MOLECULAR PHARMACOLOGY, 16, 234-241

The Reaction of Bovine and Rat Liver Monoamine Oxidase with [^{14}C]-Clorgyline and [^{14}C]-Deprenyl

JAMES I. SALACH[1] AND KRISTINA DETMER

Molecular Biology Division, Veterans Administration Hospital, San Francisco, California 94121

MOUSSA B. H. YOUDIM

Technion, Faculty of Medicine, Department of Pharmacology, Bat-Galim, Haifa, Israel

(Received September 19, 1978)
(Accepted January 12, 1979)

SUMMARY

SALACH, J. I., K. DETMER, AND M. B. H. YOUDIM: The reaction of bovine and rat liver monamine oxidase with [^{14}C]-clorgyline and [^{14}C]-deprenyl. *Mol. Pharmacol.* **16**, 234-241 (1979).

Highly purified monoamine oxidase from beef and rat liver has been compared with regard to the presence of A and B type enzymes. The beef enzyme oxidizes B and A-B type substrates rapidly and A type substrates slowly in both membrane and soluble preparations. Deprenyl, a selective inhibitor of the B type enzyme, inhibits it at very low concentrations and the I_{50} for inhibition is about the same with A, B, and A-B type substrates. Clorgyline, a selective inhibitor of the A type enzyme, inhibits only at high concentrations, and the I_{50} value is again independent of the substrate used. This shows that only the B type enzyme occurs in beef liver. Rat liver, in contrast, contains both A and B type of monoamine oxidase. Deprenyl inhibits the oxidation of B substrates at low concentrations, and clorgyline inhibits oxidation of A substrates at low concentrations, while biphasic inhibition is seen with A-B substrates upon titration with either inhibitor. Deprenyl and pargyline are stoichiometrically bound to the beef and rat liver enzymes, but clorgyline is bound to the beef enzyme in amounts in considerable stoichiometric excess indicating nonspecific binding. The enzyme from both sources binds these acetylenic suicide inhibitors at N-5 of the covalently bound flavin in a flavocyanine linkage. No conversion of the A to the B type of enzyme on extraction of the enzyme from the membrane and removal of lipids has been noted.

INTRODUCTION

Mitochondrial monoamine oxidase preparations from many tissues contain multiple bands on polyacrylamide gel electrophoresis which have been considered to be isoenzymes (1). There is also a considerable body of evidence for the existence in some tissues of two forms of MAO[2] differing in substrate specificity and sensitivity to inhibitors. One form, which has been called A, is extensively inhibited by clorgyline (2), is relatively insensitive to deprenyl (3), and is highly active on serotonin and norepinephrine, while the other, the B form, is sensitive to deprenyl, relatively resistant to

This work was supported in part by NIH Grant FR-05546 and NIH Grant NS14222 to J. Salach, administered by the Friends Medical Science Research Center, Inc.

[1] To whom reprint requests should be addressed.

[2] The abbreviations used are: MAO, monoamine oxidase.

0026-895X/79/040234-08$02.00/0

clorgyline, and oxidizes phenylethylamine and benzylamine rapidly. MAO preparations which are thought to contain both A and B forms give biphasic titration curves with clorgyline and deprenyl (4).

There seems to be little agreement, however, concerning the physicochemical basis of the different substrate and inhibitor specificities of the two types of MAO. McCauley and Racker (5), while cautioning that such differences are an insufficient basis to conclude that two forms of the enzyme exist, concluded that two distinct forms of the enzyme occur in bovine brain, since they appeared to be physically separable by immunoprecipitation. Gomes *et al.* (6), on the other hand, found that two MAO components may be isolated from beef liver which differ in apparent molecular weight but not in substrate specificity. These authors suggested, therefore, that the multiple forms may be aggregates of the same enzyme. Tipton (4) proposed that the differences between the A and B forms may be a function of the lipid material attached, because harsh treatment of a rat liver membrane preparation with detergents or chaotropic agents abolishes the biphasic titration curves. This view is not supported by the findings of Ekstedt and Oreland (7), since complete delipidation of rat liver mitochondria leads to inactivation of the A type enzyme, without any evidence of conversion to the B type.

