AMPHOPTERICIN B HAS VERY DIFFERENT EFFECTS ON THE GLUCAGON- AND FLUORIDE-STIMULATED ADENYLATE CYCLASE ACTIVITIES OF RAT LIVER PLASMA MEMBRANES

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1. Introduction

The glucagon receptor and catalytic unit of adenylate cyclase are separate entities [1,2] able to undergo free lateral diffusion in the plane of the bilayer [3,4]. Upon addition of hormone they interact to form a multicomponent system spanning the bilayer membrane [3].

The activity of the catalytic unit in the absence of hormone is only sensitive to those lipid phase separations occurring in the inner half of the bilayer, whereas in the presence of glucagon, the coupled receptor–catalytic unit senses lipid phase separations occurring in both bilayer halves [5–7].

Cholesterol is a major constituent of the lipids of the plasma membrane (e.g., see [8]) and it has been suggested that the phase separations observed in such membranes must occur in relatively cholesterol free areas [9] due to its lateral segregation by specific lipids [10].

The water soluble polyene antibiotic, amphotericin B interacts specifically with cholesterol with great avidity [11]. This study demonstrates it has a marked effect on Arrhenius plots of adenylate cyclase activity, and it differentially affects the fluoride- and glucagon-stimulated activities.

2. Materials and methods

Rat liver plasma membranes were prepared and stored as in [12].

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Adenylate cyclase was assayed as in [12], ensuring that linear timecourses were obtained under all conditions [5,9,12] and assay pH remained constant. Lubrol solubilised adenylate cyclase was prepared as in [13]. Glucagon was a gift from Dr W. W. Bromer, Eli Lilly and Co., Indianapolis. Amphotericin B was a kind gift from E. R. Squibb and Sons, Liverpool. Creatine phosphate and creatine kinase were from Sigma. Cyclic AMP, ATP and triethanolamine–HCl were from Boerhinger. Lubrol PX was a gift from ICI Ltd, Macclesfield, Cheshire. All other chemicals were of A.R. quality from BDH Chemicals, Poole, Dorset.

3. Results and discussion

Amphotericin B progressively inhibited the activity of fluoride-stimulated adenylate cyclase to ~50% of its original activity at 10^{-6} M (fig.1). In contrast the glucagon-stimulated activity was increased by some 30% at 10^{-9} M amphotericin B. As the amphotericin B concentration was increased this activity fell, to reach a plateau of activity similar to that of the original (fig.1). These changes in activity exhibited no apparent time dependence as assays over 10 min were linear, and preincubation of assays (without ATP) on ice for 30 min had no effect on their form. These effects could be reversed by extensive washing of the membranes (×4) with 1 mM $KHCO_3$ (pH 7.2) to remove bound amphotericin B.

Using a Lubrol-solubilised preparation, amphotericin B had no effect on adenylate cyclase activity

