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EFFECTS OF NONDENATURATING ZWITTERIONIC DETERGENT CHAPS, 3-[(3-CHOLAMIDO PROPYL) DIMETHYL AMMONIO]-1-PROPANESULFONATE, ON RAT LIVER MITOCHONDRIAL AND MICROSOMAL MONOAMINE OXIDASE

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Abstract—It has been reported that monoamine oxidase (MAO) activity (EC1.4.3.4) and, in general, enzymes possessing cationic substrates, were activated and inhibited by anionic and cationic detergents, respectively. In order to examine this hypothesis, the effect of the zwitterionic detergent CHAPS 3-[(3-cholamido propyl) dimethyl ammonio]-1-propanesulphonate was studied in comparison with the effects of cationic, anionic, and non-ionic detergents.

The non-denaturating zwitterionic detergent CHAPS was used to solubilise rat liver monoamine oxidase MAO (EC1.4.3.4) of mitochondrial and microsomal origin; the solubilisation conditions, purification, inhibition and kinetic studies were then determined. These results are compared with those previously obtained with the non-ionic detergent Triton X-100, which would also be expected to have no net charge, and are interpreted in terms of specific ionic effects.

Monoamine oxidase, MAO (amine: oxygen oxidoreductase, EC1.4.3.4), has been found up to the present in many animal tissues [1, 2] and is predominantly located in the mitochondrial outer membrane [3]. This enzyme is responsible for the deamination of aromatic amines in peripheral tissues and in the brain, and has two forms. MAO-A is defined as being sensitive to inhibition by clorgyline [4], and MAO-B as being selectively inhibited by (−)-deprenyl [5]. These two forms have also been characterised in terms of the specificities of their substrates [6–9]. The enzyme has also been localised in the microsomal fraction [10–12] and some authors have suggested that this may be due to mitochondrial contamination arising during the homogenation process [13]. It has, however, also been suggested that microsomal MAO could be a precursor form of the mitochondrial enzyme [14]. Other authors [15] have suggested that MAO activities present in both subcellular organelles could be closely related forms. Wojtczak [18] has reported the activity of MAO to be critically dependent on the surface charge of the mitochondrial outer membranes, and thus to respond in defined ways to detergents of different charge characteristics. The present studies using anionic, zwitterionic, uncharged and cationic detergents, were designed to test this hypothesis. The membrane proteins are difficult to solubilise unless vigorous procedures, such as sonication freezing/thawing or detergents (see 16, 17), are used.

For quite some time, the most common non-ionic detergent used to solubilise MAO activity has been Triton X-100 (see 19). Nevertheless, it binds strongly to the protein, which can alter the lipid microenvironment and modify the catalytic properties of the enzyme [20–22]. Other common detergents used are bile salts, which can alter the charge properties of the soluble enzyme.

A new non-denaturating zwitterionic detergent, the 3-[(3-cholamidopropyl)-dimethyl-amonio]-1-propanesulfonate (CHAPS) [23, 24], which combines the useful properties of both the sulfobetaine-type detergents as well as the bile salt anions, was selected to solubilise MAO activity in rat liver mitochondria and microsomes. This new detergent presents a high critical micellar concentration (CMC value), is easy to remove by dialysis, and has been reported to be effective in solubilising membrane proteins in a non-denaturating, disaggregated and electrically neutral state [23, 24].

MATERIALS AND METHODS

5-Hydroxy (side-chain-2-^{14}C) tryptamine creatinine sulphate was purchased from Amersham International (Amersham, U.K.); phenylethylamine hydrochloride β-(ethyl-1-^{14}C) was purchased from New England Nuclear (Boston, MA). The 3-[(3-cholamidopropyl)-dimethyl-amino)]-1-propane sulfonate (CHAPS), sodium deoxycolate, sodium taurocholate, sodium dodecyl sulfate, and lauryl-D-maltoside were all purchased from Fluka A.G. (Germany). Tetradecyltrimethylammonium bromide and β-D-octylglucoside were obtained from Sigma (St. Louis, MO). Cetyltrimethylammonium bromide was purchased from Serva (Germany). [^{3}H]-Pargyline was obtained from New England Nuclear (Boston, MA). All other compounds were standard analytical grade laboratory reagents.

