Table 1. Activities of amylase and glycyl-prolyl dipeptidyl-aminopeptidase in serum and homogenates of pancreas and liver in rats

Enzymatic activities	Control group	Ethanol group	Significance*
Amylase			
Serum (U/l)	7443 ± 1195† (10)‡	4474 ± 1295 (6)	$P < 0.001$
Pancreas (U/g tissue)	244 ± 77 (7)	116 ± 103 (7)	$P < 0.05$
Glycyl-prolyl dipeptidyl-aminopeptidase			
Serum (mU/ml)	68.4 ± 12.9 (8)	65.3 ± 19.2 (6)	NS
Pancreas (mU/g tissue)	179 ± 65 (7)	410 ± 96 (6)	$P < 0.001$
Liver (mU/g tissue)	1602 ± 174 (4)	1418 ± 397 (4)	NS

* Significance was measured by Student's *t*-test. NS = not significant.
† Values are expressed as means ± S.D.
‡ Figures in parentheses refer to number of animals.

therefore, may afford some evidence of raised collagen metabolism in the pancreas, but not in the liver, suggesting an initiation of pancreatic fibrosis. The result may also explain the clinical fact that ethanol consumption gives rise to chronic pancreatic lesion within several years, whereas it takes over 10 years for the development of liver cirrhosis [1].

In summary, ethanol administration to rats for 4 weeks caused no change of glycyl-prolyl dipeptidyl-aminopeptidase activity in the liver, but a significant increase in the pancreas, where a concomitant decrease in amylase activity occurred.

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Lipid substitution of mitochondrial monoamine oxidase can lead to the abolition of clorgyline selective inhibition without alteration in the A/B ratio assessed by substrate utilisation

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The mitochondrial monoamine oxidase activity of rat liver can be divided into two categories, MAO A and MAO B, based upon their substrate specificity and the effect of reversible and irreversible inhibitors [2–4]. The model accounting for the rat liver system [2] does not appear to be universally applicable as tissues can vary in the substrate specificity exhibited and their sensitivity to selective inhibitors [3, 4]. Recently MAO A and B have been shown to be different protein species [5–7] with their active sites exposed asymmetrically at the mitochondrial outer membrane [8] and with sialic acid residues playing an important role in the functioning of MAO A[9]. Accepting that MAO A and MAO B may be different protein species there would appear to be a significant heterogeneity within this classification (see [3, 4, 10]). This may result from the binding of membraneous material to the enzymes, a view origiginally proposed by Gorkin and collaborators [11] and for which there is considerable experimental support [3, 12]. Houslay [13] suggested that the selective effects of the irreversible inhibitor clorgyline® (N-methyl-N-propargyl-3-[2,4-dichlorophenoxy] propylamine) may not be directly related to the substrate specificity of the enzyme species but merely resulting from differences in the nature of the environment of the inhibitor binding site. It was proposed [13] that differences in the membrane environment of the propargylamine inhibitor site on MAO A and MAO B accounted for the selective effects of clorgyline®. To test such an hypothesis the, endogeneous lipids of rat liver mitochondrial outer membranes have been replaced by a defined synthetic lipid, dimyristoyl phosphatidyl choline. Such a technique has been successfully used to evaluate the role of the lipid environment in the functioning of other membrane bound enzymes [14, 15].

Rat liver mitochondrial membranes from 200–300 g male Sprague–Dawley rats were purified as described previously [16] with the modification as in [9]. A radiochemical assay

Table 1. Ratio of MAO A to MAO B in native and lipid-substituted mitochondrial outer membranes as assessed by 5-hydroxytryptamine and 2-phenylethylamine oxidation

$$\frac{\text{MAO A}}{\text{MAO B}} = \frac{\text{5-hydroxytryptamine oxidation}}{\text{2-phenylethylamine oxidation}}$$

	Native	Dimyristoyl phosphatidyl choline substituted
Expt. I	0.56	0.58
Expt. II	0.52	0.46
Expt. III	0.58	0.57
Average	0.55 ± 0.03	0.54 ± 0.07

The average is given as ± S.D. Assays of membrane preparations were carried out in triplicate and the averages of such values were taken to calculate these ratios.

[17] was used routinely with 1-[^{14}C]-2-phenylethylamine (from NEN, W. Germany) at a final concentration of 0.5 mM or 1 mM and [^{3}H]-5-hydroxytryptamine and [^{3}H]-tyramine (both from Amersham, U.K.) both at final concentrations of 1 mM although in some instances a coupled spectrophotometric assay was used [12]. Incubations were carried out at 30° and rates were taken from linear time-courses. Treatment with clorgyline was as described previously [2]. Lipid substitution was carried out using established methods [14, 15]. Briefly this entailed rapidly mixing at 4°, 1 ml of 10 mM potassium phosphate (KP_i) buffer pH 7.2 containing mitochondrial outer membranes (3.8 mg protein) with 1 ml of a mixture of dimyristoyl phosphatidyl choline (50 mg) and K cholate (25 mg) and incubating on ice for 30–60 min. The K cholate was purified as described previously [14]. The detergent–lipid dispersion was prepared [14, 15] by dissolving the lipid (50 mg) in 1 ml $CHCL_3$: MeOH (2:1) in a 10 ml glass scintillation vial, evaporating off solvent with a stream of N_2 and removing residual solvent by leaving 3–4 hr under vacuum prior to resuspending this film in 1 ml of a cocktail containing K cholate (25 mg), 0.25 M sucrose, 1.0 M KCl and 0.05 M KP_i buffer final pH 8.0. After incubation this mixture was loaded onto a discontinuous sucrose density gradient of 0.3 ml 80% sucrose and 1.3 ml 30% sucrose both containing 10 mM KP_i pH 7.2. Centrifugation was carried out on an MSE 65 using a 6 × 4.2 ml rotor at 160,000 $g_{av.}$ for 20 hr at 4°. The substituted mitochondrial outer membranes collected at the 80–30 per cent sucrose interface. The isolated pellet was then either washed by centrifugation [9] or treated as before [14, 15] to remove any residual detergent prior to study. Lipid and cholate determinations were all as described previously [14].

