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The effect of lipid-depletion on the kinetic properties of rat liver monoamine oxidase-B

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The extraction of lipids from rat liver mitochondrial membranes by 2-butanone treatment inhibited the activity of membrane-bound monoamine oxidase -A but not -B. For the -B form, the apparent Michaelis constants of the enzyme towards oxygen and the maximum molecular turnover numbers obtained when β-phenethylamine and benzylamine were used as substrates were not significantly changed by the lipid-depletion procedure, but the values of the Michaelis constant towards benzylamine was significantly increased after lipid-depletion. The differential sensitivity of β-phenethylamine and benzylamine oxidation to inhibition by Tris-HCl was not changed after lipid-depletion. The results are consistent with the hypothesis that the mitochondrial membrane lipids, while essential for the activity of the -A form of the enzyme in rat liver, play a more subtle modulatory role in the activity of the -B form.

Monoamine oxidase (MAO, monoamine O_2: oxidoreductase, EC 1.4.3.4) is thought, on the basis of the sensitivity to inhibition by substrate-selective inhibitors, such as the acetylenic compounds clorgyline and (−)-deprenil, to exist as two forms, termed MAO-A and MAO-B (for review, see Fowler et al 1978). The activity of MAO-A is inhibited by low concentrations of clorgyline, but is fairly resistant to inhibition by (−)-deprenil, whereas the reverse is true for MAO-B (Johnston 1968; Knoll & Magyar 1972). In the rat liver, 5-hydroxytryptamine (5-HT) is metabolized by MAO-A alone, β-phenethylamine and benzylamine by MAO-B alone, and tyramine by both forms of the enzyme (Hall et al 1969; Houslay & Tipton 1974). Furthermore, clorgyline and (−)-deprenil, which act as 'suicide' inactivators of MAO (for review, see Rando 1974), are so potent as inhibitors of the respective forms, that they have been used to 'titrate' the concentrations of the MAO-A and -B in a variety of tissues (Egashira et al 1976b; Fowler & Callingham 1979; Oreland & Fowler 1979).

Treatment of mitochondrial membranes with 2-butanone, involving a two-step extraction procedure (Hollunger & Oreland 1970) produces, in the case of pig liver, a 40% release of MAO activity into the high-speed supernatant (Oreland 1971; Severina 1973), which is also found for the rat liver, albeit to a lesser extent. If only the first part of the extraction procedure is used, an effective removal of lipids from the mitochondrial membranes is achieved without release of the MAO into the high-speed supernatant (Oreland & Olivecrona 1971; Olivecrona & Oreland 1971). This procedure, however, inactivates selectively the -A form of the enzyme (Ekstedt & Oreland 1976a,b).

In this study, lipid-depletion of rat liver mitochondrial membranes by use of the first step of the 2-butanone extraction procedure has been undertaken in an attempt to determine whether or not the kinetic properties of MAO-B are dependent upon the lipid environment of the enzyme. A preliminary report of this work has been presented (Fowler & Oreland 1979).

METHODS

The radioactive substrates for MAO, 5-hydroxytryptamine[side chain-2-^{14}C]binoxalate, tyramine-[ethyl-1-^{14}C]hydrochloride and β-phenethylamine-[ethyl-1-^{14}C]hydrochloride were obtained from New England Nuclear, Boston, Mass., USA. Benzylamine-[methylene-^{14}C]hydrochloride was obtained from ICN Pharmaceuticals Inc., Irvine, CA, USA. All non-radioactive substrates, apart from benzylamine, were bought as salts, as their purity over a long period could be guaranteed better than the free bases, and substrate contamination has been shown to produce inaccuracies in kinetic experiments (see Tipton et al 1977). Benzylamine was converted to its hydrochloride by dissolution of the free base in ethyl acetate and addition of concentrated hydrochloric acid. The insoluble hydrochloride was recrystallized in ethanol–ethyl acetate.

Clorgyline hydrochloride was a gift from May and Baker Ltd., Dagenham, U.K. (−)-Deprenil hydro-

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chloride was a gift from Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary. Human serum albumin, desiccated before use, was obtained from Kabi AB, Stockholm, Sweden. All other reagents were of analytical grade wherever possible. Male Sprague-Dawley rats were obtained from Anticimex AB, Stockholm, Sweden.