Male Sprague–Dawley rats (150–250 g), starved

overnight, were killed by stunning followed by decapitation, and the liver was subsequently removed. The tissue was homogenized (20 ml/g tissue) in sucrose (0·25 M) and centrifuged twice for 10 min at 600 *g* in order to remove unbroken cells and cellular debris. The supernatant was centrifuged again at 8000 *g* for 10 min in order to sediment the mitochondrial fraction (Mt). The resultant supernatant was then centrifuged at 20,000 *g* for 10 min, and the pellet was discarded. The supernatant was centrifuged again at 200,000 *g* for 30 min in order to obtain the microsomal fraction (Mc). Mitochondria and microsomes were washed twice by suspension and centrifugation and finally re-suspended in phosphate buffer (50 mM pH 7.2), to give a final protein concentration of 10–15 mg/ml, and then stored at −20° for at least 48 hr.

For the enzyme solubilisation studies, mitochondria and microsomes were mixed with different concentrations of CHAPS and gently stirred for 30 min at 4°, before centrifugation of the samples for one hour at 100,000 *g* in the case of Mt and at 200,000 *g* in the case of Mc. The precipitates were re-suspended in phosphate buffer (50 mM pH 7.2), and both precipitate and supernatant fractions were assayed for MAO activity and protein concentration. In some cases, as indicated in the results, the supernatants were dialysed overnight against 100 vol. of buffer with one change after 2 hr. The dialysates were centrifuged at 100,000 *g* for 60 min and the pellets were re-suspended in a small volume of buffer and stored at −20°.

Protein concentration was determined by the Hartree method [25] with bovine serum albumin (Sigma) as standard.

MAO activity was assayed radiometrically by the method of Otsuka and Kobayashi [26] with 100 μM (^{14}C-5-hydroxytryptamine, 500 μCi/mmol) as substrate for MAO A and 20 μM (^{14}C-2-phenylethylamine, 2500 μCi/mmol) as substrate for MAO B, in a final volume of (225 μl) of phosphate buffer (50 mM pH 7.2) at 30°. The reaction was terminated by the addition of citric acid (2M) (100 μl), the products were extracted into toluene/ethylacetate (1:1 v/v) containing 0.6% (w/v) PPO, and were then counted in a Beckman LS 8000 liquid scintillation counter. In all cases reaction times were chosen to ensure that product formation remained linear. Times of 4–6 min and 10–20 min were used for determination of activities towards PEA and 5-HT, respectively.

Inhibition studies were carried out by pre-incubating membrane-bound and soluble samples at 30° for 30 min with appropriate dilutions of the inhibitors clorgyline and deprenyl.

The Lineweaver–Burk plot of CHAPS inhibition of 5-HT, as well as PEA deamination by mitochondrial and microsomal MAO, were determined using different concentrations of CHAPS as stated in the figures. The reaction was initiated with the addition of the enzyme to the substrate mixture containing the detergent.

In order to determine K_m values, solutions containing exclusively A or B type enzymatic activity were prepared. Membrane-bound and soluble enzymes, extracted by CHAPS (0.75% w/v) under optimal conditions: final protein concentration 4 mg/ml and overnight dialysis, were incubated for 2 hr at 30° with deprenyl or clorgyline (3×10^{-7} M) in order to inhibit MAO B or MAO A activity, respectively. After this, centrifugation was performed at 200,000 *g* for 45 min, the pellets were resuspended in phosphate buffer (50 mM, pH 7.2), and the MAO activity towards 5-HT and PEA was measured against a range of different substrate concentrations.

The reversibility of the CHAPS induced inhibition of MAO activity in Mc and Mt fractions was investigated by the dilution method as described by Fowler *et al.* [27]. The sample and detergent mixture was diluted to reduce the concentration of the enzyme and detergent. If the inhibition proved to be reversible, dissociation of the inhibitor would occur, and the specific activity would increase and have the same value as when incubation was performed with the diluted concentration of the detergent.

In order to carry out the binding assay with [^{3}H]-labelled pargyline, samples (25 μl) of mitochondria and microsomes containing 325 μg and 525 μg of protein, respectively, were incubated with 10 μl of clorgyline or deprenyl (1.5×10^{-6} M) for 60 min at 37° in a total volume of 50 μl in order to obtain MAO B and MAO A forms, respectively. Control samples were incubated under identical conditions with 5×10^{-3} M clorgyline to allow non-specific binding to be determined. After this, samples (50 μl) were incubated for 60 min at 37° with ^{3}[H]-pargyline containing 200 pmol for studies with the A-form. The samples were filtered and washed on Millipore filters and added to 4 ml of scintillation mixture. Other samples were solubilised with CHAPS 0.75% for 30 min and centrifuged at 200,000 *g* for 60 min. The supernatant was precipitated with TCA 20% and then was filtered and the distribution of the picomoles of pargyline bound to each fraction after solubilisation was determined.

