5-HYDROXYTRYPTAMINE: A SUBSTRATE FOR RAT LIVER MITOCHONDRIAL MONOAMINE OXIDASE-A AND -B*

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Accepted June 17, 1982

On the basis of substrate specificities and inhibitor sensitivities, mitochondrial monoamine oxidase (MAO) (EC 1.4.3.4) has been classified as two forms, MAO-A and MAO-B (1). In rat liver, serotonin is preferentially oxidized by MAO-A, which is highly sensitive to inhibition by clorgyline but less so to deprenyl (1, 2). Benzylamine and β -phenylethylamine (PEA) are preferentially oxidized by MAO-B, and tyramine is oxidized by both forms of MAO (1, 2). However, these specificities are not always observed (3) and it has also been found that MAO-B in some tissues can oxidize serotonin to some extent (4-8). Our previous studies on rat brain MAO revealed that PEA, long regarded as a specific substrate for MAO-B, is also oxidized by MAO-A and that the ratio of MAO-A to MAO-B activity with PEA is concentration-dependent (9-13).

Here, we investigated the concentration-dependence of the oxidation rates of serotonin by the two forms of MAO in rat liver mitochondria. We also examined whether the oxidation with MAO-A of rat liver has a "ping-pong" or double-displacement reaction mechanism like that shown for MAO-B in a variety of tissues (14-18).

Rat liver mitochondria were prepared by differential centrifugation of a homogenate in

0.25 M sucrose-0.01 M phosphate buffer, pH 7.4. MAO activity was determined radiochemically at 37°C and pH 7.4 as described previously (18). Unless otherwise specified, in inhibition studies, mitochondria were preincubated for 30 min with either clorgyline or deprenyl at 37°C before adding substrate. The remaining activity was then determined radiochemically (18). In experiments in which the concentration of oxygen (54.3-542.5 μ M) was changed, the reaction mixture was bubbled for 5 sec with different mixtures of oxygen and nitrogen.

The protein concentration was determined by the modified biuret method (19) with bovine serum albumin as standard.

The inhibitions by clorgyline and deprenyl of MAO activity towards different concentrations of serotonin were studied and results with clorgyline are shown in Fig. 1. The curves for inhibition by both inhibitors differed with the concentration of serotonin. In accordance with the concept that serotonin is a preferential substrate of MAO-A (1, 2), at low concentrations (0.01-0.05 mM), single-sigmoidal inhibition curves with a higher sensitivity to clorgyline than deprenyl were obtained. In contrast, at higher concentrations of serotonin (0.5 and 5 mM), inhibition curves gradually became double-sigmoidal (Fig. 1). These results are analogous to those reported for PEA, which was found to be oxidized differentially by the two forms of MAO in a concentration-

*This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

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Fig. 1. Effect of clorgyline on MAO activity in untreated, clorgyline-treated and deprenyl-treated rat liver mitochondria. For A, mitochondria (1 mg/ml protein) were preincubated at 37°C for 30 min with clorgyline before estimation of activity. For B, mitochondria were first preincubated with either 2 nM (Δ , $\blacktriangle$) or 3.3 nM ($\circ$, $\bullet$) clorgyline or 3.3 nM ($\square$, $\blacksquare$) deprenyl for 60 min, and then samples were further preincubated with different concentrations of clorgyline for an additional 30 min. (A); MAO activity in untreated mitochondria was estimated with 0.01 (Δ), 0.05 ($\blacktriangle$), 0.5 ($\bullet$), or 5 mM ($\circ$) serotonin as substrate and expressed as a percentage of the control activity in the absence of clorgyline. (B); MAO activity was estimated with 0.5 ($\circ$, Δ , $\square$), or 5 mM ($\bullet$, $\blacktriangle$, $\blacksquare$) serotonin as substrate and expressed as a percentage of the activity in preparations preincubated for 90 min in the presence of 2 or 3.3 nM clorgyline or 3.3 nM deprenyl. Each point represents the mean of triplicate determinations of activity in three groups of mitochondrial preparations, each derived from the livers of four rats.

dependent manner (9–13, 20).

None of the clorgyline-resistant activity towards serotonin at high concentrations was due to soluble amine oxidase, which is reversibly inhibited by clorgyline (21) or to

amine oxidase, which is insensitive to clorgyline and deprenyl (22), since KCN and semicarbazide (1 mM), inhibitors of these two enzymes, did not change the double-sigmoidal inhibition curves of activity and the activity could be inhibited completely by either clorgyline or deprenyl.

