Biochemical Pharmacology, Vol. 17, pp. 2137–2141. Pergamon Press. 1968. Printed in Great Britain

# THE HOMOGENEITY OF PIG BRAIN MITOCHONDRIAL MONOAMINE OXIDASE

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(*Received* 4 *April* 1968; *accepted* 13 *June* 1968)

**Abstract**—The time-courses of inhibition of the activity of pig brain mitochondrial monoamine oxidase by 2-bromo-2-phenylacetaldehyde toward the substrates tyramine, tryptamine, 5-hydroxytryptamine, adrenaline and *m-O*-methylnoradrenaline have been found to be the same. Mixed substrate experiments gave $K_m$ values in agreement with those calculated by the usual methods, and the loss of activity resulting from partial heat denaturation of the enzyme was the same for all substrates. Harmine was shown to be a competitive inhibitor, and the $K_i$ was shown to be independent of the substrate used. These findings are interpreted as indicating that the majority of the monoamine oxidase activity in pig brain mitochondria is due to a single enzyme.

ALTHOUGH the mitochondrial enzyme monoamine oxidase (monoamine: $O_2$ oxidoreductase (deaminating) (E.C. 1.4.3.4.) ) is generally beleived to have a broad substrate specificity[1] a considerable amount of evidence has indicated that there are more than one enzyme. Differences in the temperature stabilities[2] and inhibitor sensitivities[3–5] of monoamine oxidase activity towards different substrates, and in the protection of thiol groups in the enzyme by different substrates[6] have been interpreted in terms of multiplicity of mitochondrial monoamine oxidases. This view has been strengthened by the partial separation of rat liver mitochondrial monoamine oxidases chromatographically by Gorkin[7] and electrophoretically by Youdim and Sandler,[8] and recently Johnston has reported an inhibitor which apparently discriminates between two monoamine oxidase activities in rat brain.[9]

In this study the homogeneity of pig brain mitochondrial monoamine oxidase was investigated, using five different substrates, by means of mixed substrate experiments, partial temperature denaturation and the use of inhibitors. The results indicate that in this source, the monoamine oxidase activity is associated with a single enzyme.

## MATERIALS AND METHODS

### *Chemicals*

D-L-*m-O*-methylnoradrenaline HCl, L-adrenaline HCl and harmine HCl were obtained from Koch-Light Ltd, Triton X-100 from Lennig Chemicals Ltd, bovine serum albumin from Armour Chemicals Inc. and Tris from Sigma Ltd. All other chemicals were obtained either from British Drug Houses Ltd or Hopkin and Williams Ltd. and were of the highest quality available. 2-Bromo-2-phenylacetaldehyde was synthesized by the method previously reported.[10] Distilled water was passed through a Permutit mark 2 deionizer before use.

*Preparation of mitochondria*

Brains were removed from freshly killed pigs and kept in ice until use. They were defatted, and mitochondria were prepared by the method of Garbus.[11] The mitochondrial preparation was washed twice in 250 mM sucrose containing 1·0 mM Tris (pH 7·6) and were finally suspended in 10 mM phosphate buffer (pH 7·6) and stored frozen until use.

*Assays*

The oxygen uptake when substrates were oxidized was followed by the method previously reported.[12] The assay mixture contained in a total volume of 2·4 ml: 200 $\mu$mole of sodium phosphate buffer (pH 7·0), 20 $\mu$mole of semicarbazide, 2$\mu$mole of KCN, 100 units of catalase, and mitochondria. The mixture was equilibrated in air at 30° and the reaction was started by the addition of 100 $\mu$l of substrate.

The protein concentration of mitochondrial preparations was determined using the microbiuret method;[13] samples were made 0·6% with the detergent Triton X-100 to assist solubilization and bovine serum albumin was used as a standard.

## RESULTS

*Inhibition by 2-bromo-2-phenylacetaldehyde*

This compound, which has been shown to be a potent inhibitor of yeast alcohol dehydrogenase,[10] was synthesized as an analogue of monoamine oxidase products containing a single aromatic ring. If this compound were to function as a substrated directed inhibitor it was hoped that an enzyme acting on an aromatic substrate would be inhibited more rapidly than one acting on a heterocyclic substrate such as tryptamine. Inhibition of the mitochondrial monoamine oxidase activity was time dependent and was not reversed by dialysis. The time courses of inhibition of monoamine oxidase activity were determined at 30° and it was found that, at the concentration of inhibitor used, inhibition was a first order process. A semi-logarithmic plot of the loss of activity toward five substrates is shown in Fig. 1, from which it can be seen that the first order rate constant for loss of activity is the same for all substrates.

