radioactivity of the $^{14}CO_2$ was measured in a Tri-Carb liquid scintillation spectrometer. Corrections for quenching were made using the channel ratios method.[14] The amount of radioactive substrate incubated was estimated by separately counting 20 μl of the substrate mixture in scintillator solution containing 20% methanol, the result being corrected for quenching.

RESULTS AND DISCUSSION

The two most generally employed methods of estimating 5HTPD activity are based on manometric determination of CO_2 production,[2] or fluorometric estimation of 5HT.[6] The availabilty of ^{14}C-COOH labelled 5HTP allows the use of a much more sensitive direct method for determining the activity of this enzyme.

(1) The effect of PP on 5HTPD activity.

Fig. 1 shows the effect of various concentrations of PP on the decarboxylation of 5HTP. The presence of PP at concentrations as low as 10^{-8}M increased enzyme activity. Maximum activity occurred when PP concentration was 10^{-5}M, higher concentration of 10^{-4}M resulted in lower activity.

These findings are in agreement with those of Lovenberg *et al.*[15] who also found maximum enzyme activity when added PP concentration was between 10^{-6} and 10^{-5}M decreased enzyme activity with higher concentrations of PP.

Enzyme activity was dependent upon the length of the preincubation period and in the absence of PP declined to 85 per cent of the original value at the end of 2 hr; the presence of 10^{-5} or 10^{-6}M PP prevented this fall in activity (Fig. 2.)

Lovenberg *et al.*[15] found that preincubation of 5HTPD for 30 min in the absence of PP resulted in a 50 per cent fall in activity; in the presence of PP (7×10^{-5}M)

enzyme activity decreased by approximately 10 per cent during the same period. Since the activity of 5HTPD is related to PP concentrations, a possible explanation of this difference with our findings is that the amount of PP bound to the apoenzyme may vary with the enzyme preparation.

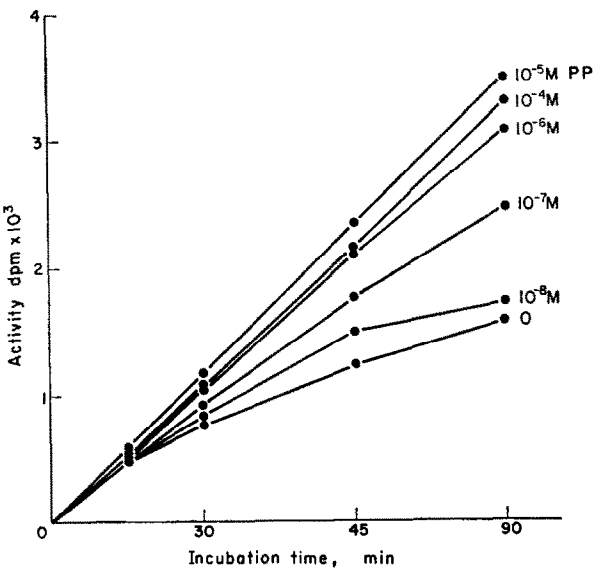

FIG. 1. Effect of PP on 5HTPD activity. The enzyme was preincubated for 15 min with PP, after addition of substrate, the incubation times were as indicated. Final substrate concentration of L-5HTP was $2{\cdot}5 \times 10^{-4}$M.

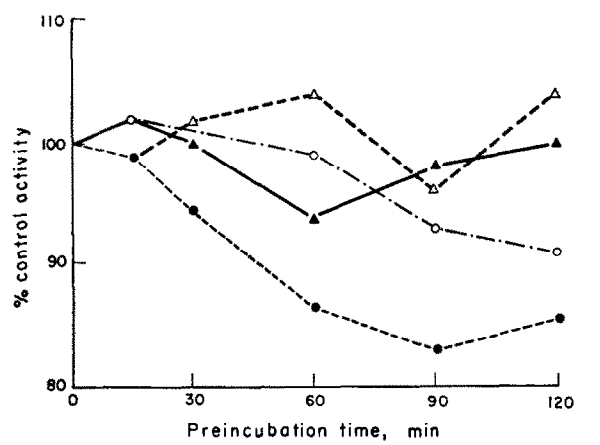

FIG. 2. Effect of preincubation time on enzyme activity in the absence of PP (● ---- ●); and with 10^{-7}M PP (○ - · - · ○) 10^{-6}M PP (△ - - - △); and 10^{-5}M PP (▲ — ▲).

(2) The effect of 5HT on 5HTPD activity. Inhibition studies were found to be independent of whether the incubation flasks were flushed through with nitrogen or not. Analysis of the enzyme preparation showed it to be free of monamine oxidase activity when kynuramine was used as the substrate.[16]

Table 1 shows the effect of 5HT on 5HTPD activity. Significant inhibition of enzyme activity was observed when 5HT (5×10^{-4}M, and 10^{-3}M) was preincubated for 15 min with the enzyme in the absence of PP, the degree of inhibition being related to the concentration of 5HT. When the enzyme was preincubated for 15 min without added PP, and 5HT (10^{-3}M) was added together with the substrate, no inhibition occurred. This was also found to be the case when the enzyme was preincubated for 15 min with 5HT (10^{-3}M) and PP (10^{-7}, 10^{-6} or 10^{-5}M).