RESULTS

Effects of detergents on MAO activity

The effects of unchanged (β-D-octylglucoside, lauryl-D-maltoside), anionic (sodium dodecyl sulphate, sodium deoxycholate, sodium taurocholate), zwitterionic (CHAPS), and cationic (cetyltrimethylammonium bromide, tetradecyltrimethylammonium bromide) detergents on the MAO activities in Mt and Mc fractions were studied. Two ranges of detergent concentrations were considered: one interval (0–100 μM) below the detergents critical micellar concentrations (CMC); another range (1–25 mM), which included the corresponding (CMC) values. Different sets of results were obtained in each case. In the first case, MAO A and MAO B activities present in Mt and Mc fractions were found to be preserved at detergent concentrations below their critical micellar concentration (data not shown). In the concentration range that spanned CMC values (1–25 mM), a differential effect of those detergents on MAO activities was found to occur.

As can be seen in Fig. 1A, the effect of surfactants on MAO B activity present in the Mt and Mc fractions depended on the detergent's charge. Cationic detergents at concentrations of 1 mM totally inhibited MAO B activity. The anionic detergent

sodium dodecyl sulphate produced, however, an activation of 400% in Mt and 300% in Mc fractions.

Nonetheless, the effect of the anionic detergent sodium deoxycholate was similar to that of the cationic detergents and produced total inhibition at a concentration of 10 mM. Sodium taurocholate was a poor inhibitor, concentrations above 25 mM being required to produce total inhibition. Non-ionic detergents, such as lauryl-D-maltoside, β-D-octylglycoside, and the zwitterionic detergent CHAPS, were poor inhibitors of MAO B activity, and detergent concentrations above 25 mM were required to produce total inhibition.

The influence of various detergents on MAO A is shown in Fig. 1B. Cationic detergents produced a total MAO A inhibition at concentrations of 1 mM. The anionic detergent sodium dodecyl sulfate produced an activation of 200% in both cases, but the other anionic detergents produced no significant activating effects. With the exception of the non-ionic detergent β-D-octyglycoside, where concentation of 25 mM was required to give total inhibition of MAO A, the bile salts and the zwitterionic detergent caused an inhibitory effect on MAO A activity in the range of 5–10 mM. It can be summarised that the cationic detergents inhibit both forms of MAO present in both fractions. The anionic detergent sodium dodecyl sulphate triggered both activities in the Mt and Mc fractions, but sodium deoxycholate and sodium taurocholate inhibited them. Non-ionic and zwitterionic detergents affected the MAO activities differentially, MAO A being more sensitive than

