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Developmental changes in the form of the Arrhenius plots of rat liver plasma membrane adenylate cyclase and 5'-nucleotidase activities

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Summary: Arrhenius plots of both adenylate cyclase and 5'nucleotidase activity in liver plasma membranes from rats 6 weeks of age, and older, exhibit a single well-defined break at around 28°C with an activation energy at temperatures above the break point that is lower than that found at temperatures below the break. In contrast, animals at 4 weeks of age, whilst exhibiting a similar break temperature, have activation energies for these enzymes which are higher at temperatures above the break than below.

Introduction. Adenylate cyclase and 5' nucleotidase are integral proteins associated with the liver plasma membrane (1,2), whose activities are sensitive to changes in membrane fluidity (3-7). In rat liver plasma membranes a number of investigators have demonstrated that the activity of both of these enzymes is influenced by a thermotropic lipid phase separation occurring in the membrane at around 28°C. This causes Arrhenius plots of their activities to exhibit a well-defined break at this temperature (5,6,8-12). However, in contrast to all other investigators, Kreiner et al. (8) observed that the activation energy for the reaction of both of these enzymes was greater at temperatures above the break point, at around 28°C, than it was at temperatures below this point. In this study we resolve this apparent anomaly by demonstrating that the form of the Arrhenius plots of both glucagon-stimulated adenylate cyclase and 5'-nucleotidase changes upon development of the rats.

Materials and Methods. Rat liver plasma membranes from male, Sprague-Dawley rats were prepared and stored as described previously (9). Assays of adenylate cyclase and 5' nucleotidase methods for carrying out Arrhenius plots using initial rates of enzyme activity were as described in full previously (9,13). All biochemicals were from Sigma (U.K.) Ltd. except for cyclic AMP, creatine phosphokinase and triethanolamine HCl which were from Boerhinger (U.K.) Ltd. All other chemicals were of A.R. quality from B.D.H., Poole, U.K.

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Results and Discussion. Arrhenius plots of fluoride-stimulated adenylate cyclase in rat liver plasma membranes are linear. This is because no thermotropic lipid phase separation occurs in the inner half of the bilayer, over a temperature range of 3-42°C, where this enzyme is believed to exist (4,5,9,14-18). It is possible to induce one there either by using the polyene antibiotic, amphoptericin B (15) or by adding anions such as calcium (16) or prilocaine (17), whereupon Arrhenius plots of fluoride-stimulated adenylate cyclase activity exhibit a well-defined break at this temperature. However, the activity of this catalytic unit of adenylate cyclase, which faces the cell cytosol (18), can be induced to sense the lipid phase separation, occurring at around 28°C, in the external half of the bilayer when glucagon triggers the association of its receptor with the catalytic unit to form a transmembrane complex (14,18). On the other hand the ectoenzyme 5'-nucleotidase, whilst sensing the lipid phase separation occurring at 28°C in the outer half of the bilayer, appears to be insensitive to perturbations of the inner half of the bilayer, at least in rat liver plasma membranes (19). These breaks in the Arrhenius plots are indeed lipid-mediated as they can be affected by bilayer fluidising agents (3,4,10,14,17,19); lipid substitution (6,20) and by detergents (21,22). Furthermore the lipid phase separation at 28°C can be detected by a variety of different physical techniques (3,5,10).

In rats that are six weeks old (figs.1,2) or older (table 1; 5,6,9-12) then plots of 5' nucleotidase activity and glucagon-stimulated adenylate cyclase activity exhibit a well-defined break at around 28°C and exhibit activation energies that are greater at temperatures below the break than above the break (table 1). In contrast, the Arrhenius plots of the activities of these enzymes from rats of 4 weeks old, which have recently finished weaning, whilst still exhibiting a break at around 28°C, show instead activation energies which are smaller at temperatures below the break than above the break (fig.1,2, table 1). Thus the apparent anomaly of the data of Kreiner et al. (8), who observed lower activation energies below the break temperature in contrast with other workers (5,6,9-12) who observed the opposite, is not due