TABLE 1. EFFECT OF 5HT ON 5HTPD ACTIVITY WHEN (a) 5HT IS PREINCUBATED WITH THE ENZYME WITH OR WITHOUT PP AND (b) 5HT IS ADDED TOGETHER WITH THE SUBSTRATE. IN BOTH CASES PREINCUBATION WAS FOR 15 MIN AND SUBSTRATE CONCENTRATION WAS $2{\cdot}5 \times 10^{-4}$M

5HT concentration (M)	Pyridoxal phosphate concentration (M)	Enzyme activity in presence of 5HT expressed as % of control activity (a)	(b)
5×10^{-4}	0	82·5	—
5×10^{-4}	10^{-5}	97·5	—
10^{-3}	0	75	96·5
10^{-3}	10^{-7}	97	100·5
10^{-3}	10^{-6}	99·5	96
10^{-3}	10^{-5}	98	—

The degree of inhibition was increased by lengthening the preincubation time of 5HT (10^{-3}M) with the enzyme in the absence of PP. At least 60 min preincubation of 5HT with enzyme was necessary before any inhibition occurred in the presence of 10^{-7}M PP. This inhibition increased progressively with preincubation periods of 90 and 120 min. Inhibition appeared to be significant after 120 min preincubation of enzyme and 5HT in the presence of 10^{-6}M PP (Fig. 3).

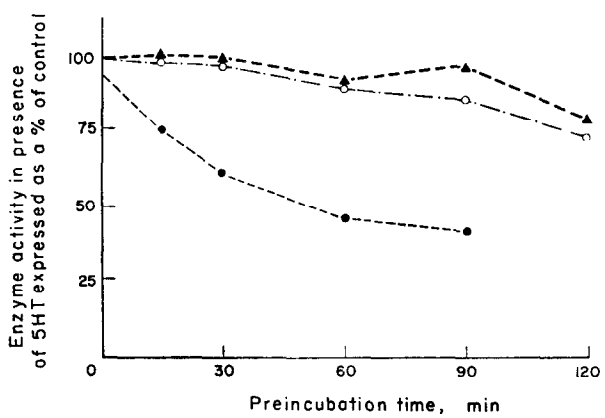

FIG. 3. Relationship between enzyme inhibition and preincubation time of 5HT with enzyme in the absence of PP (● - - - ●); and with 10^{-7}M PP (○ - · - · ○); and 10^{-6}M PP (△ - - - △).

The nature of inhibition ot 5HTPD by 5HT was investigated by varying the substrate concentrations. Double reciprocal plots[17] of enzyme activity against substrate concentration are shown in Fig. 4.

Mixed inhibition (competitive and non-competitive) was observed when 5HT was preincubated with the enzyme in the absence of PP for 15 min and inhibition was much greater at 90 min (Fig. 4a and b). When 5HT was added simultaneously with the substrate, the plots indicated that inhibition was competitive (Fig. 4c).

In a parallel series of experiments with identical substrate concentrations, no significant inhibition occurred when 5HT was simultaneously preincubated with enzyme in the presence of optimal concentration of PP (10^{-5}M) for 15 and 90 min, or, when 5HT was added together with the substrate to the enzyme and PP.

These experiments show that in certain conditions the decarboxylation of 5HTP can be inhibited by addition of 5HT, the product of the reaction. Yuwiller *et al.*[5] investigated the effect of many compounds on the decarboxylation of 5HTP and DOPA by a hog kidney enzyme preparation and found no inhibition of activity by 5HT in the presence of added PP, when the molar ratio of 5HT to substrate was 1. The present study confirms that 5HTPD is not inhibited by 5HT in the presence of optimal concentrations of PP. However, the enzyme is inhibited by 5HT in the absence of PP (Table 1) and if the enzyme is preincubated with 5HT (Fig. 3). Inhibition also occurs in the presence of sub-optimal concentrations of PP provided the enzyme, PP, and 5HT are preincubated together (Fig. 3).

It is of interest that the comparable biological amines adrenaline and noradrenaline, have been shown to inhibit 5HTP decarboxylation and the inhibition was even more marked in the absence of PP.[5] Preincubation of adrenaline with 5HTPD for 60 min was found to be a necessary factor for inhibition in the absence of PP; with noradrenaline, inhibition was obtained without preincubation.[5] Catechol amines and DOPA are also known to inhibit DOPA decarboxylation by binding pyridoxal phosphate.[18] Buzard and Nytch[11] who reported noradrenaline to be a competitive inhibitor of rat kidney 5HTPD in the presence of PP, showed that inhibition was achieved by removal of added PP through non-enzymic reaction between PP and noradrenaline to form a substituted tetrahydroisoquinoline derivative. The formation of similar derivatives between PP and histidine, histamine, tryptophan, phenylalanine and phenylethylamine containing a meta-hydroxy substituent has been demonstrated.[18] It would seem likely, therefore, that the inhibition of 5HTPD by 5HT which is related to PP concentration and preincubation times of enzyme with 5HT, occurs by a similar mechanism, i.e. removal or masking of enzyme bound PP by interaction with 5HT. Spectrophotometric studies of the reaction between PP and 5HT or 5HTP have shown that 5HT and 5HTP both rapidly form Schiffs base complexes with PP. The rate of formation of Schiffs base complex between 5HTP and PP was shown to be greater than that between 5HT and PP.[4]