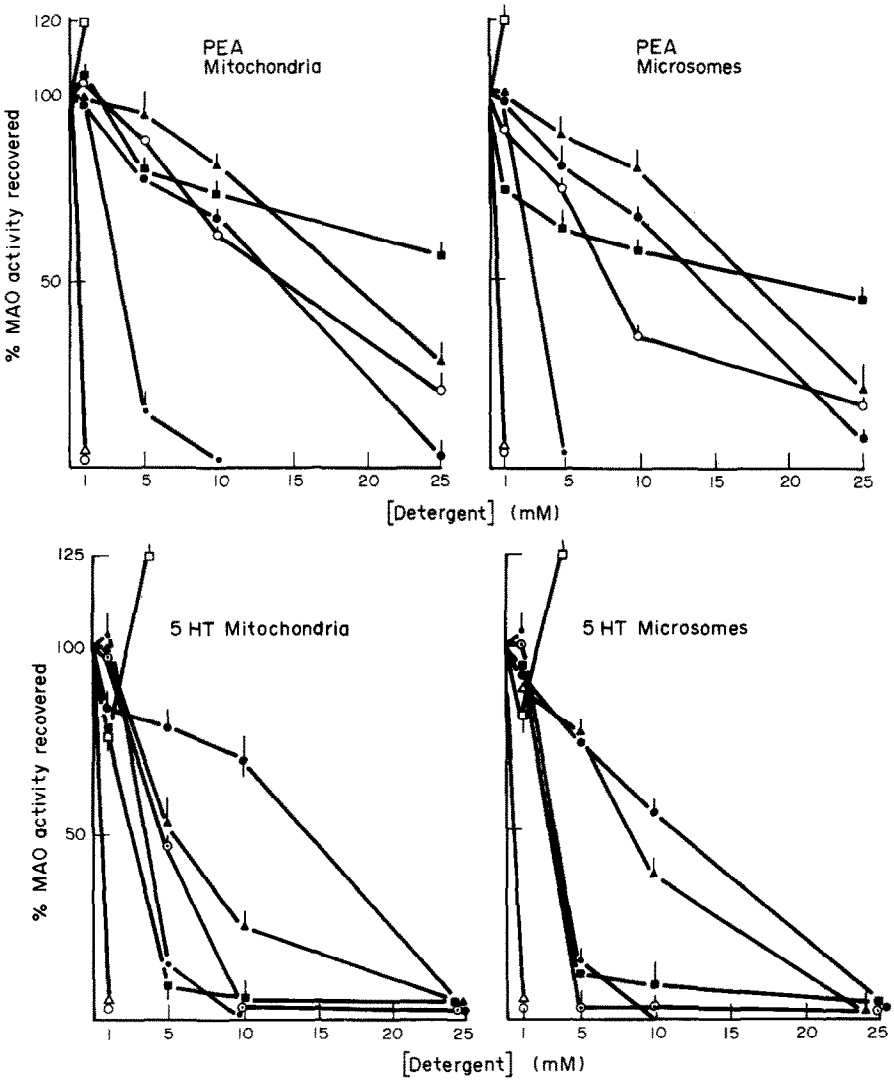

Fig. 1A, B. MAO A and MAO B activity in Mt and Mc fractions in presence of various detergents. Activity assays, were performed in 50 mM phosphate buffer, pH 7.2 with a final protein concentration of 1.3 mg/ml and in the presence of varying detergent concentrations. Reaction velocities were determined after incubating for 4 and 20 min for PEA and 5-HT, respectively. Enzyme activity in presence of cetyltrimethyl-ammonium bromide (△), tetradecyltrimethyl-ammonium bromide (○), sodium dodecyl sulphate (□), sodium deoxycholate (•), sodium taurocholate (▲), β-D-octylglycoside (●), Lauryl-D-maltoside (■), CHAPS (⊙). The activity of the enzyme, incubated in absence of detergent was taken as 100%.

Table 1. IC_{50} values for the inhibition of rat liver MAO in mitochondria and microsomes by detergents*

	IC_{50} (mM)				
	PEA (Mt)	PEA (Mc)	5HT (Mt)	5HT (Mc)	CMC (mM)
Ce Me_3N Br^-	0.5	0.5	0.5	0.5	—
Tetradecyl Me_3N Br^-	0.5	0.5	0.5	0.5	4–5
SDS†	—	—	—	—	8–2
DOC	3.3	2.9	3.4	3.4	4–6
Na-taurocholate	19	17.9	5.4	8.6	10–15
OG	13.9	14.3	14	11.6	25
Lauryl-D-maltoside	>25	20.2	3.6	3.1	0.16–0.19
CHAPS	14.5	8	5	3.5	1.4

* Six point curves were drawn through the means (± SD) of duplicate determinations of the per cent of MAO activity remaining in the presence of the detergents at varying concentrations. IC_{50} values were derived from these curves (see Fig. 1A, B). The assay procedure is described in the legend of the same graphics.

† The effect of sodium dodecyl sulphate on MAO activity was activation and consequently it was not possible to calculate the IC_{50} values.

MAO B. The corresponding IC_{50} values are shown in Table 1.

Effect of CHAPS on MAO activity

The effect of various concentrations of CHAPS on the relative MAO activity present in rat liver mitochondria and microsomes is shown in Fig 2.

In rat liver, the deamination of 5-HT is a measure of MAO A activity, whereas the deamination of PEA is used to determine MAO B activity [28].

The presence of an increasing concentration of CHAPS had different effects on MAO A and MAO B activities in both organelles. Both MAO activities decreased with the increase of CHAPS concentration, but the MAO A activity was more sensitive to the effects of the detergent in both Mt and Mc. The corresponding IC_{50} values determined are shown in Table 2. Preincubation with the detergent for 30 min at 37° prior to activity determination had little effect on the inhibition of MAO A, but increased that of the B form (Table 2).