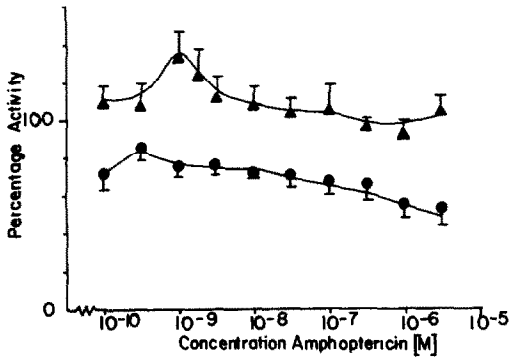


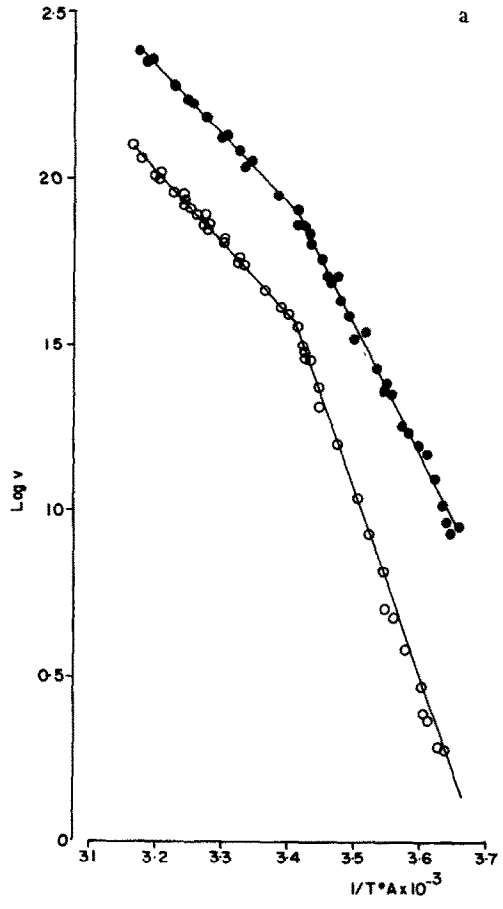
Fig.1. The effect of amphoptericin B on adenylate cyclase activity. Experiments were carried out at 30°C following fluoride-stimulated activity (•) and glucagon-stimulated activity (▲). Errors are SD using 4 plasma membrane preparations.

or the form of Arrhenius plots of this activity, suggesting that the effects observed were due to an interaction of the drug at the level of the intact membrane. That a chemically very different compound, filipin, that also interacts efficaciously with cholesterol in the membrane, achieves similar effects on the glucagon and fluoride-stimulated activities [14] would support this contention. This being so we examined the effects of 10^{-9} M and 10^{-6} M amphoptericin B on the Arrhenius plots of glucagon- and fluoride-stimulated adenylate cyclase. In native plasma membranes the break at 28°C observed in Arrhenius plots of glucagon-stimulated adenylate cyclase [12] has been localised to the external half of the bilayer [6,7]. The fluoride-stimulated activity exhibited a linear Arrhenius plot, there being no lipid phase separation event occurring in the inner half of the bilayer over 0–42°C.

Figure 2 clearly demonstrates the marked effect amphoptericin B has on the Arrhenius plots of both activities. At 10^{-9} M amphoptericin B the Arrhenius plots of both stimulated activities exhibited a single break at around 20°C (fig.2a). At 10^{-6} M amphoptericin B, Arrhenius plots of fluoride-stimulated activity showed a single break at 10°C, whereas those of glucagon-stimulated activity exhibited two breaks at around 23°C and 9°C (fig.2b).

There is ample evidence that the break at 28°C is due to a lipid phase separation occurring in the bilayer [6,13,15,16] and this can be manipulated by incorporation of defined synthetic lecithins into the bilayer [5] or by agents increasing bilayer fluidity such as the local anaesthetic, benzyl alcohol [13].

Amphoptericin B can interact with cholesterol with high avidity to form a complex; this complex is preferred to that between cholesterol and phospholipids [11]. Thus upon addition of amphoptericin B to liver plasma membranes, we might well expect to see a lateral redistribution of lipid species. Presumably those phospholipids which preferentially interacted with cholesterol [10] may be freed to mix with other lipids in those relatively cholesterol free areas occupied by enzymes. If the physical properties of lipids previously interacting with cholesterol were different from those of the lipids in the original cholesterol-depleted areas, then we might well expect to see changes in the lipid phase separations experienced. Upon this model, at 10^{-6} M amphop-