Table 1

Enzyme	Age of Animal (weeks)	Break Point(°C)	Activation Energy (KJ mol^{-1}) above break	below break
Fluoride-stimulated adenylate cyclase	4	Linear	69.7	
	6	Linear	66.7	
Glucagon-stimulated adenylate cyclase	4	27.6	81.7	36.1
	6	27.4	32.8	61.5
5'-nucleotidase	4	27.8	57.4	44.5
	6	26.4	62.8	91.8

to assay conditions but to the weanling rats used by Kreiner et al. (8) in their study. That both 5'-nucleotidase and glucagon-stimulated adenylate cyclase are similarly affected suggests that it is a lipid-mediated effect. Furthermore, it is presumably localised to the external half of the bilayer. This is because the activity of 5'-nucleotidase is apparently insensitive to perturbations of the inner half of the bilayer (19) and the activation energies of the linear fluoride-stimulated adenylate cyclase activity, which monitors this half of the bilayer (14-18) are similar in membranes from both 4 and 6 week old animals (table 1).

Alterations in the physical properties of the lipid bilayer affect the conformational flexibility of integral proteins, and hence their activity (23). Thus increases in bilayer fluidity augment enzyme activity and reduce the activation energy of the reaction (10,24). On this basis it is tempting to suggest that, in the case of the membranes from the 6 week and older animals, cooling the membrane below the lipid phase separation causes these enzymes to find themselves in a lipid domain that is more rigid than at temperatures above 28°C. However in the case of the membranes from the four week old rats then at temperatures below 28°C these enzymes would find themselves in a more fluid lipid domain. As marked changes in lipid composition occur over the weaning period (see 23) then it is not perhaps too untoward to expect that thermotropic perturbations of the complex domain structure occurring in these membranes (see 4,16,17) will lead to different effects. It would seem there-

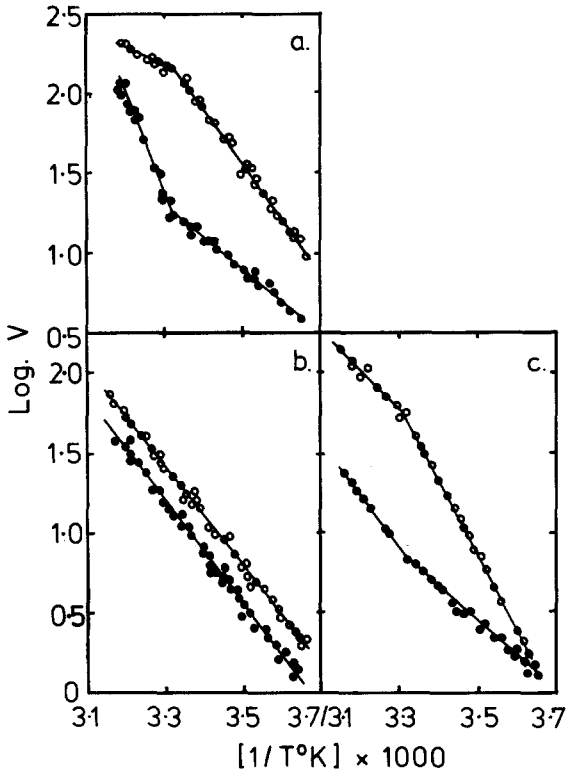

Figure 1 Arrhenius plots of adenylate cyclase and 5'-nucleotidase activity in liver plasma membranes

a) glucagon-stimulated adenylate cyclase. Activity was measured in the presence of 10^{-6}M glucagon + 5×10^{-7}M GTP to initiate Mobile Receptor Activation kinetics yielding a transmembrane complex (16,18,24). Rats of 4 weeks (●) and 6 weeks (o) of age.
b) fluoride-stimulated adenylate cyclase. Activity was measured in the presence of 15 mM-fluoride. Rats of 4 weeks (●) and 6 weeks (o) of age.
c) 5'-nucleotidase activity. Rats of 4 weeks (●) and 6 weeks(o) of age.
Log V, the activity of these enzymes is expressed in μunits/mg protein for adenylate cyclase and munits/mg protein for 5'-nucleotidase.

fore that the same enzymes can yield very different Arrhenius plots dependent on the nature of the lipid environment of the protein. Thus the ability of animals to alter their membrane lipid composition may act as a potent regulator of the functioning of integral membrane proteins.

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