The effect of CHAPS on MAO B and MAO A activities which had been solubilised under optimal conditions were also studied. The results obtained are summarised in Table 2, by which it can be seen that the solubilised preparations were still sensitive to inhibition, the MAO B form being less sensitive.

Solubilisation of MAO activity by CHAPS

MAO activity present in Mt and Mc fractions was solubilised as described in Materials and Methods. Figure 3(a) shows that mitochondrial MAO activity in the precipitate was gradually lost as the CHAPS concentration was increased, and there was also a concomitant increase in MAO B activity (PEA

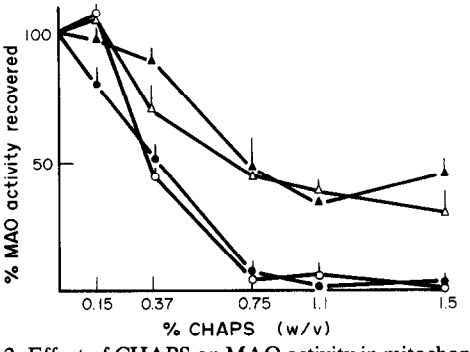

Fig. 2. Effect of CHAPS on MAO activity in mitochondria and microsomes. MAO activity was measured in presence of different concentrations of CHAPS. Using PEA (△▲) and 5HT (○●) as substrates. Activity assays were performed in 50 mM phosphate buffer, pH 7.2, with a final protein concentration of 4 mg/ml of mitochondria Mt (▲●) and microsomes Mc (△○) and 0.15, 0.37, 0.75, 1.1 and 1.5% (w/v) CHAPS in the reaction mixture. The reaction was started by the addition of enzyme. Activity values were expressed relative to control samples, containing no detergent and assayed under the same conditions.

Table 2. IC_{50} values for the inhibition of MAO activity in microsomes and mitochondria by detergent CHAPS*

	IC_{50} (mM)			
	Mitochondria		Microsomes	
	5HT	PEA	5HT	PEA
Crude enzyme	6.3	11.8	5.7	11.2
Solubilized enzyme	3.9	13.30	3.2	24
30 min 37° incubation	6.5	9.2	4.8	7.8

* Five point curves were drawn through the means (± SD) of duplicate determinations of the per cent MAO activity remaining in the presence of CHAPS detergent at varying concentrations. IC_{50} values were derived from these curves (see Fig. 2). The assay procedure is described in the legend of these graphics.

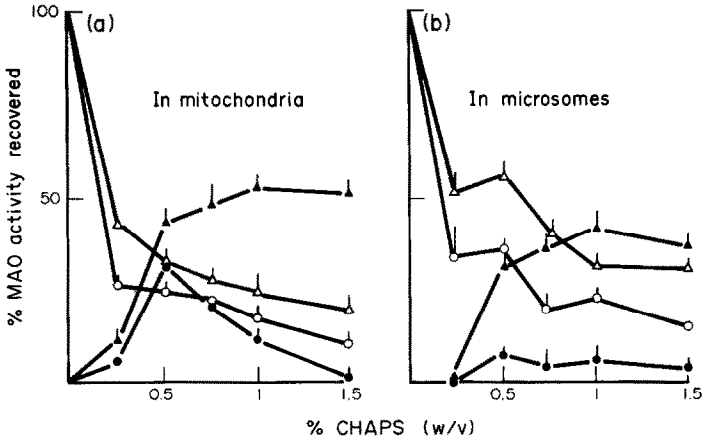

Fig. 3. Distribution of MAO activity after solubilisation with CHAPS. Samples of mitochondrial (a) and microsomal fractions (b), at a final protein concentration of 4 mg/ml in 50 mM-phosphate buffer, pH 7.2, were treated at 4° with 0.25, 0.5, 0.75, 1.0, 1.5% (w/v) CHAPS for 30 min, and centrifuged at 100,000 *g* (Mt) and 200,000 *g* (Mc) for 1 hr. Supernatant (▲●) and precipitate fractions (△○) were assayed for MAO A activity (●○), with 5HT as substrate, and MAO B activity (▲△) with PEA as substrate, in 50 mM phosphate buffer, pH 7.2, at 30°. The amount of protein used per reaction mixture ranged from 50 μg to 200 μg depending on the fraction being assayed. Activity is expressed as a percentage of the total activity found in untreated fractions assayed under the same conditions.

deamination) present in the supernatant. MAO A activity, against 5-HT as substrate, also showed an initial increase in the supernatant fraction of up to 0.5% CHAPS, after which the activity diminished.