The non-competitive inhibition of 5HTPD by 5HT (Figs. 4a and b) occurs when the enzyme and 5HT are preincubated together, presumably as a result of 5HT complexing with the bound PP and preventing it from acting as the co-enzyme for the reaction. On the other hand the competitive inhibition observed (Fig. 4c) when 5HT and 5HTPD are added simultaneously would depend on the rates of reaction of 5HT and 5HTP with enzyme bound PP. These rates may well differ from those occurring during the non-enzymic reactions between free PP and 5HT or 5HTP.

The concept of feed-back mechanisms regulating biosynthetic processes is now firmly established,[19, 20] and its application to the elucidation of enzyme, substrate, co factor and end-product interactions is becoming increasingly apparent. The possibility

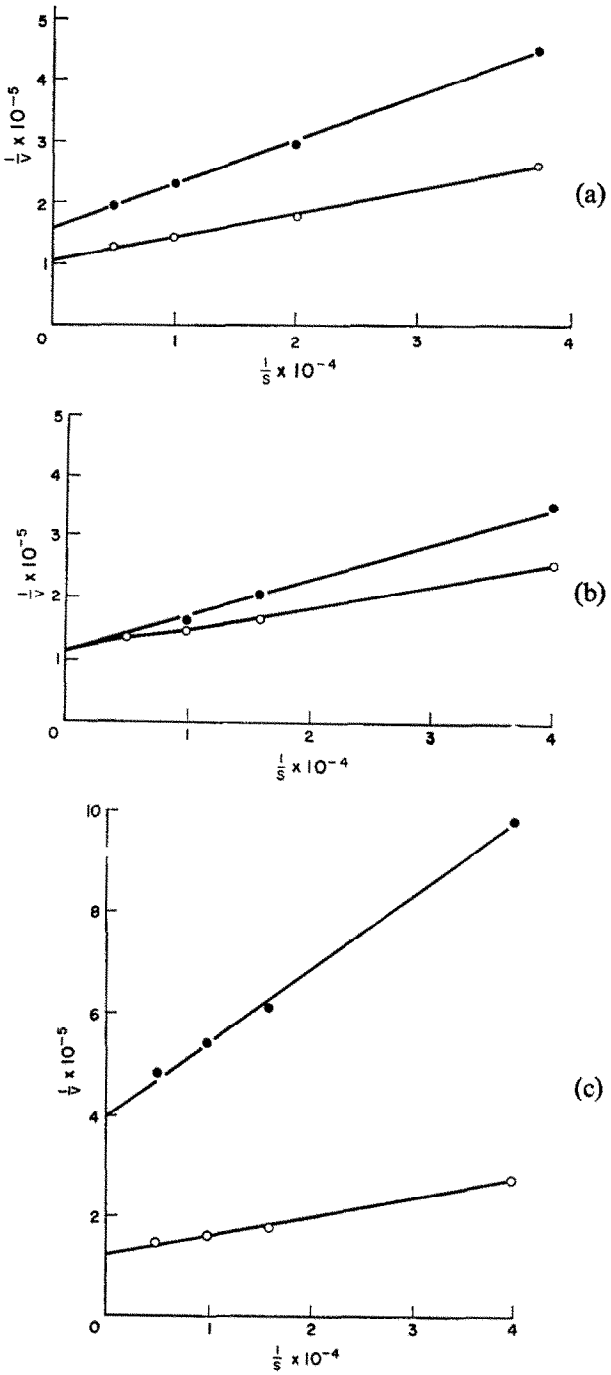

FIG. 4. Double reciprocal plot of enzyme activity against substrate concentration in the absence (○——○) and presence (●——●) of 10^{-8}M 5HT (a) 15 min preincubation of 5HT and enzyme, (b) 90 min preincubation of 5HT and enzyme, (c) 15 min preincubation of enzyme, 5HT was added simultaneously with the substrate. The incubation period for the estimation of enzyme activity was 30 min.

of 5HT regulating its own production by such a mechanism is an exciting one especially if one believes that it has an important biological function to fulfil. Certainly, its accumulation resulting in a build up to toxic levels in tissues could cause adverse effects at different levels in the animal.[21] The tissue levels of 5HT depend not only on its rate of production but also on the rapidity with which it is metabolised. It would be of interest to study the metabolism of 5HT in those conditions such as thyrotoxicosis where a definite decrease of tissue levels of monoamine oxidase activity has been shown.[22]

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