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Lysolecithin is a selective reversible inhibitor of mitochondrial monoamine oxidase

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It is now well established that in rat liver and many other tissues there are two forms of mitochondrial monoamine oxidase, which differ in their substrate specificity for amines [1]. These have been called the A-enzyme and the B-enzyme, a designation originally based on their differential sensitivity to the irreversible inhibitor clorgyline® (*N*-methyl-*N*-propargyl-3-(2,4-dichlorophenoxy) propylamine) as shown by Johnston [2]. These two enzyme species appear to have immunologically identical active sites, the differences in inhibitor sensitivity and substrate specificity observed in the membrane bound state presumably arising from lipid modification of a single protein species [3–5]. This report demonstrates that lysolecithins show a selective reversible inhibition of the A and B forms of mitochondrial monoamine oxidase. Rat liver mitochondrial outer membranes were purified as described previously, except that the final dialysis was against 10 mM K phosphate buffer, pH 7.2 [6]. Pure preparations of synthetic myristoyl lysolecithin (C14) and palmitoyl lysolecithin (C16) were isolated as products of the action of cabbage phospholipase A_2 on pure dimyristoyl lecithin and dipalmitoyl lecithin respectively as described in detail [7].

The activity of the B-enzyme was followed spectrophotometrically using benzylamine HCl as the substrate and the activity of the A-enzyme using a radio-assay for [^{3}H]-5-hydroxyindole acetaldehyde production using [^{3}H]-5-hydroxytryptamine as substrate [6, 8]. All assays were carried out in 50 mM K phosphate buffer, pH 7.2, as Tris buffers have been found to inhibit the A and B enzymes selectively [9]. Assays were carried out at 30° under air saturating conditions. Time courses were linear under all conditions, and initial rates of reaction were measured. All chemicals were of A.R. purity.

The oxidative deamination of both benzylamine and 5-hydroxytryptamine was inhibited in a fully reversible fashion by both of the lysolecithins, and exhibited no time dependence. The reversibility could be demonstrated by dilution, or by centrifuging the membranes (100,000 for 1 hr) and resuspending them in fresh 10 mM K phosphate buffer, pH 7.2. The recovered activity was > 95 per cent, and was all in the pellet. Double reciprocal plots of the steady state kinetic data for palmitoyl lysolecithin inhibition of benzylamine (B-enzyme) and 5-hydroxytryptamine (A-enzyme) oxidations are shown in Fig. 1. The inhibition is mixed in form, altering both the apparent V_{max} and K_m for substrate (yielding two K_i values), a similar result being obtained for myristoyl lysolecithin. Dixon replots, slope and intercept replots of this data were linear in all cases, and the various inhibition constants (K_i) demonstrating affinity of the lysolecithins for the two enzyme species are listed in Table 1.

That the inhibition was freely reversible, instantaneous and subject to steady state kinetic analysis with no anomalies, suggests that the lysolecithins interact freely with a site on the enzyme's surface exposed to the aqueous environment, rather than with one buried in the bilayer. This is not too surprising, as experiments have indicated extensive hydrophobic areas in the area of the active site of monoamine oxidase [10, 11]. Both the palmitoyl and myristoyl lysolecithins appear to be equally efficacious as inhibitors of the two enzyme species, however there appears to be a distinct preference for their interaction with the B enzyme (benzylamine as substrate). This is demonstrated by a 4–8 fold lower value for the K_i (slope) and 6–7 fold lower value for the K_i (intercept) shown towards the B-enzyme.

This report demonstrates that lipids can selectively modulate the activity of mitochondrial monoamine oxidase, albeit in a reversible fashion. This selective modulation may function *in vivo*, for the normal concentration of lysolecithin in rat liver has been estimated as 1.5 mg/ml (approx. 2.9 mM) considering a uniform distribution

Table 1

Inhibitor / Substrate	Myristoyl lysolecithin 5-Hydroxy-tryptamine	Myristoyl lysolecithin Benzyl-amine	Selectivity ratio K_i (A-enzyme) / K_i (B-enzyme)	Palmitoyl lysolecithin 5-Hydroxy-tryptamine	Palmitoyl lysolecithin Benzyl-amine	Selectivity ratio K_i (A-enzyme) / K_i (B-enzyme)
K_i (slope) μM	300	36	8.3	152	34	4.5
K_i (intercept) μM	420	60	7	304	53	5.7

Steady state inhibition constants from linear secondary replots of primary double reciprocal plots are shown in μM. The ratio of K_i (slope) *or* K_i (intercept) for the A-enzyme and the B-enzyme is shown as a measure of selectivity.

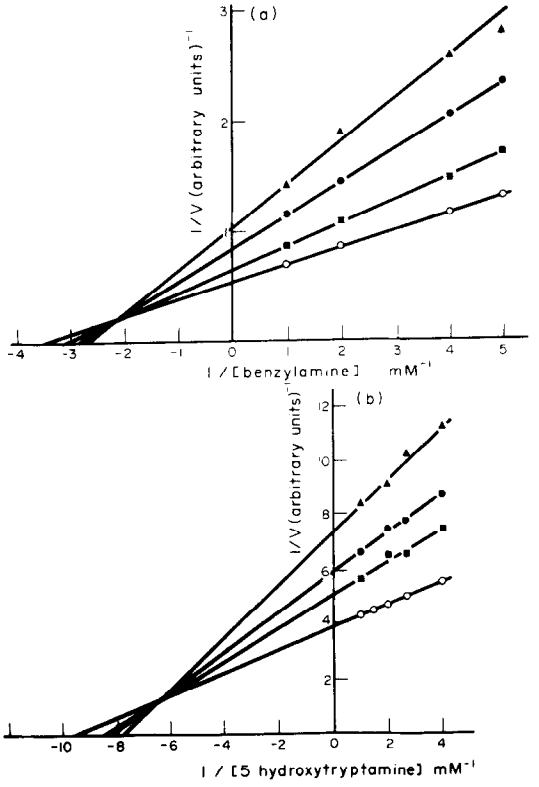

Fig. 1. Reciprocal plots of initial velocities against substrate concentrations at various fixed palmitoyl lysolecithin concentrations are shown. (a) With benzylamine as the varied substrates, the palmitoyl lysolecithin concentrations were (○) zero, (■) 19 μM, (●) 38 μM, and (▲) 57 μM. (b) With 5-hydroxytryptamine as varied substrate, the palmitoyl lysolecithin concentrations were (○) zero, (■) 80 μM, (●) 160 μM, and (▲) 240 μM.

throughout the tissue [12, 13]. Whilst it is unlikely that this represents a true concentration of lysolecithin available to interact with monoamine oxidase, it is conceivable that the activity of monoamine oxidase *in vivo* may be selectively modulated by lysolecithin levels. Indeed significant concentration of lysolecithin must be available in the vicinity of mitochondrial outer membranes, as the acyl transferase enzymes responsible for converting lysolecithin back to lecithin are located there, as well as in the endoplasmic reticulum and plasma membranes [14].

However, we must be aware that as lysolecithins are reversible inhibitors, the actual inhibition observed will not only relate to its concentration and K_i value shown for a particular enzyme species, but also to the free amine level and the respective K_m for the enzyme. Indeed if lysolecithins were to achieve an immediate selective increase in the ratio of amine substrates for the B-enzyme to those for the A-enzyme, one would expect inhibition of the A-enzyme to result from the binding of B-enzyme substrates as reversible inhibitors. This somewhat complicated situation has been discussed in full elsewhere [15, 16].

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