Preparation of mitochondrial membranes

Six rats, body weight 250–256 g, were killed by a blow to the head, and the livers rapidly removed, blotted on filter paper, weighed, and homogenized 1 : 8 (w/v) in 'sucrose buffer' (0·25 M sucrose, 10 mM potassium phosphate, pH 7·8) in an MSE Atomix blender at setting 1 for 10 s. The homogenates were centrifuged at 600 *g* for 15 min to remove nuclei and cell debris, and the resulting supernatants then centrifuged at 6500 *g* for 20 min. The pellets were washed once by resuspension followed by recentrifugation at 6500 *g* for 20 min. The washed pellets were resuspended in sucrose buffer, and pooled to make three preparations, each derived from the livers of two rats ('control mitochondrial membranes'). This procedure has been shown to produce a preparation of mitochondrial membranes with only moderate contamination with microsomal, lysosomal and mitochondrial matrix marker enzymes (Fowler 1978), presumably due to the shearing action of the Atomix homogenizer.

Preparation of lipid-depleted membranes

Lipid-depletion of the mitochondrial membranes by the first step of the 2-butanone extraction procedure was carried out essentially as described by Hollunger & Oreland (1970). Briefly, 3 ml of each control mitochondrial membrane preparation was centrifuged at 12000 *g* for 10 min, and the pellets (approximately 9 mg protein) resuspended in sucrose buffer to a volume of 1 ml. To each suspension was added, at 4 °C with stirring, 8 ml of 2-butanone over 5 min. The 2-butanone was then discarded to leave a residue which was then resuspended in 10 ml of sucrose buffer, centrifuged at 12000 *g* for 10 min, and the pellets resuspended in 3 ml sucrose buffer to give the lipid-depleted mitochondrial membrane preparations. Both control and lipid-depleted preparations were stored frozen until used for assay.

Monoamine oxidase assay

Monoamine oxidase was assayed radiochemically by the method of Callingham & Laverty (1973), with toluene instead of benzene in the organic layer, and sucrose buffer instead of 200 mM potassium phosphate, pH 7·8, as the buffer in the incubation medium (see Fowler et al 1979). Incubation times were chosen so that in all cases the measured reaction velocity was as near as possible to the initial velocity. All experiments, unless otherwise stated, were carried out under an atmosphere of air. In experiments where the concentration of oxygen was varied, the assay mixtures (100 μl final volume in 15 ml centrifuge tubes) were gassed with different mixtures of oxygen and nitrogen (the partial pressure of the oxygen ranging from 15–100% of the gas mixture) at a flow rate of 55 ml s^{-1} for 3 s before stoppering the tubes with rubber stoppers, and allowing the gas and liquid phases to equilibrate. The oxygen concentrations in the membrane preparations were taken to be directly proportional to the partial pressure of oxygen in the gas phase, the concentration of oxygen in distilled water at equilibrium with air at 37 °C (0·217 mM) being taken as reference (Dixon & Kleppe 1965; Della Corte 1975).

When clorgyline and (−)-deprenil were used to inhibit the enzyme activity, they were preincubated with the enzyme preparations for 20 and 240 min, respectively, at 37 °C before addition of substrate. These preincubation times allow for the selective inhibition of MAO-A by clorgyline and MAO-B by (−)-deprenil to reach the irreversible phase of the enzyme-inhibitor reaction (Egashira et al 1976a). After the preincubation step, no further inhibitor was added to the reaction mixture to eliminate the possibility of further enzyme-inhibitor interactions taking place. Thus the concentrations of clorgyline and (−)-deprenil given throughout refer to those at preincubation. After 240 min of preincubation, there was a small loss of activity in the samples, but the degree of the loss was the same for both β-phenethylamine and benzylamine, and was not changed after lipid-depletion. In experiments where Tris-HCl was used to inhibit the activity of MAO, no preincubation was necessary (Browne et al 1973; Fowler 1978).

It has been reported for rat brain, heart, liver and vas deferens, that at high substrate concentrations there is a significant oxidation of β-phenethylamine by the -A form of MAO (Suzuki et al 1979; Kinemuchi et al 1979; Dial & Clarke 1979). Oxidation of 5-HT by MAO-β at high substrate concentrations has been found for pig liver (Ekstedt 1979) but not human liver (R. J. Mayer and C. J. Fowler, K. F. Tipton unpublished results). At the highest concentration of β-phenethylamine used in the present study (100 μM) at least 85% of the deamination of this substrate was brought about by MAO-B alone.