The results obtained with the microsomal fraction are shown in Fig. 3(b). PEA and 5-HT activities in the precipitate fraction diminished with increasing CHAPS concentrations. PEA activity in the corresponding supernatant increased, but 5-HT activity in this fraction remained very low. The presence of 0.5 M KCl in the incubation mixture with CHAPS increased the % of MAO activity recovered in the supernatant (See Table 3).

Purification of MAO activity after CHAPS solubilisation

Mitochondria and microsomes, at a final concentration of 4 mg/ml of protein in the solubilisation mixture, were treated at 4° with CHAPS in the concentration range of 0.25–1.5% (w/v) for 30 min, and centrifuged as described in Materials and Methods. After overnight dialysis, samples were centrifuged again at 100,000 *g* for 60 min, and the specific activity, purification and yield were calculated with PEA and 5-HT as substrates. Optimal results were obtained in the range of 0.5–1% CHAPS with both Mt and Mc, and are summarised in Table 3.

Binding studies with [^{3}H]*-pargyline*

In order to investigate whether the differential effects of CHAPS on MAO A and MAO B activities were due to the differential release from Mt and Mc, or to differential inhibitory effects, these fractions were reacted with [^{3}H]-pargyline, as described in Methods, and the radioactivity distribution in pellet and supernatant after solubilisation by CHAPS was calculated. The results obtained are summarised in Table 4. In the case of Mt, both forms were solubilised to the same degree, and so the sensitivity of MAO A to CHAPS was due to the differential inhibitory effect on this form. In Mc, however, there was a different release of the two forms with this detergent, the release of MAO B being greater than that of MAO A.

Table 3. Purification of MAO from rat liver mitochondria and microsomes by CHAPS*

	% Activity solubilized		Purification factor after dialysis		% Recovery after dialysis	
	A	B	A	B	A	B
Mitochondria						
CHAPS 0.75% − KCl 0.5 M	25 ± 3.2	50 ± 6.1	2.03	11.40	4.18 ± 0.2	22.3 ± 1.8
CHAPS 0.75% + KCl 0.5 M	40 ± 3.8	90 ± 11.2	0.99	6.82	19 ± 1.7	34 ± 2.4
Microsomes						
CHAPS 0.75% − KCl 0.5 M	10 ± 1.2	35 ± 2.8	1.02	3.70	2.68 ± 0.3	9.7 ± 0.8
CHAPS 0.75% + KCl 0.5 M	40 ± 3.1	60 ± 4.2	1.49	3.36	17 ± 1.1	37 ± 2.7

* The assay procedure is described in Materials and Methods. Values represent means ± SD of three experiments.

Table 4. Recoveries of [^{3}H]-pargyline in the process of solubilization by CHAPS*

	pmol bound (^{3}H)-pargyline		MAO amount (%)	
	MAO A	MAO B	MAO A	MAO B
Mitochondria	0.9	1.35	100	100
CHAPS-treated residue	0.49	0.74	54	55
Soluble fraction after CHAPS treatment	0.28	0.51	31	38
Microsomes	0.53	0.54	100	100
CHAPS-treated residue	0.24	0.14	45	26
Soluble fraction after CHAPS treatment	0.09	0.21	17	39

* The assay procedure is described in Materials and Methods.

Sensitivity to the clorgyline inhibitor

The effect of CHAPS on rat liver mitochondrial and microsomal MAO sensitivity to the specific irreversible inhibitor, clorgyline (MAO A), with PEA and 5-HT as substrates, is shown in Fig. 4(a) and (b). The small differences observed in Fig. 4(b) between membrane-bound and soluble MAO will presumably not be significant. A possible explanation might be that the alteration of the lipidic microenvironment by the solubilisation process could increase the affinity of MAO A towards the inhibitor clorgyline. Houslay [29] described a similar effect.

Reversibility of CHAPS inhibition

The reversibility of MAO activity inhibition by CHAPS was investigated by dilution experiments. These were carried out without previous incubation of the sample with the detergent, and after preincubation for 30 min at 4°. The results showed that in both cases the inhibition was a reversible process. However, when the preincubation of enzyme and inhibitor was performed at 37° for 30 min, the inhibition became irreversible.

