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SHORT COMMUNICATIONS

The inhibition of rat liver mitochondrial monoamine oxidase by 2-bromo-2-phenylacetaldehyde

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2-BROMO-2-PHENYLACETALDEHYDE has been shown to be a powerful inhibitor of yeast alcohol dehydrogenase[1] and pig brain mitochondrial monoamine oxidase.[2] Rat liver mitochondrial monoamine oxidase appears to exist in a number of forms which differ in their electrophoretic mobilities[3,4] and inhibitor sensitivities.[5,6] Since 2-bromo-2-phenylacetaldehyde is an analogue of the aldehyde products given by substrates containing a single aromatic ring, such as benzylamine and phenylethylamine, it was hoped that this compound might be capable of distinguishing between the enzymes acting on benzylamine and those acting on double ring substrates such as 5-hydroxytryptamine. The observed inhibition of monoamine oxidase activity by its aldehyde products[7,8] indicates that these can be bound to the enzyme.

Rat liver mitochondrial were prepared by the method of Hogeboom, Schneider and Palade[9] and were suspended in 0·01 M phosphate buffer (pH 7·6) and frozen and thawed before use. 2-Bromo-2-phenylacetaldehyde was synthesized by the method of Yanovskaya and Teren'tev.[10] Monoamine oxidase activity was assayed by measuring oxygen consumption with an oxygen electrode using the method previously reported.[11] The reaction mixture contained in a total volume of 2·4 ml : 200 μmole of sodium phosphate buffer (pH 7·0), 100 units of catalase, and mitochondria. The mixture was equilibrated in air at 30° and the reaction was started by the addition of 100 μl of 0·025 M substrate. The rates of inhibition of monoamine oxidase activities were determined by incubation of the reaction mixtures at 30° with 2-bromo-2-phenylacetaldehyde before the addition of substrate.

The time courses of inhibition are shown as semi-logarithmic plots in Fig. 1. A straight line is obtained for the inhibition of activity towards 5-hydroxytryptamine indicating that the inhibition obeys a first-order rate law, and the first order rate constant was calculated to be 0·022 min^{-1}. The graphs obtained with tyramine and benzylamine as the substrates were more complicated, but each can be interpreted as being composed of two first-order curves. The first order rate constants obtained from the later parts of the curves were 0·021 min^{-1} and 0·022 min^{-1} for loss of activity towards benzylamine and tyramine respectively. The rate constants for the more rapid initial inhibitions were obtained by extrapolating the slower portions of the curves to zero time and subtracting values along the extrapolated lines from the initial portions of the curves,[12] as shown in Fig. 1. The first order rate constants calculated in this way were 0·31 min^{-1} and 0·29 min^{-1} for the initial rapid destruction of activity towards benzylamine and tyramine respectively.

The proportion of the enzyme acting on tyramine which was less sensitive to the inhibitor was found, by extrapolating the slower rate of inhibition to zero time, to be 77 per cent, similarly with activity towards benzylamine the less sensitive enzyme accounted for 62 per cent of the total activity.

The inhibitor *N*-methyl-*N*-propargyl-3-(2,4 dichlorophenoxy) propylamine hydrochloride (M & B 9302) has been shown to give biphasic curves for the inhibition of rat liver and brain monoamine oxidase activity towards tyramine.[5] Figure 2 shows the sensitivities of rat liver mitochondrial monoamine oxidase towards this inhibitor with tyramine as the substrate, both before and after pretreatment with 2-bromo-2-phenylacetaldehyde for 5 min. Preincubation with 2-bromo-2-phenylacetaldehyde resulted in a 28 per cent reduction in total enzyme activity and a 41 per cent reduction in the amount of enzyme that was not inhibited by 10^{-6}M M & B 9302.

Johnston[5] interpreted his results with M & B 9302 as indicating that there were two species of monoamine oxidases; one which oxidized tyramine and was relatively insensitive to the inhibitor, and the other which oxidized tyramine and 5-hydroxytryptamine and was considerably more sensitive to the inhibitor. Recently it has been shown that activity towards benzylamine is confined to the less sensitive enzyme species.[13] The results reported in this work suggest that the M & B 9302-insensitive