One reason that it has been difficult to arrive at a consensus in this field is that different species and different tissues have been used in the various laboratories. There is evidence that while in one organ of a given species only the B form exits, another appears to contain both the A and B forms (5). Moreover, as documented below, liver mitochondria from two mammalian species show a similar divergence.

The availability of a rapid and relatively mild procedure for isolating the bulk of MAO activity from beef liver and of a chemical procedure, based on analysis for cysteinyl flavin, for measuring MAO content regardless of the form or degree of inactivation of the enzyme (8) prompted the present investigation. One of our aims was to compare, under essentially identical conditions, the inhibitory effects and binding of clorgyline and deprenyl in rat and bovine liver preparations. Another aim of this study was to confirm our expectation that, as proposed by Knoll (9), the two acetylenic "suicide inhibitors," clorgyline and deprenyl, form the same flavocyanine adduct with N-5 of the cysteinyl flavin active site of MAO, as that formed during inactivation of the enzyme with N, N-dimethylpropynylamine (10).

MATERIALS AND METHODS

Materials. Dopamine, tryptamine, tyramine, phenylethylamine, and L-norepinephrine as the hydrochlorides, kynuramine hydrobromide, 5-hydroxytryptamine creatinine sulfate, NAD^+, benzylamine, and L-epinephrine were obtained from Sigma Chemical Co. Benzylamine was stabilized and benzaldehyde present removed by crystallization from ethanol as the hydrochloride. Sephadex G-200 and G-50, fine, were from Pharmacia Fine Chemicals, and [2-^{14}C]-5-hydroxytryptamine creatinine sulfate from California Bionuclear Corp., [1-^{14}C]-5-hydroxytryptamine creatinine sulfate was obtained from Amersham, [1-^{14}C]-phenylethylamine, and [1-^{14}C]-dopamine from New England Nuclear, U. S. A.

Clorgyline, unlabelled and [^{14}C]-labelled in the ring (3.80 mCi/mmole), were generously supplied by May and Baker, Ltd., Essex, Great Britain; unlabelled, as well as [^{14}C]-labelled, deprenyl (2.46 mCi/mmole) were the gifts of Prof. J. Knoll and Dr. K. Magyar, Budapest, Hungary, and [7-^{14}C]-pargyline of Dr. R. L. Sonders, Abbott Laboratories, North Chicago, Ill.

Enzyme preparations. Outer membranes from beef liver (11) and highly purified, soluble MAO from the same source (8) were prepared as previously described. Aldehyde dehydrogenase was isolated by a combination of published procedures, as detailed elsewhere.[3]

Soluble MAO from rat liver was extracted from mitochondria obtained from 1 kg of fresh liver (12) by sonic oscillation

[3] Salach, J. I. (1979) Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties, and determination of its cysteinyl flavin content. *Arch. Biochem. Biophys.* **192**, 128–137.

and treatment with Triton X-100 (1.5%, v/v), as previously described (13). The extracted enzyme was precipitated with solid ammonium sulfate (30 to 60% saturation at 4°) at pH 7.4 and further purified by chromatography on DEAE-cellulose, Sephadex G-200, and finally on a hydroxylapatite column. The specific activity of the enzyme by the spectrophotometric kynuramine assay (cf. below) was 5870 units.

Estimation of MAO activity. During isolation of the enzyme, MAO oxidase activity was followed by adaptations of the spectrophotometric method of Tabor *et al.* (14), using benzylamine as substrate in the case of the beef liver enzyme, as described (8) and kynuramine with the rat liver enzyme. To be consistent with previous reports on the enzyme from these two sources, the specific activity of the beef liver enzyme is expressed as μmoles substrate oxidized per min per mg of protein, while that of the rat liver enzyme denotes units (0.001 A change at 316 nm) per min per mg. In the experiments presented below MAO activity was assayed either radiochemically (15, 16) or by following the reduction of NAD^+ in the presence of aldehyde dehydrogenase (17), as specified in the legends of tables and figures.