Native mitochondrial outer membranes have a lipid/protein ratio of 0.6 and the amount of exogenous lipid added should effect a 96 per cent substitution if equilibriation occurred. Indeed the membranes obtained had lipid–protein ratios between 0.5–0.6 and contained approx. 95 per cent synthetic dimyristoyl phosphatidyl choline, indicating that substitution had indeed occurred. The recovery of MAO A and MAO B as assessed by 5-hydroxytryptamine and β-phenylethylamine oxidation was in the region of 60–70 per cent and all of this activity was membrane bound as it was sedimented by centrifugation at

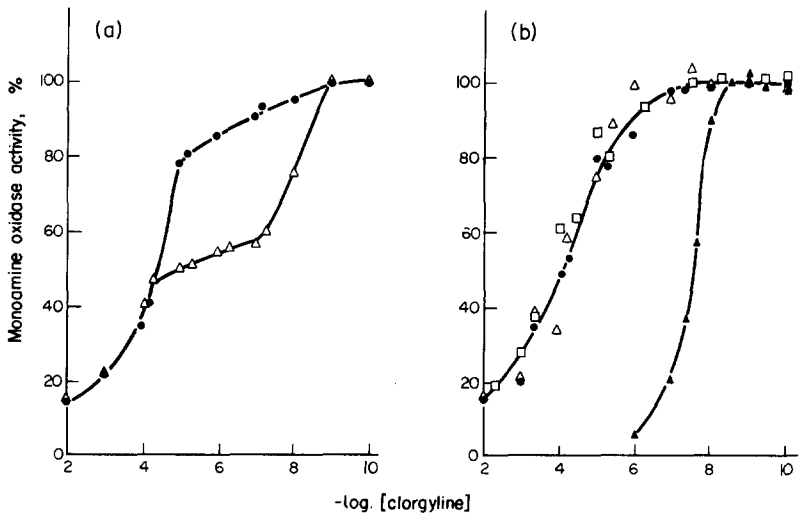

Fig. 1. The sensitivity of the monoamine oxidase activity of native and lipid substituted mitochondrial outer membranes to inhibition by clorgyline. (a) Tyramine oxidation. Tyramine is a substrate for both MAO A and MAO B [1–3]. Native membranes (△) and lipid substituted membranes (●). (b) 5-hydroxytryptamine (5HT) and 2-phenylethylamine (PEA) oxidation. Under the assay conditions used in native membranes both 5HT (▲) and β-PEA (□) behaved as pure MAO A and MAO B substrates respectively as we have described in detail previously [2]. 5HT (△) and β-PEA (●) in substituted membranes.

100,000 *g* for 1 hr. As Table 1 demonstrates, the ratio of MAO A:MAO B as assessed by these two substrates was hardly affected by the lipid substitution procedure. However, the ratio of MAO A:MAO B as assessed by the sensitivity of tyramine, a substrate for both enzymes, to clorgyline inhibition indicated the loss of MAO A activity (Fig. 1a). In fact, as Fig. 1b demonstrates, the MAO A activity in the substituted membranes as monitored by 5-hydroxytryptamine oxidation, exhibited a MAO B type sensitivity to clorgyline. Thus whilst the substrate specificity had apparently remained unaltered the enzymes placed in the defined lipid environment exhibited identical sensitivities to clorgyline.

Unlike detergent solubilisation [3, 12, 18] or organic solvent extraction [19] which effect changes in both the relative rates of substrate utilisation and sensitivity to clorgyline, presumably by denaturing the enzyme species, this procedure discriminates between these two processes. Thus substitution of outer membranes with dimyristoyl phosphatidylcholine to 95 per cent yields a membrane preparation, which contains two enzyme proteins [5–7], expressing the substrate specificity of the native membrane, yet exhibiting the clorgyline sensitivity of MAO B only. Substrate specificity and sensitivity to inhibition by clorgyline can thus be separated and so it would appear that the type of membrane lipid environment can determine the sensitivity to inhibition by clorgyline. On this basis one might well find that tissues which express activity to both MAO A and MAO B substrates but show identical sensitivities to clorgyline still contain two enzyme species. This study does not, however, rule out the possibility that specific endogeneous lipids may modulate enzyme function as the molar concentration of MAO in this tissue is small and such species may be able to segregate out sufficient of these lipids for function.

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