Specific activities of MAO, corrected for the

estimated efficiency of extraction of the deaminated metabolites into the organic layer used in the assay, are expressed throughout as nmol (of substrate deaminated) mg protein^{-1} min^{-1}. Protein content of the membrane preparations was determined by a modified Lowry procedure (Markwell et al 1978), as this method is not affected by high concentrations of sucrose. Human serum albumin was used as standard.

RESULTS

Lipid-depletion of the mitochondrial membranes produced a marked inhibition of the activity of MAO-A with little effect on the activity of MAO-B. The activity towards 5-HT (at a concentration of 100 μM) was reduced from 15·3 $\pm$ 0·5 to 3·2 $\pm$ 0·6 nmol mg protein^{-1} min^{-1} by the lipid-depletion procedure, whereas the activity towards β-phenethylamine at the same concentration was 27·5 $\pm$ 2·0 and 25·7 $\pm$ 1·1 nmol mg protein^{-1} min^{-1} for the control and lipid-depleted membranes, respectively. The activity towards benzylamine was 16·6 $\pm$ 1·3 and 20·0 $\pm$ 0·6 nmol mg protein^{-1} min^{-1} for the control and lipid-depleted membranes, respectively. The deamination of tyramine was also affected, but not to the same degree as 5-HT. The inhibition by clorgyline of the oxidation of tyramine by control and lipid-depleted membranes indicated that, while both forms of the MAO were present in the control membranes, the metabolism of tyramine was brought about by MAO-B alone in the lipid-depleted membranes (Fig. 1).

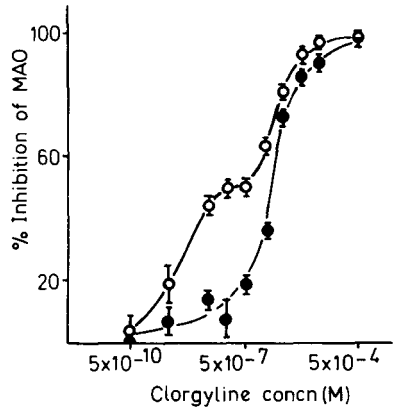

FIG. 1. The effect of clorgyline upon control and lipid-depleted membranes. Each point represents the mean $\pm$ s.e.m. of duplicate determinations of the % inhibition of MAO activity in 3 membrane preparations. Tyramine (100 μM) was used as substrate. Control (○) and lipid-depleted (●) membrane preparations were preincubated with clorgyline for 20 min at 37 °C before addition of substrate to assay for activity.

In both control and lipid-depleted membrane preparations, the oxidation of β-phenethylamine was inhibited to the same degree by Tris-HCl. For example, the inhibition of the oxidation of this substrate by 20 mM Tris-HCl was 51 $\pm$ 2% and 54 $\pm$ 3% for the control and lipid-depleted membranes, respectively. The oxidation of benzylamine was inhibited by the concentrations of Tris-HCl used, but to a much smaller extent (19 $\pm$ 1% and 16 $\pm$ 5% for 20 mM Tris-HCl in control and lipid-depleted membranes, respectively).

When both control and lipid-depleted membrane preparations were preincubated with different concentrations of (−)-deprenil for 240 min at 37 °C, the inhibition of MAO activity towards either β-phenethylamine or benzylamine increased in a manner linear with increasing concentrations of (−)-deprenil (Fig. 2A and B). From the concentrations of inhibitor required to produce 100% inhibition of enzyme activity, the concentrations of MAO-B in the two fractions could be determined, and are shown in Table 1. The enzyme concentrations in the lipid-

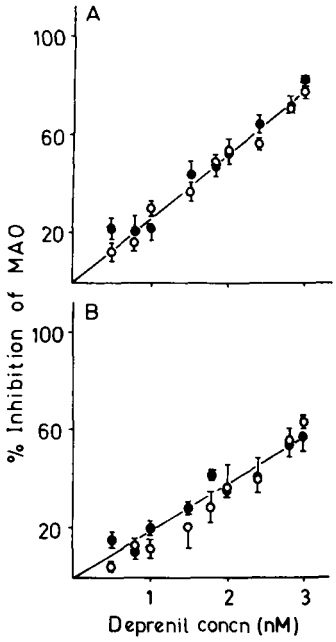

FIG. 2. The effect of (−)-deprenil upon the activity of MAO-B in A, control; B, lipid-depleted membrane preparations. Each point represents the mean $\pm$ s.e.m. of duplicate determinations of the % inhibition of MAO-B activity in three preparations. The enzyme preparations were preincubated with inhibitor for 240 min before addition of substrate. The protein concentration of the fractions at preincubation was set to 0·25 mg ml^{-1}. Substrates used: 100 μM β-phenethylamine (○); 100 μM benzylamine (●).