In separate experiments in which mitochondria were preincubated with 3.3 or 2 nM clorgyline, concentration-dependent inhibitions of 50–90% of the serotonin oxidation were observed. When 0.01 mM serotonin was used and the remaining activity was then inhibited by clorgyline, the curve was also single-sigmoidal, and shifted to lower concentrations of clorgyline (data not shown), since, in the clorgyline-pretreated preparation, less of the active enzyme remained and so it could be titrated with less clorgyline (23). Thus, at this concentration of serotonin, all oxidation was catalyzed by MAO-A, even though MAO-A had been partially and selectively inhibited. When 0.5 and 5 mM serotonin was used, double-sigmoidal curves were obtained with a higher proportion of the less clorgyline sensitive enzyme (MAO-B) activity (Fig. 1B).

In similar experiments with a preparation pretreated with 3.3 nM deprenyl and with 0.5 and 5 mM serotonin as substrate, all the inhibition curves with additional clorgyline were single-sigmoidal, with no appreciable MAO-B activity, and showed higher sensitivity to clorgyline (Fig. 1B). These results reinforced evidence that, in rat liver mitochondria, serotonin at the high concentrations is oxidized by both forms of MAO but with a high proportion of MAO-A activity, since after partial inhibition of MAO-A activity by clorgyline, the shape of the inhibition curves changed considerably and a trace of the MAO-B component was apparent with these concentrations of serotonin.

Lineweaver-Burk plots showed that Km

values for serotonin were $60\text{ }\mu\text{M}$, $40\text{ }\mu\text{M}$ and 2 mM in untreated, deprenyl-treated (MAO-A) and clorgyline-treated mitochondria (MAO-B), respectively, in agreement with the values reported previously (5). The plots with serotonin as substrate for MAO-B in the clorgyline-treated preparation were found to be linear, but the plots for the deprenyl-treated preparation (MAO-A) and for the untreated preparation were not linear, deviating with substrate inhibition. These results with serotonin as substrate are similar to those for rat brain MAO with PEA (10, 11, 13).

Fig. 2. Lineweaver-Burk plots of MAO-A activity in rat liver mitochondria assayed at different concentrations of oxygen. Ordinate, $1/\text{activity}$, expressed as $\text{cpm} \times 10^{-4}$; abscissa, $1/\text{mM}$ serotonin. Before assay, mitochondria were preincubated with 33 nM deprenyl for 4 hr, and then MAO activities towards different concentrations of serotonin were assayed under atmospheres of 54.3 ($\square$), 108.5 ($\bullet$), 217.0 ($\circ$), and 542.5 ($\triangle$) μM oxygen. All points shown are the means of triplicate determinations of activity in three groups of mitochondrial fractions, each derived from the livers of four rats.

MAO in a variety of tissues has been shown to follow a "ping-pong" or double-displacement reaction mechanism (14–18). However, to our knowledge, this mechanism has been demonstrated only for MAO-B, while the mechanism for MAO-A is still unknown. When the MAO-A activity in mitochondria pretreated with deprenyl (33 nM 4 hr), which completely and irreversibly inhibited MAO-B activity towards low concentrations of PEA (10–12), was assayed using low concentrations (0.013 – 0.2 mM) of serotonin with decreasing concentrations (542.5 – $54.3\text{ }\mu\text{M}$), of oxygen results showed uncompetitive inhibition giving parallel plots with a K_m value of $144.9\text{ }\mu\text{M}$ for oxygen (Fig. 2). Similar parallel lines were obtained when the oxygen concentration was varied at a series of fixed serotonin concentrations, indicating that MAO-A reacts according to the double-displacement reaction mechanism previously shown for MAO-B.

The preferential oxidation of low concentrations of serotonin by MAO-A is presumably due to its lower K_m value ($40\text{ }\mu\text{M}$), than that of MAO-B (2 mM). With increasing concentrations of serotonin, the proportion of MAO-B activity will increase, since oxidation by MAO-A, which is already at the V_{max} and which becomes subject to substrate inhibition, cannot increase. The data presented here and previously (9–13, 20) show that the concentrations of substrates must be taken into account when the substrate specificities and inhibitor sensitivities of MAO to MAO inhibitors are investigated.

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