*Mixed substrate experiments*

The Michaelis constants for the oxidation of the five substrates were determined by reciprocal plots[14] of initial rate measurements at a series of substrate concentrations. These values were compared with those calculated from mixed substrate experiments. If a single enzyme catalyzes the oxidation of more than one substrate, the velocity will not be the sum of the rates given by each substrate alone, and the following relationship has been calculated:[15]

$$\frac{K_a}{K_b} = \frac{V_a - V_m}{V_m - V_b}$$

where $K_a$ and $K_b$ are the Michaelis constants for the two substrates and $V_a$, $V_b$ and $V_m$ are the maximum velocities obtainable with the two substrates separately and with the mixture respectively. This method only gives accurate results if the difference between the two Michaelis constants is small and the difference between $V_a$ and $V_b$ is large. The Michaelis constants for the five substrates were calculated from initial rate

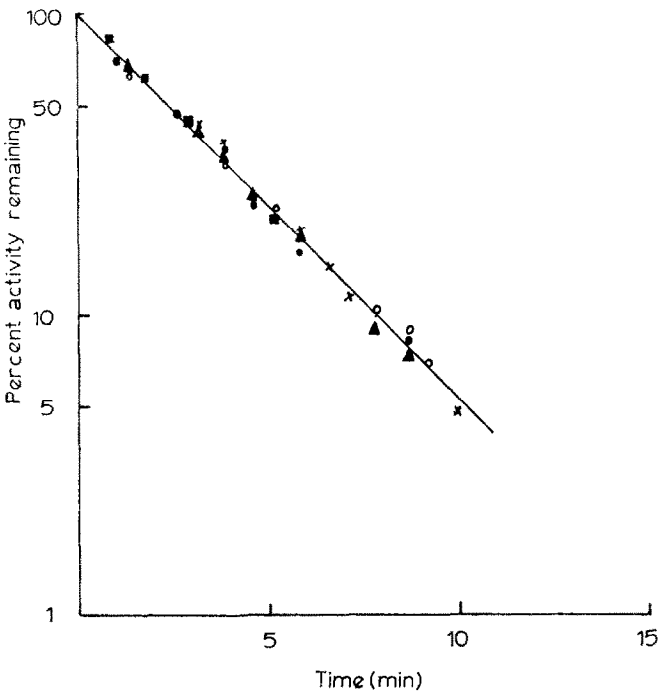


FIG. 1. The Inhibition of monoamine oxidase by 2-bromo-2-phenylacetaldehyde. The mitochondrial preparation was preincubated at 30° in the normal assay medium with 3·9 $\mu$M 2-bromo-2-phenylacetaldehyde. The reaction was then started by the addition of substrate. Five different substrates were used: tyramine (×), tryptamine (○), 5-hydroxytryptamine (●), D-L-*m*-*O*- methylnoradrenaline (▲) and L-adrenaline (■) at a concentration of 1 mM in each case. Specific activity is defined as m$\mu$ atoms of oxygen consumed, mg mitochondrial protein$^{-1}$, min.$^{-1}$

measurements using high substrate concentrations (2·0 mM) and mixtures of pairs of substrates. Michaelis constants calculated in this way are compared with those calculated from reciprocal plots in Table 1.

TABLE 1. KINETIC CONSTANTS FOR THE OXIDATION OF SEVERAL SUBSTRATES BY MONOAMINE OXIDASE AND ITS INHIBITION BY HARMINE

| Substrate | *Km* ($\mu$M) | | $K_i$ ($\mu$M) for harmine inhibition |
|---|---|---|---|
| | From reciprocal plots | From the mixed substrate method | |
| Tyramine | 120 | — | 9·1 |
| Tryptamine | 115 | 110 | 9·8 |
| L-Adrenaline | 85 | 98 | 9·0 |
| 5-Hydroxytryptamine | 280 | 320 | 9·9 |
| D-L-*m*-*O*-methylnoradrenaline | 28 | 37 | 10·0 |

Details of the assay method are given in the text. The $K_m$ values derived by the method of mixed substrates were calculated with respect to tyramine.

*Temperature denaturation*

Oswald and Strittmatter[2] found considerable differences in the temperature stabilities of the monoamine oxidase activities of rat liver mitochondria toward different substrates, and Dixon[16] has pointed out that parallel rates of heat inactivation are a sensitive criterion for the identity of enzymes acting on several substrates. Table 2 shows that partial heat denaturation of pig brain mitochondrial monoamine oxidase affects the activity towards the five substrates to the same extent.