Table 1. Concentrations and maximum molecular turnover numbers of MAO-B from control and lipid-depleted membranes. Enzyme concentrations were determined from the amount of (−)-deprenil required to produce 100% inhibition of activity, calculated from the lines of best fit (with correlation coefficients in all cases higher than r = 0·91) to duplicate determinations of the inhibition of MAO activity at 9 concentrations of (−)-deprenil, and divided by the protein concentration. Preincubation time: 240 min. 100 μM substrate was used to assay for activity after preincubation. Preincubation protein concentration was set to 0·25 mg ml^{-1}. Max molecular turnover numbers were calculated as V_{max} (data summarized in Table 2)/enzyme concentration. All values in the Table are means ± s.e.m. of determinations in three membrane preparations. Values for the enzyme concentrations for either control or lipid-depleted membranes when β-phenethylamine was used as substrate were not significantly different from when benzylamine was used ($P > 0·10$, two-tailed paired t-test).

	Enzyme concn (pmol mg protein^{-1})	Max molecular turnover number (mol mol MAO^{-1} min^{-1})
β-Phenethylamine		
control	15·8 ± 0·1	6719 ± 654
lipid-depleted	20·3 ± 1·4*	5898 ± 949
Benzylamine		
control	15·2 ± 0·4	2734 ± 456
lipid-depleted	22·3 ± 2·3	2621 ± 519

* significantly higher than the value for control membranes ($P < 0·05$, two-tailed paired t-test).

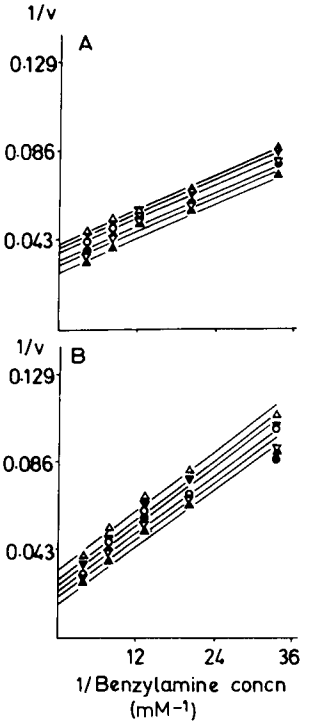

FIG. 3. Double reciprocal plots of the activity of rat liver MAO-B towards benzylamine in A, control; B, lipid-depleted membrane preparations. Abscissae: 1/benzylamine concentration in mM; ordinates: 1/initial velocity (in nmol mg protein^{-1} min^{-1}). Each point represents the mean of duplicate determinations of activity in three preparations of membranes. Oxygen concentrations were: 152 (△), 217 (▼), 271 (○), 434 (●), 651 (▽) and 1085 μM (▲).

depleted fractions were higher than in the control membranes, and did not depend upon the MAO-B substrate used to assay for activity.

In both control and lipid-depleted membrane preparations, the activity of MAO-B was increased in an apparent uncompetitive manner with increasing concentrations of oxygen (see Figs 3 and 4, A and B). Similar uncompetitive increases in activity with increasing amine concentrations were also found when these data were replotted as 1/initial velocity against 1/oxygen concentration. The kinetic parameters derived from these experiments are summarized in Table 2. In control membranes, the values of the apparent Michaelis constants towards oxygen were significantly higher ($P < 0·05$, two-tailed paired t-test) when β-phenethylamine was used to assay for enzyme activity than when benzylamine was used. A similar result was found for the lipid-depleted preparations, although there was a larger scatter of the points when β-phenethylamine was used as substrate. There were no significant differences in the K_0 values for either substrate between the control and lipid-depleted membrane preparations. Maximum molecular turnover numbers, calculated as V_{max}/enzyme concentration, for either substrate, were unchanged after lipid-depletion (Table 1).