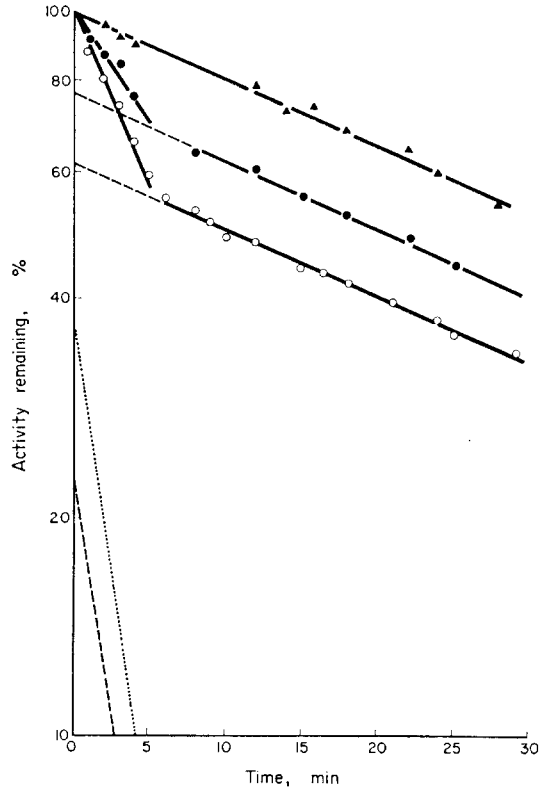

FIG. 1. The inhibition of monoamine oxidase by 2-bromo-2-phenylacetaldehyde. The mitochondria were incubated at 30° in the normal assay medium with 4·0 μM 2-bromo-2-phenylacetaldehyde for fixed times and the activity was then assayed using tyramine (●), benzylamine (○), or 5-hydroxytryptamine (▲) as substrate. The rates of inhibition of the more sensitive enzymes are shown for benzylamine (. . . . .) and tyramine (– – – – –).

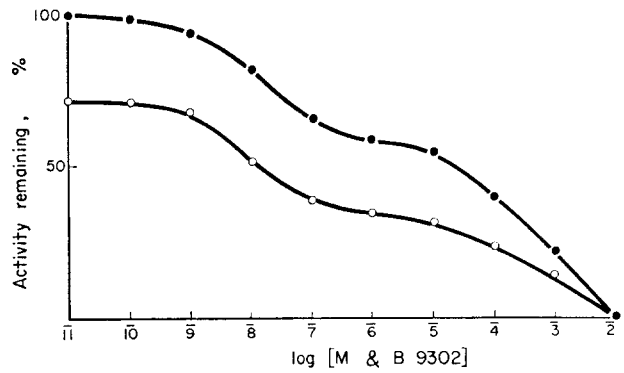

FIG. 2. The effect of 2-bromo-2-phenylacetaldehyde on the sensitivity of monoamine oxidase towards M & B 9302. The mitochondria were incubated at 30° with either water (●) or 4·0 μM 2-bromo-2-phenylacetaldehyde (○) for 5 min. After this time 1 μmole of cysteine was added followed by M & B 9302 to give the appropriate final concentration and incubation was continued for a further 15 min before the monoamine oxidase activity was assayed with tyramine. The activity of the mixture in the absence of M & B 9302 and 2-bromo-2-phenylacetaldehyde was taken as 100%.

enzyme may itself be composed of two components differing in their reactivities towards 2-bromo-2-phenylacetaldehyde although the possibility of two reactive groups on the enzyme being responsible for this differential loss of activity cannot be completely ruled out. The presence of more than two monoamine oxidases in rat liver mitochondria is in agreement with the results of electrophoretic separations.[3,4]

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Stimulation *in vitro* of microsomal aniline hydroxylation by 2,2′-bipyridine

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IN A PREVIOUS report[1] it was shown that acetone markedly stimulated the microsomal hydroxylation of aniline *in vitro* when added directly to incubation mixtures. It was also shown that acetone produced significant alterations in the kinetics of aniline hydroxylation and in susceptibility to inhibition by SKF 525-A and piperonyl butoxide. Furthermore, it appeared that acetone produced its enhancing effect by a mechanism different from that of ethyl isocyanide, a compound known to stimulate the microsomal hydroxylation of aniline.[2]

In a study of the oxidation of drugs by fishes, Buhler and Rasmusson[3] observed that 2,2′-bipyridine markedly stimulated the hydroxylation of aniline but produced a slight inhibition of the *O*-dealkylation of phenacetin. Since the magnitude of the stimulation of aniline hydroxylation by 2,2′-bipyridine was similar to that produced by acetone it suggested that both compounds, though chemically unrelated, might produce their stimulatory effects on aniline hydroxylation by similar mechanisms. The results presented in this paper suggest that acetone and 2,2′-bipyridine produce their stimulation of aniline hydroxylation by a similar mechanism and, furthermore, that this stimulation is not the result of an inhibition of microsomal lipid peroxidation.