Kinetic studies of CHAPS inhibition

Kinetic studies of CHAPS inhibition were carried out in the presence of different detergent concentrations. The Lineweaver–Burk plots of CHAPS inhibition of the PEA and 5-HT deamination caused by the mitochondrial preparation are presented in Fig. 5. Mixed-type inhibition with respect to the deamination of both substrates was observed in all cases.

The K_m values of membrane-bound and solubilised enzyme preparations are shown in Table 5. These results demonstrate that the solubilisation by CHAPS caused no significant alteration in the K_m values of MAO activity towards 5-HT and PEA as substrates.

DISCUSSION

The surface charge of membranes is formed by polar groups of phospholipids and membrane proteins. It is well known that some insoluble enzymes can be activated or inhibited by changes in

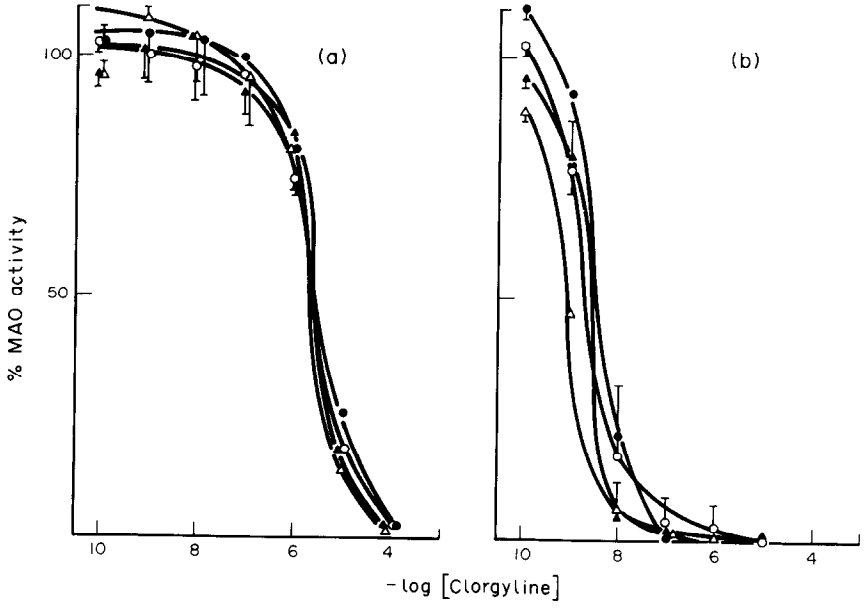

Fig. 4. Effect of CHAPS solubilisation on the susceptibility to clorgyline. Inhibition of membrane-bound MAO from mitochondria (▲) and microsomes (●), and solubilised MAO from mitochondria (△) and microsomes (○) by clorgyline using PEA (a) and 5HT (b) as substrates. MAO was assayed for activity following 60 min preincubation at 30° with different clorgyline concentrations.