Titration with inhibitors. Soluble enzyme, from beef liver, (0.47 mg, specific activity = 1.4 μmole per min-mg) was incubated for 30 min at 37° in small test tubes containing 10 mM Tris, 30 mM glycine, pH 8.2 at 23°, and the desired concentration of inactivator in 0.5 ml final volume. Following incubation, tubes were chilled to 0° and the remaining activity was measured using the coupled assay with liver aldehyde dehydrogenase (17). All substrates were 3.3 mM in the assay.

Beef liver mitochondrial outer membrane (3.6 mg, specific activity = 0.095 μmole/min-mg) in 50 mM NaP_i, pH 7.2, was incubated with inactivator and activity remaining following incubation was measured as for the soluble enzyme, except that the oxidation of 5-hydroxytryptamine was followed by the radioisotope assay, and that the benzylamine was followed by the spectrophotometric assay. Both substrates were 1 mM. Activity on dopamine, tryptamine and tyramine (3.3 mM) was measured with the coupled assay.

The purified enzyme from rat liver (0.18 mg, specific activity = 5875 and 5600 units/mg in the spectrophotometric kynuramine assay) was incubated in a series of tubes containing 50 mM P_i buffer, pH 7.4, and the desired concentration of inhibitor in 0.5 ml total volume for 60 min at 25°. Residual enzyme activity was then measured using the radioisotope assay.

Binding studies. Equilibrium binding of [^{14}C]-labelled pargyline and of [^{14}C]-labelled clorgyline to soluble beef liver MAO was studied using the method of Penefsky (18). After incubation with the inhibitor, 100 μl aliquots of the enzyme were centrifuged at room temperature through a Sephadex G-50 (fine) bed, equilibrated with 50 mM Na P_i, pH 7.2 at 30°, and packed in 1 ml disposable syringes, as described. Following centrifugation, a 100 μl aliquot of buffer was centrifuged through the bed as a wash. This wash permitted recovery of 85–90% of the protein applied to the gel. No radiolabel was found to be eluted by this wash procedure in control experiments using [^{14}C]-pargyline or [^{14}C]-clorgyline alone. In order to obtain sufficient amounts of enzyme for measurement of activity in the spectrophotometric assay with benzylamine, and to determine the extent of radiolabel binding as well, the enzyme eluted from four such columns was pooled for each sample. To correct for variability in protein recovery in each sample pool, proteins were determined by the Lowry method (19) and data were corrected to a constant value.

Binding of [^{14}C]-deprenyl to the purified soluble MAO of rat liver was measured by inhibiting the enzyme (0.28 mg) with varying concentrations of deprenyl exactly as in the titration experiment. Perchloric acid, 6% (v/v), 1 ml, was added to each tube following incubation with the inhibitor and the precipitate obtained following centrifugation at 4000 × *g* for 10 min was washed by suspending it in 1% (w/v) trichloroacetic acid and centrifuging as before. The washed precipitate was dissolved in 1 ml of 1 N NaOH and the radioisotope content determined.

Other methods. Cysteinyl flavin content,

a chemical measure of the concentration of the enzyme, was determined fluorometrically as described elsewhere.[3] Protein was measured by either the biuret reaction (20) or by the method of Lowry (19). Following the inactivation of soluble MAO with clorgyline or deprenyl, the flavin peptide-inhibitor adducts were isolated and their spectra recorded as in previous work (10).

RESULTS

Inhibition of beef liver MAO by clorgyline and deprenyl. The effect of varying concentrations of clorgyline and deprenyl on the oxidation of A type, B type, and A-B type substrates by outer membranes and purified MAO of beef liver are shown in Fig. 1. Regardless of the type of substrate being oxidized, the activity was inhibited by very low concentrations of deprenyl (I_{50} for soluble preparations = $\sim 10^{-6}$ M, for outer membranes ~3 to 4 × 10^{-7} M) while clorgyline inhibited the enzyme only at very much higher concentrations. No indication of a biphasic titration curve was noted with the A-B type substrates.