When benzylamine was used as substrate, the value of the apparent Michaelis constant of the enzyme towards the amine substrate (K_{am}) was significantly higher ($P < 0·05$, two-tailed paired t-test) in the lipid-depleted membranes than in the control membranes (Table 2, Fig. 3A and B). A similar pattern was also found when β-phenethylamine was used as substrate (Table 2, Fig. 4A and B).

DISCUSSION

Monoamine oxidase has been shown to be functionally dependent for its activity upon its mitochondrial membrane environment (for review, see Houslay et al 1976). In rat hepatoma mitochondria, introduction of phosphatidylcholine into the mitochondria has been shown to increase the activity of MAO,

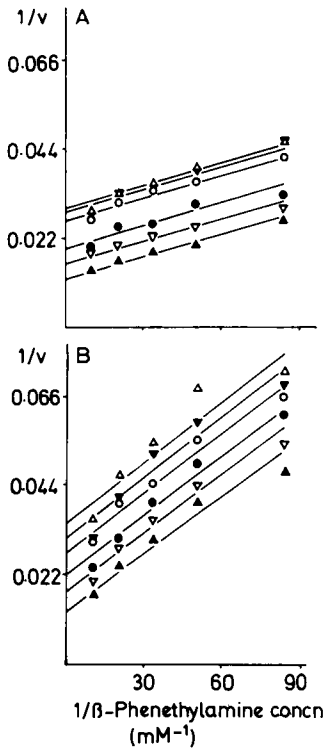

FIG. 4. As for Fig. 3, with β-phenethylamine as substrate instead of benzylamine.

Table 2. Kinetic paramteers of control and lipid-depleted MAO-B. K_{am} values were determined by linear regression analysis of the initial velocities at infinite oxygen concentration for each amine substrate concentration, plotted as S/v against S. K_o values were determined in the same way from plots of initial velocities at infinite amine concentration for each oxygen concentration. V_{max} values obtained from the two methods were in all cases within 7% of each other, and the average value was taken. Initial velocities at the infinite first or second substrate concentration were determined by linear regression analysis from duplicate determinations of activity at a fixed concentration of one substrate and a varying concentration of the other, plotted as S/v against S. In all cases, 6 oxygen and 5 amine concentrations were used, and the correlation coefficients for the linear regression analysis were higher than r = 0·93. The values shown in the Table represent means ● s.e.m. of determinations in 3 membrane preparations. Concentration ranges used were: 152–1085 μM (oxygen), 12–100 μM (β-phenethylamine) and 30–250 μM (benzylamine). In the control membranes, the K_o values for β-phenethylamine as substrate were significantly higher than for benzylamine as substrate ($P < 0·05$, two-tailed paired t-test).

	V_{max} (nmol mg prot^{-1} min^{-1})	K_{am} (μM)	K_o (μM)
β-Phenethylamine			
control	106 ± 10	18 ± 5	351 ± 32
lipid-depleted	122 ± 27	57 ± 24	447 ± 109
Benzylamine			
control	41 ± 6	68 ± 12	142 ± 13
lipid-depleted	56 ± 5	123 ± 4*	141 ± 10

* Significantly higher than control value ($P < 0·05$, two-tailed paired t-test).

whereas phosphatidylethanolamine, phosphatidylserine and cardiolipin were without effect (Dyatlovitskaya et al (1977). After 2-butanone extraction of pig brain mitochondria, the sensitivity of MAO to inhibition by Δ-tetrahydrocannabinol is lost, but is regained after addition of phosphatidylcholine to the lipid-depleted mitochondria (Schurr et al 1978). Furthermore, palmitoyl- and myristoyl-lysolecithin have been demonstrated to act as MAO-B selective inhibitors in the rat liver (Houslay 1978). The lipid-dependence of MAO has also been demonstrated in vivo: there is a decreased MAO activity in the brains of lipid-deficent rats, which can be restored to normal levels upon the addition to the diet of linoleic and linolenic acids (Bernsohn & Spitz 1974). Phospholipids also appear to be important for the binding of MAO to lipid-depleted mitochondrial membranes (Olivecrona & Oreland 1971; Oreland & Olivecrona 1971; Ekstedt et al 1975).