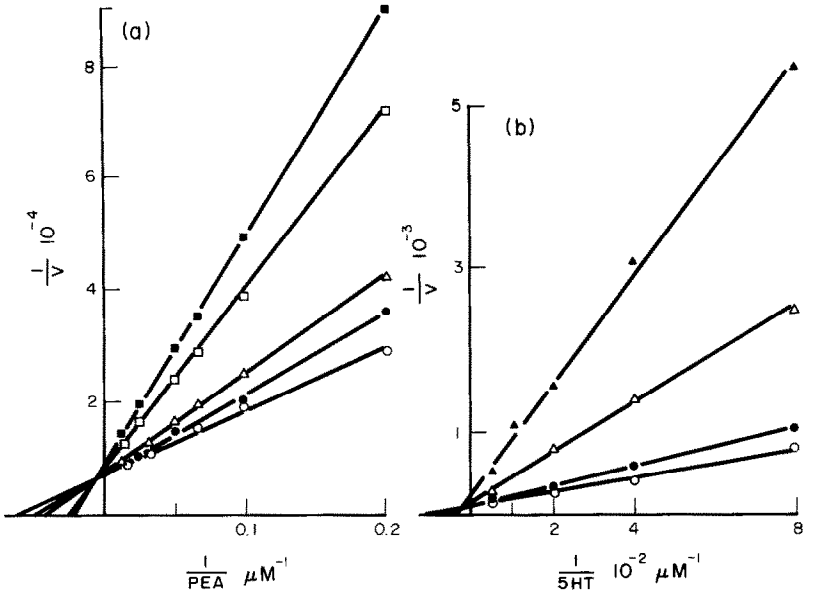

Fig. 5. Lineweaver–Burk plot of inhibition of monoamine oxidase in mitochondria. Lineweaver–Burk plot of the initial velocities against different PEA concentration (a) and 5HT concentration (b) at several fixed CHAPS concentrations which were zero (○), 0.25% (●), 0.5% (△), 0.75% (▲), 1.0% (□) and 1.5% (■). Mitochondria were incubated for 2 hr at 30° in the presence of 3×10^{-7} M of clorgyline or deprenil in order to obtain B or A enzymatic activity. After this, samples were centrifuged at 200,000 *g* for 45 min and the pellet was preincubated in presence of different CHAPS concentrations for 5 min before starting the enzyme reaction by addition of different PEA or 5HT concentrations. Velocity is expressed in pmol oxidized (mg protein)$^{-1}$ min^{-1}.

charge density. This can be affected by the presence of ions, amphiphiles or alterations in the pH of the medium.

Wojtczak [18] reported that monoamine oxidase activity and other membrane-bound enzymes that have cationic substrates were activated and inhibited by anionic and cationic detergents, respectively. These authors suggested that the presence of the membrane is necessary for the maintenance of the surface charge, which modulates the enzymatic activity.

The effect of the zwitterionic detergent CHAPS was studied in comparison with the effects of cationic, anionic and non-ionic detergents. The results obtained were interpreted in terms of specific ionic effects predicted by Wojkczak [18].

The cationic surfactants such as cetyltrimethylammonium bromide and tetradecyltrimethylammonium bromide produced total inhibition of MAO A and MAO B. The anionic detergent sodium dodecyl sulphate produced a significant activation of both forms in the proximity of its CMC values. These results are in agreement with Wojtczak's [18] hypothesis. Nevertheless, the results obtained with other anionic detergents such as sodium taurocholate and sodium deoxycholate do not support the said hypothesis.

Non-ionic and Zwitterionic detergents would not be expected to have any effect on MAO activity with respect to alterations of the charge density of the membrane; however they did inhibit MAO activity in different degrees, MAO A being more labil in some cases.

These results differ from those reported by Wojtczak [18], and it is clear that the effects of detergents used in this work arose not only from the alteration of the surface charge and that additional factors must be involved.

The effects of CHAPS concentrations on MAO B and MAO A activities were not similar, the latter being more susceptible to CHAPS inhibition. This inhibitory difference was diminished by preincubation at 37° for 30 min. Similar behaviour has been reported for the effect of Triton X-100 on MAO activity [22], where MAO A was more sensitive to the detergent than MAO B. Some authors [30] have

Table 5. Apparent K_m (μM) values of rat liver monoamine oxidase*

Source	Substrate	Membrane-bound	Soluble
Mitochondria	PEA	12.0 ± 4.07	9.15 ± 1.77
	5HT	91.71 ± 4.65	81.53 ± 0.5
Microsomes	PEA	7.28 ± 0.38	6.69 ± 0.81
	5HT	152.14 ± 59.21	148.5 ± 48.79

* The assay procedure is described in Materials and Methods.

produced evidence to suggest that the B form of the enzyme is surrounded by tightly bound lipids that stabilise its activity. If that were the case, the increased temperature would have the effect of breaking the interactions between MAO B and the lipids, increasing accessibility to this form by CHAPS.

The kinetic studies revealed a mixed inhibition pattern, altering the apparent V_{max} and K_m. However, replots of the slopes and intercepts against detergent concentration were parabolic, suggesting the presence of multiple interacting binding sites on the enzyme rather than a specific effect involving a single site.

After solubilisation in the pellet and supernatant, the different distribution of MAO activity shows a differential inhibiting effect on MAO A in the case of mitochondria and a differential release of MAO B in the case of microsomes, as demonstrated by the labelling studies with [^{3}H]pargyline.

The fact that both the sensitivities towards irreversible suicide inhibitors such as clorgyline and deprenil as well as the apparent K_m values for PEA and 5-HT were not altered during the solubilisation process demonstrated that the native properties of the monoamine oxidase were preserved after CHAPS treatment.

These results would suggest that the non-denaturing zwitterionic detergent CHAPS is more suitable for solubilising mitochondrial rather than microsomal MAO. It is also more efficient in solubilising MAO B, MAO A being more sensitive to inhibition by this compound. Thus, use of CHAPS as a solubilising agent should be followed by exhaustive dialysis to avoid inhibition by the detergent, which may become irreversible at elevated temperatures.

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