Binding of clorgyline and pargyline to beef liver MAO. Pargyline, an acetylenic suicide inhibitor, relatively specific for B type MAO (21), was bound to the highly purified beef liver enzyme stoichiometrically, with parallel loss of activity (Fig. 2), as expected from previous work on the kidney enzyme (22) and from the nature of the adduct formed between the beef liver enzyme and the acetylenic suicide inhibitors (10). In contrast, clorgyline, although a suicide inhibitor of MAO of the acetylenic type, was bound in substantial excess of the stoichiometric amount of enzyme, and binding of the labelled inhibitor did not parallel the loss of catalytic activity (Fig. 3). These binding ratios were confirmed by direct comparison of the content of bound radioactive inhibitor and cysteinyl flavin in the flavin peptide fractions obtained after proteolysis of the inhibited enzyme. In one experiment 2.8 mg of enzyme, containing 20.4 nmoles of cysteinyl flavin, was inhibited with an excess of [^{14}C]-pargyline, precipitated, extensively washed with trichloroacetic acid to remove unreacted pargyline, and digested with trypsin chymotrypsin (8). The deproteinized digest contained 20.4 nmoles of radioactivity. In the companion experiment 21.8 nmoles soluble

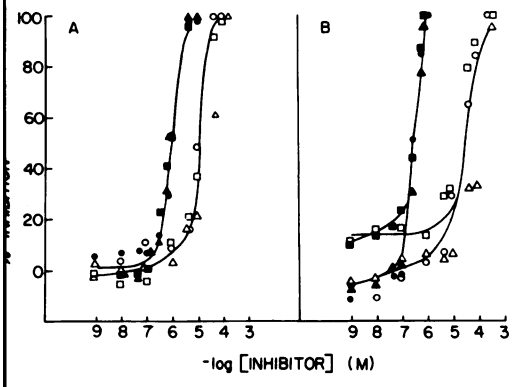

FIG. 1. *Inhibition by clorgyline (open figures) and deprenyl (solid figures) of soluble beef liver MAO (A) and of beef liver outer mitochondrial membrane fragments (B)*

○, ● benzylamine; □, ■ dopamine; △, ▲ 5-hydroxytryptamine. Activity remaining after incubation with inhibitor was measured using the coupled assay with the soluble enzyme; all substrates were 3.3 mM. In titrations of outer membrane fragments the activity remaining was measured with 1 mM [2-^{14}C]-5-hydroxytryptamine in the radioisotope assay, with 1 mM benzylamine in the spectrophotometric assay, and with 3.3 mM dopamine in the coupled assay.

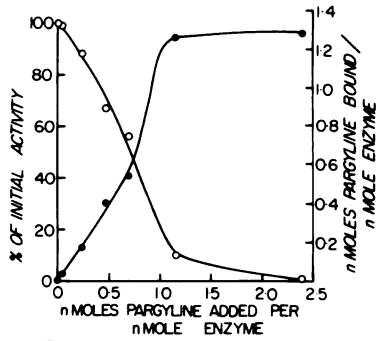

FIG. 2. *Inactivation and pargyline binding to beef liver MAO*

Purified soluble enzyme (0.88 mg, specific activity = 3.4 μmoles/min-mg, cysteinyl flavin content = 7.3 nmoles/mg) was incubated with [^{14}C]-pargyline (1.12 μCi/μmole) in 50 mM phosphate, pH 7.2 for 5 min at 30°. The inhibitor concentration ranged from 0.6 μM to 30 μM. The reaction mixture was cooled to 0°, aliquots of 100 μl were removed and rapidly centrifuged through a micro-column of Sephadex G-50, fine (see METHODS). ○ activity on 3.3 mM benzylamine in the spectrophotometric assay; ● moles inhibitor bound per mole of enzyme.

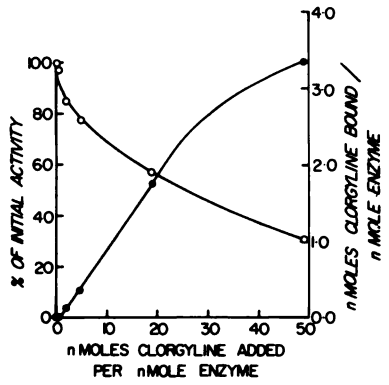

FIG. 3. *Loss of enzyme activity and binding of [^{14}C]-clorgyline to soluble beef liver MAO*

Conditions were as in Fig. 4, except that the protein concentration was 0.94 mg/ml and [^{14}C]-clorgyline (0.85 μCi/μmole) concentration ranged from 3.3 μM to 330 μM. Activity was measured spectrophotometrically. ○ residual activity; ● [^{14}C]-clorgyline bound.

enzyme, based on cysteinyl flavin content, were incubated with [^{14}C]-clorgyline until inhibition was complete. The preparation was then precipitated and digested as above. The digest contained 116 nmoles of radioactivity, or a 5.3-fold excess over the quantity of MAO used.