Extraction of rat liver mitochondrial membrane lipids by 2-butanone appears to inactivate MAO-A but not MAO-B (Fig. 1) as reported previously (Ekstedt & Oreland 1976a; Verevkina et al 1977; Sawyer & Greenawalt 1979), which would agree with the suggestion made earlier that the presence of membrane lipids is essential to the activity of MAO-A but not MAO-B in the rat liver (Ekstedt & Oreland 1976a). A similar conclusion can be derived from experiments with the chaotropic agent sodium perchlorate (Houslay & Tipton 1973a; Tipton et al 1976; Verevkina et al 1977). However, 2-butanone treatment does not extract all the mitochondrial membrane phospholipids to the same extent, the membrane concentration of the acidic phospholipids (in particular cardiolipin) being little affected by this procedure (Ekstedt & Oreland 1976a; Sawyer & Greenawalt 1979). Thus if the activity of MAO-B were dependent upon the presence of these acidic phospholipids alone, 2-butanone extraction would not be expected to influence the activity of this enzyme form. However, if this were the case, rigorous treatment with the chaotropic agents might be expected to inactivate the -B form of the enzyme. Such

an inactivation of MAO-B by these agents is not found (Houslay & Tipton 1973a; Tipton et al 1976; Verevkina et al 1977). The residual activity towards 5-HT found in the present study after lipid-depletion could be due to either a small remaining activity of MAO-A, or else an indication that a small proportion of the 5-HT is metabolized by MAO-B. The lipid-sensitivity of MAO-A shown here for rat liver, and also found for pig liver (Ekstedt & Oreland 1976b), is not, however, found in all tissues. For example, MAO-A from human placenta has been purified by methods that are likely to interfere with the enzyme-lipid interactions, but do not inhibit the activity of this enzyme form (Powell & Craig 1977; Zeller & Gurne 1979). The converse is also true: in human platelets, 2-butanone extraction has been shown to inactivate most of the MAO-B (Fowler et al 1979).

Tris buffers (pH 7·8) have been shown to inhibit the oxidation of 5-HT, tyramine and β-phenethylamine but not benzylamine by rat liver MAO (Browne et al 1973; Fowler et al 1977). Although the precise mode of inhibition is not fully elucidated, it is thought to be due to a direct effect of Tris on the enzyme, rather than due to complex formation or anion effects (Fowler et al 1977). The experiments reported in the present study would also suggest that the differential sensitivity of β-phenethylamine and benzylamine oxidation to inhibition by Tris buffer is not due to the presence of membrane lipids.

(−)-Deprenil belongs to a class of acetylenic inhibitors of MAO that cause inhibition by a reversible association with the active centre of the enzyme followed by a 'suicide' reaction to form a covalent adduct with the flavine prosthetic group (see Hellerman & Erwin 1968; Oreland et al 1973; Rando 1974; Maycock et al 1976). (−)-Deprenil is so potent an inhibitor of the activity of MAO-B that the concentration of the free inhibitor is depleted by the formation of the reversible and irreversible enzyme-inhibitor complexes. After 240 min of preincubation, when all reactions between enzyme and inhibitor have stopped (Egashira et al 1976a,b), (−)-deprenil effectively titrates the concentration of MAO-B, and the degree of inhibition of enzyme activity is linear with inhibitor concentration, as found in this study for both control and lipid-depleted membrane fractions (Fig. 2A and B). The concentration of enzyme active centres in the membranes can be calculated from the concentration of inhibitor required to produce 100% inhibition of enzyme activity, divided by the protein concentration (see Fowler & Callingham 1979). Under the conditions used, the degree of non-specific binding of (−)-deprenil to structures other than the active centre of MAO-B is thought to be minimal, thus allowing reasonable estimations to be made of the enzyme concentrations in the control and lipid-depleted membrane fractions (Table 1). The concentration of MAO-B active centres determined in this way is the same when β-phenethylamine is used as the substrate as when benzylamine is used, which would suggest that both substrates are metabolized by the same enzyme 'pool' in the membranes, especially as lipid-depletion appears to affect the enzyme concentration towards both substrates to the same degree (Table 1). This increased concentration of MAO-B after lipid-depletion is most likely due to the removal of membrane contaminants during the extra centrifugation steps resulting in a higher MAO : protein ratio, rather than an unmasking of previously occluded active centres by the extraction procedure. It has been demonstrated for rat liver MAO that the oxidation of β-phenethylamine is inhibited in a competitive manner by benzylamine, and vice versa, under atmospheres of either oxygen or air, with K_i values of the amines acting as inhibitors similar to their K_m values (Fowler & Callingham 1978). These observations would suggest that the two MAO-B substrates used here are metabolized by the same enzyme active centres, and that the different Tris sensitivities found towards the two substrates are not due to enzyme heterogeneity.