TABLE 2. THE EFFECT OF HEAT TREATMENT ON THE ACTIVITY OF MONOAMINE OXIDASE TOWARD SEVERAL SUBSTRATES

| Substrate | Activity (%) after preincubation at 55° | 60° | 65° |
|---|---|---|---|
| Tyramine | 77 | 65 | 17 |
| Tryptamine | 76 | 70 | 20 |
| L-Adrenaline | 78 | 64 | 22 |
| 5-Hydroxytryptamine | 75 | 67 | 16 |
| D-L-*m*-*O*-methylnoradrenaline | 73 | 65 | 22 |

Pig brain mitochondria in 10 mM phosphate buffer (pH 7·6) were incubated for 5 minutes at the temperature stated and then cooled in ice. The activities toward the various substrates (at a final concentration of 1 mM) were then assayed and are expressed as percentages of the activity of the untreated enzyme with that substrate.

*Inhibition by harmine*

Harmine is a competitive inhibitor of monoamine oxidase[17] although it differs in its potency toward enzyme preparations from different sources.[18] The reciprocal plot of tyramine oxidation in the absence and presence of harmine shown in Fig. 2 indicates that harmine is a competitive inhibitor of tyramine oxidation by pig brain mitochondrial monoamine oxidase.

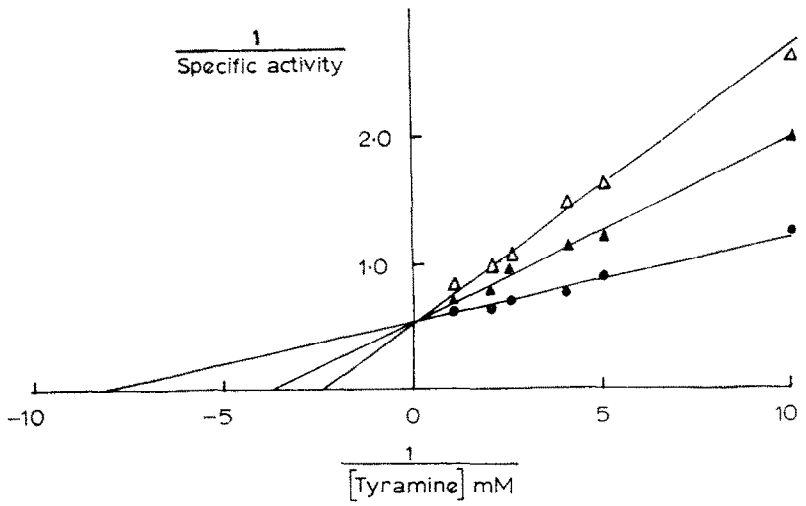


FIG. 2. The Inhibition of Tyramine Oxidation by Harmine. The following harmine concentrations were used, 20 $\mu$M (△), 10 $\mu$M (▲) and zero (●).

The inhibitor constant ($K_i$) for the oxidation of each substrate was calculated from reciprocal plots and inhibition was found to be competitive in each case. The inhibitor constant calculated for each substrate is shown in Table 1.

## DISCUSSION

The agreement between the $K_m$ values calculated from the mixed substrate experiments and from reciprocal plots indicates that the oxidation of these substrates is probably carried out by a single enzyme. This conclusion is supported by the similar time-courses of inhibition of all activities by 2-bromo-2-phenylacetaldehyde, although this inhibitor may be acting non-specifically. In contrast to results obtained with rat liver, partial heat inactivation affected the activity to all the substrates to the same extent and the $K_i$ value for harmine was independent of the substrate used. The possibility that storage of the mitochondria in the frozen state may inactivate a second enzyme is unlikely since this does not occur with the enzymes from rat liver mitochondria.[2]

These data indicate that the monoamine oxidase activity of pig brain mitochondria probably resides in a single enzyme, in contrast to results obtained with the rat liver enzyme. Recent work by Logan, Parsons and Hall[19] with the inhibitor *N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy) propylamine hydrochloride, which has been shown to be capable of discriminating between two monoamine oxidase activitiesin rat brain,[9] can detect the presence of only one monoamine oxidase activity in pig brain. The fact that the suggested multiplicity of monoamine oxidase may not be a general phenomonon must be taken into account in studies on the metabolism of the biogenic amines, and in attempts to design new inhibitors with selective action on the oxidation of different monoamine oxidase substrates.

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