Inhibition of rat liver MAO by deprenyl and clorgyline. Figure 4A shows the titration of purified rat liver MAO with deprenyl. The differences from the behavior of the beef liver enzyme (Fig. 1) are obvious. First, the apparent I_{50} value depends on the type of substrate used: it is very low (I_{50} = 0.2 μM) when phenylethylamine, a B type substrate is tested; two orders of magnitude higher (I_{50} = 30 μM) when 5-hydroxytryptamine, an A type substrate, is used; and with kynuramine, an A-B substrate, the I_{50} is 1.25 μM (25). Second, with dopamine, also an A-B substrate, the inhibition curve is biphasic showing two I_{50} values, one at 10^{-8} M and another at 25 μM. All this is consistent with the conclusion that purified MAO from rat liver acts as if it contained both the A and B type of enzyme. Figure 4B shows similar titrations with clorgyline. While the pattern is very similar to that of Fig. 4A, the relationships of inhibitor sensitivity and substrate are reversed, the I_{50} is very low (0.4 μM) when 5-hydroxytryptamine is used, two orders of magnitude higher (I_{50} = 30 μM) when phenylethylamine is the substrate, and a biphasic titration curve results when dopamine is the substrate with I_{50} values of 0.2 μM and 25 μM.

Binding of deprenyl to rat liver MAO. Figure 5 shows the binding curve of deprenyl to the purified rat liver enzyme. At the point where binding levels off, all activity on phenylethylamine is abolished, but nearly 90% of the activity on 5-hydroxytryptamine, the A-specific substrate, remains. One of us has reported elsewhere (24) that when this preparation is incubated with an excess of deprenyl and unreacted inhibitor is removed by gel filtration, nearly a stoichiometric amount of deprenyl (~1.1 mole/mole of flavin) remains bound to the enzyme. This seems to imply that, despite the presence of both A and B types of MAO activity in rat liver preparations and only of the B type in beef liver, in both cases one mole of deprenyl is bound per mole of enzyme.

Structure of the adduct formed with clorgyline and deprenyl. Although the majority of studies on the action of acetylenic suicide

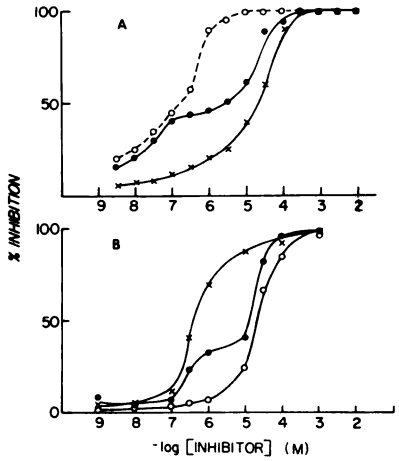

FIG. 4. *Effect of deprenyl (A) and clorgyline (B) inhibition of rat liver MAO on the oxidation of biogenic amines*

Experimental conditions are as described in METHODS. Specific activity of the enzyme was 5875 units/mg (A) and 5600 units/mg (B) in the spectrophotometric assay with kynuramine. Following inactivation residual enzyme activity was determined with [1-^{14}C]-phenylethylamine, ○——○; [1-^{14}C]-5-hydroxytryptamine, ×——×; and [1-^{14}C]-dopamine ●——●.

nhibitors on MAO have been conducted vith pargyline, clorgyline, and deprenyl, he structure of the adduct formed with the nzyme has not been elucidated in any of hese cases. It has been shown, however,

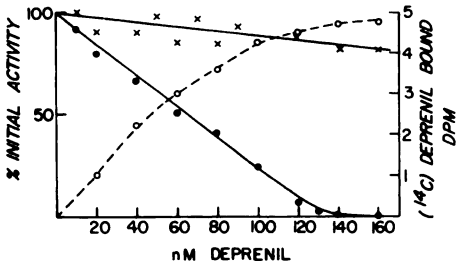

FIG. 5. *Loss of enzyme activity and binding of [14C]-deprenyl to rat liver MAO*

Titration and binding estimation were performed s described in METHODS. Activity in the radioisotope ssay with [-1 14C]-phenylethylamine, ●——●, and ith [1-14C]-5-hydroxytryptamine, ×——× binding of 14C]-deprenyl, O——O.