In the rat liver, MAO follows a 'ping-pong', or double-displacement, reaction pathway (Houslay & Tipton 1973b). The kinetic parameters can be written as:

$$v = \frac{V_{max}}{1 + \frac{K_{am}}{[amine]} + \frac{K_o}{[oxygen]}}$$

(see e.g. Houslay & Tipton 1973b)

where K_{am} and K_o are the apparent Michaelis constants of the enzyme toward the amine substrate and oxygen, respectively. Thus, V_{max} is defined as the maximum velocity attainable when the enzyme is saturated with both substrates; K_{am} and K_o are the concentrations of the appropriate substrate required to produce half-maximum velocity when the enzyme is saturated with the other substrate. It should be stressed that these constants are not dissociation constants, but convenient groups of rate constants. As a result of this reaction mechanism, the activity of MAO is increased in an uncompetitive manner with increasing concentrations of oxygen. This appears to be true for MAO-B in both control and lipid-

depleted fractions with either benzylamine or β-phenethylamine as substrate, although there was rather a large scatter of the points for the lipid-depleted fractions when β-phenethylamine was used. The K_0 values found in this study (Table 2) are similar to those found elsewhere with different oxygen concentrations (Houslay & Tipton 1973b; Fowler & Oreland 1979). Lipid-depletion of the membranes does not appear to alter either the maximum molecular turnover numbers (Table 1) or the K_0 values of the enzyme assayed with either substrate (Table 2). Furthermore, after lipid-depletion, the K_0 value when β-phenethylamine was used remained higher than when benzylamine was used (Table 2). These differences in K_0 values do not, however, indicate heterogeneity of the enzyme form, but are merely a property of an enzyme following a ping-pong reaction pathway (Fowler & Oreland 1979; Roth 1979; see also Jarabak & Westley 1974).

Lipid-depletion of the mitochondria does, however, appear to produce an increase in the K_{am} value towards benzylamine and possibly β-phenethylamine (Table 2), which would suggest that the enzyme reaction pathway has undergone some sort of change as a result of lipid-depletion. Changes in the K_m values of rat heart and ox liver MAO-A and -B, respectively, after lipid-depletion have been reported (Yu 1979), although only a single concentration of oxygen was used in this study. A change in the reaction pathway of rat liver MAO-B has also been found after disruption of lipid-protein interactions by solubilization of the enzyme with the non-ionic detergent Triton X-100 (Houslay & Tipton 1975). However, this agent has been shown irreversibly to inhibit the activity of membrane-bound MAO in a temperature-dependent manner (Fowler et al 1980).

A possibility that cannot entirely be ruled out is that 2-butanone exerts its effects in a manner unconnected with its ability to remove lipids from the mitochondrial membrane. The binding of benzylamine and β-phenethylamine is thought to take place in a hydrophobic area of the enzyme active centre (Severina 1973), and it is possible that introduction of 2-butanone into this region produces a selective alteration of the K_{am} term. Such a change could also be produced if 2-butanone acted as a competitive inhibitor with respect to the amine substrate. However, for these effects to occur, binding of 2-butanone to the enzyme would have to be of a pseudo-irreversible nature, since most of this compound is removed by the washing included in the extraction procedure. Such a pseudo-irreversible interaction would be expected to affect the maximum molecular turnover numbers of the MAO-B towards its substrates, which it clearly does not (Table 1).

In conclusion, the data presented in this study would suggest that, in the rat liver, the lipid environment is essential to the activity of MAO-A, but plays a more subtle modulatory role on the activity of MAO-B. For a simple ping-pong bi bi enzyme, a change in the Michaelis constant for the first substrate without a corresponding change in either the maximum molecular turnover number towards that substrate or the Michaelis constant towards the second substrate is the result of a change in the binding of the first, but not the second substrate to the enzyme. However, MAO follows a much more complicated reaction pathway than the ping-pong bi bi mechanism (see Houslay & Tipton 1973b), and more experimentation is clearly necessary before such a conclusion can be applied to the effect of 2-butanone extraction on the K_{am} of rat liver MAO-B towards benzylamine and β-phenethylamine found in this study.

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