ROLE OF SPECIFIC MEMBRANE LIPIDS IN MODULATING THE ACTIVITY OF ADENYLATE CYCLASE

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Adenylate cyclase is a hormonally responsive membrane-bound enzyme; many laboratories have attempted to define the role of lipids in this system (e.g., 1–8). In this communication we report that adenylate cyclase from rat brain apparently requires lysophosphatidylcholine or sphingomyelin for catalytic activity.

METHODS

Male Sprague-Dawley rats were decapitated and their brains removed; subsequent operations were performed at 0°C. The brain was homogenized in 8 vol (vol/wt) of 3 mM $MgCl_2$, 3 mM DTT, 50 mM Tris-HCl pH 8.4 and centrifuged for 10 min at 40,000 g_{max}. This procedure was repeated once and the pellet was homogenized in 8 vol (vol/wt) of 1 mM $MgCl_2$, 1 mM DTT, 10% glycerol (vol/vol), 0.5% sodium deoxycholate (DOC), 50 mM Tris-HCl, pH 8.4 (Buffer A). The homogenate was centrifuged for 45 min at 300,000 g_{max}, and the supernatant, containing solubilized enzyme, retained. Enzyme in the supernatant was separated from solubilized lipid by chromatography on Ultrogel AcA34 equilibrated with buffer A. When required, phospholipid, dissolved in chloroform:methanol (2:1 vol/vol) and dried under a stream of N_2, was dispersed in the solubilized enzyme preparation. Enzyme activity was measured at 30°C (10).

RESULTS AND DISCUSSION

Adenylate cyclase solubilized with 0.5% DOC from rat brain has essentially no enzymic activity (Table I), but full activity can be restored by the addition of phosphatidylcholine. If the mixture of enzyme and phospholipid is diluted and the lipid vesicles collected by ultracentrifugation, they are found to contain all of the adenylate cyclase activity (9). The ability of enzyme to become lipid associated has been noted previously and extensively characterized for a preparation solubilized with Nonidet P40 (10). The incorporation of solubilized enzyme into liposomes suggests either that adenylate cyclase has a hydrophobic membrane-binding domain or that it is cosolubilized with a hydrophobic protein to which it can attach. The possession of a hydrophobic region raises the possibility that lipids may be capable of regulating enzyme activity. To investigate this possibility, the effect of various phospholipids on the activity of DOC-solubilized enzyme was investigated.

In addition to phosphatidylcholine, nonionic detergent, sphingomyelin, lysophosphatidylcholine or phosphatidyl-*N*-methylethanolamine can restore full activity to the DOC-solubilized enzyme. Partial activity was restored by phosphatidylethanolamine or phosphatidyl-*N*,*N*-dimethylethanolamine (Fig. 1 *A*). All other lipids tested were without effect when added alone, and when added with phosphatidylcholine they generally inhibited reconstitution (9).

The DOC-solubilized adenylate cyclase preparation contains lipids solubilized from the brain with the enzyme, which complicates the analysis. To exclude this interference, the solubilized preparation was delipidated by gel filtration. Maximal activity was restored to this preparation by lysophosphatidylcholine. Sphingomyelin, when mixed with phosphatidylcholine, will also activate; below 75 mol % the extent of activation is directly proportional to the sphingomyelin concentration. At higher concentrations the physical state of the lipid suspension changes, becoming more particulate, which may account for the lower activity observed. Phosphatidylcholine and Triton X-100 partially reconstitute activity (Fig. 1 *B*). All other lipids tested were unable to reconstitute activity and inhibited the stimulation observed with lysophosphatidylcholine or sphingomyelin.

TABLE I
SOLUBILIZATION OF ADENYLATE CYCLASE FROM RAT BRAIN AND INCORPORATION INTO LIPOSOMES

Sample	Adenylate cyclase activity	Protein concentration
	pmol min^{-1}	*mg ml*$^{-1}$
Original brain homogenate	17.0	13.0
Deoxycholate supernatant	0.5	5.1
Deoxycholate pellet	1.3	7.9
Deoxycholate supernatant + phosphatidylcholine	36.5	5.1
Liposomes	45.5	3.0

Rat brain was homogenized and extracted with DOC as described in Methods. Phosphatidylcholine (10 mg ml^{-1}) was dispersed in the solubilized enzyme preparation, the mixture was diluted ~20–40-fold, and liposomes were collected by centrifugation (300,000 g_{max} for 1 h). All samples were resuspended to equivalent volumes, and aliquots were assayed for adenylate cyclase activity.

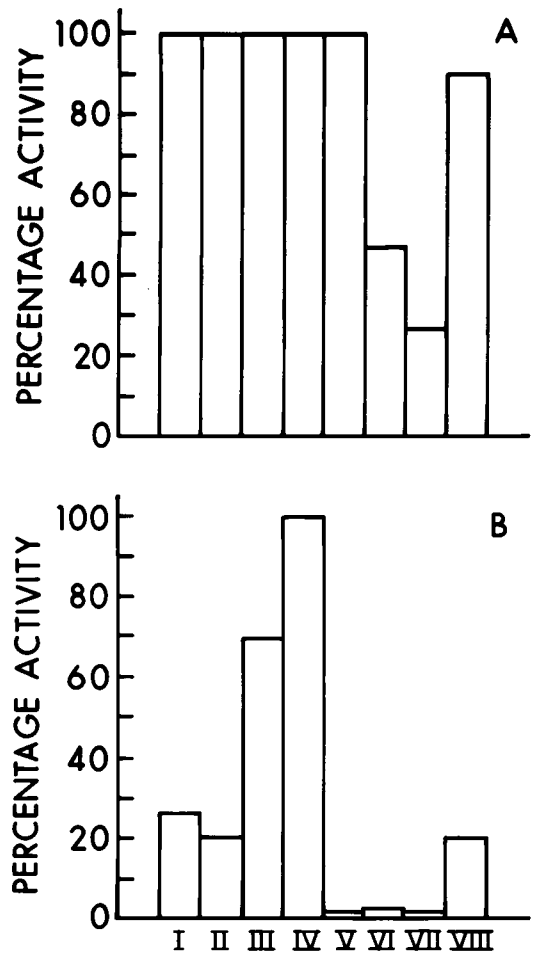

FIGURE 1 Phospholipid specificity of adenylate cyclase activation before and after delipidation. Solubilized adenylate cyclase before (*A*) or after (*B*) delipidation was assayed with phospholipid (10 mg ml^{-1} in assay) or Triton X-100.

In conclusion, these results suggest adenylate cyclase has a specific phospholipid requirement for catalytic activity. Changing the lipid environment of the enzyme might well be a mechanism whereby activity is normally modulated by hormones, by changes in the metabolic status of the cell, or during differentiation. Many possible mechanisms exist for effecting changes in the lipid environment of the enzyme. For example, exchanging an inhibitory for a stimulatory lipid by a phospholipid exchange protein or hydrolyzing phosphatidylcholine to produce lysophosphatidylcholine can both be predicted to activate adenylate cyclase. These and other possibilities are currently under investigation.

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REGULATION OF MICROSOMAL HMG-CoA REDUCTASE BY ENZYME-LIPID INTERACTIONS

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The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) catalyzes the rate-limiting reaction of hepatic cholesterol synthesis under most physiological conditions (Rodwell et al., 1976). The enzyme is unique among the early enzymes of the pathway in that it is bound to the endoplasmic reticulum in vivo. This gives rise to the potential regulation of the enzyme through interactions with the membrane lipids. Interactions between membrane bound proteins and lipid components has been the subject of several recent reviews (Lenaz

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