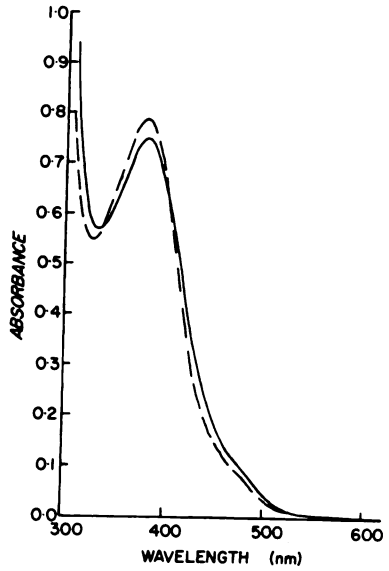

FIG. 6. *Spectra of the flavin peptides obtained om liver MAO after inactivation by clorgyline and eprenyl*

Highly purified MAO from beef liver (14.3 mg) in 0 mM phosphate, pH 7.2, was incubated with 1 μmole f unlabelled deprenyl or an excess of [14C]-clorgyline 3.7 μmoles) for 10 min at 37°. Inhibition of deprenyl as complete in 10 min; inhibition by clorgyline ached 98% at 40 min. After precipitation with an ual volume of 10% (w/v) trichloroacetic acid, both action mixtures were digested and the flavin peptide ducts isolated as in previous work (10). Solid line, orgyline adduct; dashed line, deprenyl adduct.

that a closely related acetylenic amine, N,N-dimethylpropargylamine forms a flavocyanine adduct with the cysteinyl flavin component of the enzyme (10). It was predicted that clorgyline and deprenyl would form analogous flavocyanines on inactivation of the enzyme (9). A simple test of this prediction was to isolate the flavin peptide-inhibitor adduct and measure its absorption spectrum. Flavocyanines are readily recognized by a prominent absorption peak at 380–390 nm with a very high extinction coefficient (ϵ_{380} = ~34,000) (10).

Figure 6 shows the absorption spectra of the flavin peptides isolated from beef liver MAO inactivated by clorgyline and by deprenyl. The spectra are typical of flavocyanines.

Rat liver MAO inactivated with deprenyl shows similar evidence (23, 25). Although the maximum appears to be at a somewhat higher wavelength in this case (405 nm), there seems to be little doubt that the structure is a flavocyanine in this instance also.

DISCUSSION

The results of the titration of the soluble and membrane-bound enzyme of beef liver are characteristic of the "B" type of MAO. It may also be seen that no qualitative difference was observed in the inhibition curves in outer membranes and in soluble preparations (cf. parts A and B in Fig. 1). On the other hand, deprenyl regularly gave a lower I_{50} value in membranes than with the purified soluble enzyme, but the reverse was true of clorgyline. The probable explanation is as follows. Deprenyl is a stoichiometric inhibitor binding only at one site (cf. below). Since the amount of MAO present in the outer membrane samples used for assay was considerably less than that present in the purified enzyme samples, it took less deprenyl to saturate the binding site in membranes. Contrariwise, as documented here, clorgyline binding is not stoichiometric with the enzyme; in other words, spurious binding sites unrelated to the inhibition of MAO exist in outer membranes. Hence, the much higher concentrations of impurities in outer membrane preparations are apt to reduce the effective concentration of clorgyline available for binding at

the flavin site, leading to seemingly higher I_{50} values than in highly purified samples. This pattern was confirmed with the other substrates, not shown, which included epinephrine, tyramine, phenylethylamine, norepinephrine, tryptamine, and kynuramine in the case of the soluble enzyme and tyramine and tryptamine for the outer membranes.

