RESTORATION OF GLUCAGON RESPONSIVENESS OF SOLUBILIZED MYOCARDIAL ADENYL CYCLASE BY PHOSPHATIDYLSERINE

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SUMMARY. Solubilized preparations of cat myocardial adenyl cyclase have been previously shown to be unresponsive to glucagon, norepinephrine, and thyroxine, hormones which activate the particulate adenyl cyclase. This report demonstrates that the addition of phosphatidylserine to a solubilized preparation freed of detergent, completely restores the glucagon responsiveness of adenyl cyclase. Half-maximal activity and maximal percent increase in cyclic 3',5'-AMP accumulation were virtually identical to that previously reported for the particulate preparation. Norepinephrine activates myocardial adenyl cyclase by a receptor mechanism separate and distinct from the glucagon receptor and in contrast to glucagon, norepinephrine responsiveness was not restored by phosphatidylserine. Phosphatidylserine may produce a configurational change in the adenyl cyclase which then allows glucagon binding and activation of adenyl cyclase.

We have recently described a one-step method for solubilizing the adenyl cyclase present in cat heart homogenates using a nonionic detergent, Lubrol-PX, an ethylene oxide condensate of dodecanol (1). The solubilized enzyme was not activated by glucagon, norepinephrine, or thyroxine, hormones which activate the particulate cat heart adenyl cyclase. The lack of responsiveness to hormonal stimulation was not due to the presence of the detergent since the enzyme freed of detergent by DEAE-cellulose chromatography remained unresponsive to these hormones (2). In addition, the solubilized adenyl cyclase loses 50-75 percent of its basal activity following DEAE-cellulose chromatography. This activity can in large part be restored by the addition of certain phospholipids, especially phosphatidylserine (2). The present report demonstrates that the addition of phosphatidylserine totally restores glucagon responsiveness

to the solubilized enzyme in contrast to the continued unresponsiveness of the enzyme to norepinephrine.

METHODS. Normal cats were anesthetized with pentobarbital, 25-35 mg per kg intraperitoneally, and the heart was quickly excised. The left ventricle was dissected free of endocardium and epicardium and about 300 mg of muscle was homogenized in 4.5 ml of a cold solution containing in final concentration 0.25 M sucrose, 0.01 M Tris-Cl, pH 7.7, 0.02 M Lubrol-PX, and 0.001 M EDTA-magnesium chloride. The homogenate was centrifuged at 12,000 x g for 10 minutes at 4°C. The 12,000 x g supernatant was used for DEAE column chromatography. Adenyl cyclase was assayed by the method of Krishna, Weiss, and Brodie (3). The fractions for assay, containing 0.05 to 0.12 mg protein in a total volume of 0.06 ml were incubated ay 37°C for five minutes with ATP 1.6 mM, ATP-α-^{32}P 2.5-3.5 x 10^6 cpm, theophylline 8 mM; Mg Cl_2, 2 mM; Tris-Cl, 21 mM, pH 7.7; and human serum albumin, 0.8 mg/ml. Glucagon or norepinephrine were present at concentrations stated in the text. The incubations were started by adding the enzyme at 1°C, to the other components which were at 23°C. After five minutes the incubations were stopped and the cyclic 3', 5'-AMP-^{32}P accumulated was determined as previously described (4). Phosphatidylserine was prepared for use by sonication in cold 0.01 M Tris buffer, pH 7.7 (3.0 mg/ml) and was added to the enzyme immediately before incubation.

MATERIALS. Lubrol-PX was a gift from ICI America Inc. Phosphatidylserine was from Applied Science Laboratories; DEAE-cellulose (DE-50) from Reeve-Angel; ATP-α-^{32}P, 880 mc - 4.3 c/mmole, from International Chemical and Nuclear Corp.; and cyclic 3',5'-AMP-^{3}H from Schwarz Bioresearch.

RESULTS. Effect of phosphatidylserine on the glucagon-mediated activation of solubilized adenyl cyclase. Glucagon, 1 x 10^{-5}M, and norepinephrine (NE), 5 x 10^{-5}M, maximally activate the particulate myocardial adenyl cyclase (4,5). The solubilized enzyme in the presence (1)

TABLE 1

	Picomoles Cyclic 3',5'-AMP Accumulated/5 min/mg protein		
	Control	Glucagon 1×10^{-5}M	NE 5×10^{-5}M
DEAE Fraction (1M Tris)	469 ± 27	441 ± 50	442 ± 36
DEAE Fraction (1M Tris) + phosphatidylserine, 6 µg/incubation	726 ± 28	1100 ± 35*	765 ± 56