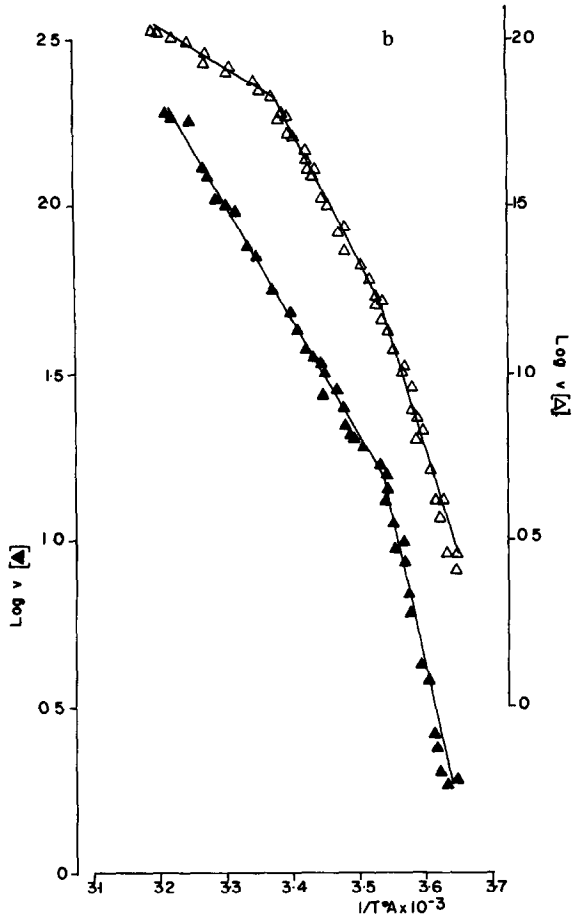


Fig.2. Arrhenius plots of adenylate cyclase activity. (a) Fluoride-stimulated activity in the presence of 10^{-9} M (•) and 10^{-6} M (○) amphoptericin. (b) Glucagon-stimulated activity in the presence of 10^{-9} M (▲) and 10^{-6} M (△) amphoptericin.

tericin B it is tempting to assign the break at 8–10°C seen in Arrhenius plots of fluoride- and glucagon-stimulated adenylate cyclase to the inner half of the bilayer. The break at 23°C seen only in the plot of the glucagon-stimulated enzyme presumably reflects that of a lipid phase separation in the external half of the bilayer. This interpretation suggests then, that the effect of amphoptericin B (10^{-6} M) in complexing cholesterol in the plasma membrane, depresses the lipid phase separation at 28°C occurring in the outer half of the bilayer by ~5°C, and causes the appearance of a lipid phase separation at 10°C in the inner half of the bilayer. At 10^{-9} M amphoptericin B, the break at 20°C in the fluoride-stimulated enzyme presumably reflects the appearance of a lipid phase separation in the inner bilayer half. The Arrhenius plot of glucagon stimulated activity exhibits but a single break around 21°C, due on this model to its sensitivity to the break at 20°C in the inner half of the bilayer and also to a lipid phase separation in the outer half of the bilayer which has been depressed below 28°C; their proximity yielding an apparent single break.

Based upon this model we would like to suggest that cholesterol is an important modulator of lipid phase separations in the liver plasma membrane. It is unlikely that it achieves this effect directly, as it would cause the smearing or abolition of lipid phase separations [10], but rather influences them by interacting preferentially with certain phospholipid species [10] achieving a lateral lipid segregation in the bilayer.

The decrease in break temperature suggests an increase in fluidity of the bilayer (at 30°C), which would be expected to increase both stimulated

Table 1
Effect of amphoptericin B on Arrhenius plot of adenylate cyclase

Stimulating ligand	Amphoptericin B concentration	Break (T°C)		Activation energy (kJ/mol)		
		Upper	Lower	Above upper break	Below upper break	Below lower break
Glucagon	10^{-9} M	−20.8 ± 1°C	–	40.3 ± 8.8	81.5 ± 14.3	–
Glucagon	10^{-6} M	23.3 ± 2.1°C	8.4 ± 1°C	23.7 ± 2.9	76.1 ± 12.6	122.2 ± 17.9
Fluoride	10^{-9} M	−19.6 ± 1°C	–	42.8 ± 8.8	78.1 ± 8	
Fluoride	10^{-6} M	−10 ± 2°C	–	61.3 ± 12.9	146.6 ± 40.2	

Errors are SD of 3 separate determinations

adenylate cyclase activities [13]. That this is only observed for the glucagon-stimulated activity suggests that the lipid species now able to interact with the enzyme, after cholesterol complexing with amphoptericin B, caused a progressive inhibition of the catalytic unit. Thus fluoride-stimulated activity is progressively inhibited, and the activation of glucagon-stimulated activity which is presumably due to an increase in fluidity of the external half of the bilayer is countered by the inhibition of the catalytic unit. This would lend further support to the contention that the headgroup nature of phospholipids can influence adenylate cyclase activity [6,17].

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