These data further show that the marked quantitative and qualitative alteration of lipids involved in the preparation of the soluble enzyme do not evoke major changes in the sensitivity of the enzyme to deprenyl or clorgyline except as already noted; hence, lipid seems to play no significant role in the B type behavior of this enzyme preparation.

However, removal of the enzyme from the membrane does alter its response to very low concentration (10^{-9}–10^{-8} M) of inhibitors.

The titrations of the outer membrane with the substrates benzylamine and 5-hydroxytryptamine showed 5–10% enzyme activation in the low inhibitor concentration range (Fig. 1B) but in the coupled assay of outer membranes with dopamine (Fig. 1B) and with tryptamine and tyramine (not shown) 10–15% inhibition was seen. This is believed to result from perturbation of the membrane by inhibitors rendering the membrane-bound MAO more exposed to the buffer and substrate. In the benzylamine and 5-hydroxytryptamine assays, all incubations were done in non-inhibiting phosphate buffers and activation resulted, while dopamine, tryptamine and tyramine assays were all done in tris/glycine buffer which is weakly inhibiting.

The experiments described leave little doubt that the beef liver enzyme is of the B type. This conclusion is confirmed by the substrate specificity of the enzyme (Table 1). It is clear that B type substrates are oxidized most rapidly. The fact that some A type substrates are also oxidized at measurable rates does not contradict the above conclusion but suggests that the distinction between A and B substrates is not absolute but is a matter of rates. With the exception of tryamine, the relative rates of substrate oxidation did not alter markedly upon solubilization (Table 1). As almost nothing is known about the alteration of the catalytic properties of MAO upon solubilization, the significance of the increased rate of tyramine oxidation remains obscure.

TABLE 1

Relative rates of oxidation of substrates by MAO from bovine liver

Coupled assay with aldehyde dehydrogenase. Substrates were present at 3.3 mM concentration.

Substrate	Type	Relative rate	
		Soluble enzyme	Outer membrane
Benzylamine	B	100	100
Tyramine	A and B	80	54
Tryptamine	A and B	45	32
2-Phenylethylamine	B	45	
Dopamine	A and B	44	43
5-Hydroxytryptamine	A	19	
Epinephrine	A	9	
Norepinephrine	A	6	

From the work reported here it is apparent that the differences in the behavior of MAO from rat and beef liver are not artifacts, but reflect a genuine biological divergence since the comparison of inhibitor response was performed under nearly identical conditions with the enzyme preparations from the two species. The results also confirm the expectation that the same type of flavocyanine adduct is formed with clorgyline and deprenyl as with the model compound N,N-dimethylpropynylamine which we had used earlier (10).

The results do not permit a decision as to whether the absence of the A type of MAO from certain soluble preparations isolated by drastic procedures (4, 7) is the consequence of selective removal of lipids, as suggested by Tipton, *et al.* (4) but denied by Oreland (4, 7); to a change in the conformation of the protein during purification by such methods (4); or to selective inactivation of the A form (7). All three possibilities remain viable alternatives, but, in our opinion, compelling evidence for none of them has been presented so far.

REFERENCES

1. Youdim, M. B. H., Collins, G. G. S. & Sandler, M. (1969) Multiple forms of rat brain monoamine oxidase. *Nature*, **223**, 626–628.
2. Johnston, J. P. (1968) Some observations upon

new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.*, **17**, 1285–1297.