Effect of phosphatydyl serine on the glucagon-mediated activation of myocardial adenyl cyclase. Each value represents the mean ± S. E. of 16-20 samples in five experiments from three cats. Approximately 1.3 ml of 12,000 x g supernatant containing the solubilized myocardial adenyl cyclase and having a protein concentration of 4 mg/ml was applied to a 1.0 x 12.0 cm DEAE-cellulose column equilibrated at 4°C in 0.01M Tris buffer, pH 7.7. The flow rate was approximately 0.25 ml/min. The column containing the enzyme was washed with 10 volumes of 0.01M Tris, pH 7.7. Adenyl cyclase was eluted with 1M Tris buffer, pH 7.7. The fraction containing adenyl cyclase activity has been shown to be totally free of detergent using Lubrol-PX labelled with ^{14}C in the ethylene oxide moiety (2).

* p value <0.01.

or absence of detergent is not activated by these hormones (Table 1). However, the enzyme, freed of detergent by DEAE-cellulose chromatography, is activated by glucagon but not by norepinephrine upon the addition of phosphatidylserine to the incubation (Table 1). A glucagon concentration response curve in the presence of phosphatidylserine is almost identical to that reported from this laboratory for the glucagon activation of particulate myocardial adenyl cyclase (4,6) (Table 2). Half-maximal activity was approximately 5×10^{-7}M. The maximal percent increase (60%) in cyclic 3',5'-AMP accumulation is also similar to that for the particles.

Phosphatidylserine, 2 µg per incubation, did not restore the glucagon responsiveness. Also, glucagon responsiveness is not restored

TABLE 2

Glucagon (M)	Picomoles Cyclic 3',5'-AMP Accumulated/5 min/mg protein
0	733 ± 50
1 x 10^{-7}M	793 ± 30
1 x 10^{-6}M	1218 ± 100
1 x 10^{-5}M	1134 ± 40
5 x 10^{-5}M	1123 ± 60

Activation of solubilized myocardial adenyl cyclase by graded doses of glucagon. Each value represents the mean ± S. E. of 6-9 samples.

if the phosphatidylserine is added to the solubilized enzyme in the presence of detergent (12,000 x g supernatant).

DISCUSSION. Sutherland, Rall, and Menon first suggested that adenyl cyclase may be bound to membrane phospholipid and could be considered a lipoprotein (7). The activity of this enzyme *in vivo* may be dependent on the integrity of the lipid-protein complex. The data previously reported showing an increase in basal adenyl cyclase activity following DEAE-cellulose chromatography by the addition of certain phospholipids to the enzyme including phosphatidylserine, and to a lesser extent phosphatidylethanolamine, and sphingomyelin are consistent with the hypothesis that adenyl cyclase is a lipoprotein (2). This solubilized preparation in the absence of phospholipid was not activated by the hormones which activate the particulate adenyl cyclase, including glucagon, norepinephrine, and thyroid hormone.

The data in the present report clearly demonstrates that the addition of phosphatidylserine to the solubilized enzyme totally restores the glucagon responsiveness noted with the particulate adenyl cyclase.

In contrast, the addition of this phospholipid does not restore the norepinephrine responsiveness of the cyclase. It is of interest that these two hormones have been previously shown to activate the adenyl cyclase by separate and distinct receptor systems in particulate adenyl cyclase preparations (4,8). Norepinephrine activation is mediated by the beta-receptor and hence can be abolished by propranolol and glucagon by a non-beta receptor mechanism which is not blocked by beta blocking agents.

The fundamental physical characteristics of hormone receptors are poorly understood. Whether they are separate, functional units of the cell membrane or part of the adenyl cyclase itself is not clear. The loss of hormone responsiveness of the solubilized adenyl cyclase in conjunction with a fully intact fluoride responsiveness was previously interpreted as being consistent with the former (1,2). Whatever the case, it seems likely that the receptors are responsible for the hormonal specificity of the cyclase. The data in this report indicate that phosphatidylserine serves an important role in mediating the glucagon activation of myocardial adenyl cyclase. Whether phosphatidylserine is the actual receptor for glucagon cannot be stated. Perhaps phosphatidylserine induces some specific configurational change in the cyclase molecule which then permits glucagon-binding and activation of adenyl cyclase. In this regard, adenyl cyclase activation by glucagon in isolated liver membranes and binding of ^{125}I-glucagon to these membranes can be significantly diminished by digitonin treatment of the membranes (9). It is of considerable interest that glucagon binding and activation of adenyl cyclase can be partially restored in this system upon the addition of a phospholipid fraction extracted from liver membranes consistent with phosphatidylserine and by pure phosphatidylserine (9).

The effect of phosphatidylserine on the histamine and thyroxine-mediated activations of cat myocardial adenyl cyclase as well as the

effect of other phospholipids on the hormonal activation of the myocardial cyclase are currently under investigation.

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