3. Knoll, J. & Magyar, K. (1972) Some puzzling pharmacological effects of monoamine oxidase inhibitors, in *Advances in Biochemical Pharmacology*, Vol 5. (I. Costa and P. Greengrad, eds.). Raven Press, New York, 393–408.
4. Tipton, K. F., Houslay, M. D. & Mantle, T. J. (1976) The nature and locations of the multiple forms of monoamine oxidase, in *Monoamine Oxidase and its inhibition.* Ciba Foundation Symposium 39 (new series). Elsevier, Amsterdam, 5–15.
5. McCauley, R. & Racker, E. (1973) Separation of two monoamine oxidases from bovine brain. *Molec. Cellular Biochem.*, **1**, 73–81.
6. Gomes, B., Igaue, I., Kloepfer, H. G. & Yasunobu, K. T. (1969) Isolation and characterization of the multiple beef liver amine oxidase components. *Arch. Biochem. Biophys.*, **132**, 16–27.
7. Ekstedt, B. & Orland, L. (1976) Effect of lipid depletion on the different forms of monoamine oxidase in rat liver mitochondria. *Biochem. Pharmacol.*, **25**, 119–124.
8. Salach, J. I. (1978) Preparation of monoamine oxidase from beef liver mitochondria, in *Methods in Enzymology* Vol. 53. (S. Fleisher and L. Packer, eds.). Academic Press, New York, 495–501.
9. Youdim, M. B. H. (1975) Monoamine oxidase active site; the binding to and titration of monoamine oxidase with [^{14}C]-selective inhibitors. *Brit. J. Pharmacol.* **56**, 375.
0. Maycock, A. L., Abeles, R. H., Salach, J. I. & Singer, T. P. (1976) The structure of the covalent adduct formed by the interaction of 3-dimethylamino-l-propyne and the flavin of mitochondrial amine oxidase. *Biochemistry,* **15**, 114–125.
1. Kearney, E. B., Salach, J. I., Walker, W. H., Seng, R. L., Kenney, W., Zeszotek, E. & Singer, T. P. (1971) The covalently bound flavin of hepatic monoamine oxidase. *Eur. J. Biochem.*, **24**, 321–327.
2. Hawkins, J. (1952) The localization of amine oxidases in the liver cell. *Biochem. J.*, **50**, 577–581.
3. Youdim, M. B. H. & Collins, G. G. S. (1971) The dissociation and reassociation of rat liver mitochondrial monoamine oxidase. *Eur. J. Biochem.*, **18**, 73–78.
4. Tabor, C. W., Tabor, H. & Rosenthal, S. M. (1954) Purification of amine oxidase from beef plasma. *J. Biol. Chem.*, **208**, 645–661.
15. Jain, M., Sands, F. & Von Korff, R. W. (1973) Monoamine oxidase measurements using radioactive substrates. *Anal. Biochem.*, **52**, 542–554.
16. Tipton, K. F. & Youdim, M. B. H. (1976) Assay of monoamine oxidase, in *Monoamine Oxidase and its Inhibition.* Ciba Foundation Symposium 39 (new series). Elsevier, Amsterdam, 393–403.
17. Houslay, M. D. & Tipton, K. F. (1973) The nature of the electrophorectically separable multiple forms of rat liver monoamine oxidase. *Biochem. J.*, **135**, 173–186.
18. Penefsky, H. S. (1977) Reversible binding of Pi by beef heart mitochondrial adenosine triphosphatase. *J. Biol. Chem.*, **252**, 2891–2899.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
20. Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) Determination of serum proteins by means of the buiret reaction. *J. Biol. Chem.*, **177**, 751–766.
21. Neff, N. H. & Fuentes, J. A. (1976) The use of selective monoamine oxidase inhibitor drugs for evaluation of pharmacological and physiological mechanisms, in *Monoamine Oxidase and its Inhibition.* Ciba Foundation Symposium 39 (new series). Elsevier, Amsterdam, 163–173.
22. Chuang, H. Y. K., Patek, D. R. & Hellerman, L. (1974) Mitochondrial monoamine oxidase. *J. Biol. Chem.*, **249**, 2381–2384.
23. Youdim, M. B. H. (1976) Rat liver mitochondrial monoamine oxidase-an iron requiring flavoprotein, in *Flavins and Flavoproteins.* Proceedings of the Fifth International Symposium. (T. P. Singer, ed.). Elsevier, Amsterdam, 593–604.
24. Youdim, M. B. H. & Sourkes, T. L. (1972) The flavin prosthetic group of purified rat liver mitochondrial monoamine oxidase, in *Advances in Biochemical Pharmacology* Vol. 5. (E. Costa and P. Greengard, eds.). Raven Press, New York, 393–408.
25. Youdim, M. B. H. & Salach, J. I. The active site of monoamine oxidase, binding with [^{14}C]-acetylenic and non-acetylenic inhibitors, in *Enzyme-Activated Irreversible Inhibitors* (N. Seiler, M. J. Jung, and J. Koch-Weser, eds.). Elsevier/North Holland Biomedical Press, Amsterdam, 235–251.