SOLUBILIZATION OF MYOCARDIAL ADENYL CYCLASE

Gerald S. Levey
Cardiology Branch, National Heart Institute,
National Institutes of Health, Bethesda, Maryland

Received October 28, 1969

SUMMARY

The detergent, Lubrol-PX, solubilized approximately 90-100% of the particulate adenyl cyclase in cat left ventricular homogenates. The activity in the 12,000 x g supernatant was not sedimented at centrifugal speeds as great as 105,000 and 250,000 x g for 2 hours. Electronmicroscopy revealed a virtual absence of particulate material in the ultracentrifuge supernatants. Adenyl cyclase activity was not filterable by a 0.22 μ millipore filter. Sephadex chromatography indicated a molecular weight of approximately 100,000 to 200,000. The solubilzed enzyme was activated by sodium fluoride but not by the hormones which activate the particulate adenyl cyclase, norepinephrine, glucagon or thyroxine.

INTRODUCTION

Adenyl cyclase is a membrane-bound enzyme thought to mediate the effects of a variety of hormones on their respective target tissues (1). The purification of adenyl cyclase has been impaired because of its association with particulate cellular components and the instability of the enzyme in broken cell preparations. Sutherland et al (2) reported partial solubilization of the enzyme with Triton in brain, heart, skeletal muscle, and liver following a multi-step procedure. The recoveries of adenyl cyclase were low and the Triton itself caused loss of enzyme activity.

This report describes a one step method for solubilizing virtually all the adenyl cyclase present in cat heart homogenates using a non-ionic detergent, Lubrol-PX. The adenyl cyclase in this preparation is fully activated by fluoride but is unresponsive to the hormones which activate particulate myocardial adenyl cyclase, norepinephrine, glucagon, and thyroxine.

MATERIALS AND METHODS

Lubrol-PX was a gift from ICI America Inc., adenosine 5'-triphosphate disodium salt (ATP) from P-L Biochemicals; norepinephrine bitartrate from

Mann Research Laboratories, crystalline glucagon was a gift from Eli Lilly and Co., L-thyroxine from Sigma; [^{32}P] ATP 880-1.5 mCi/mMole from International Chemical and Nuclear Corporation; and cyclic 3',5'-[^{3}H] AMP from Schwarz Bioresearch.

Left ventricular muscle was obtained from normal cats, and a single cat was used for each experiment. After anesthesia with pentobarbital, 25-35 mg per Kg intraperitoneally, the heart was quickly excised. The left ventricle was dissected free of endocardium and epicardium and approximately 300-350 mg of muscle was homogenized in 4.5 ml of a cold solution containing in final concentration 0.25 M sucrose, 0.20 M tris-Cl, pH 7.4, 0.02 M Lubrol-PX,and EDTA-magnesium chloride, 0.001 M. The homogenate was centrifuged at 12,000 x g for 10 minutes at 4° C. The supernatant was assayed for adenyl cyclase activity or subsequently centrifuged at 105,000 x g or 250,000 x g. The precipitates from the centrifugations were washed twice with cold 0.25 M sucrose pH 7.0 and resuspended, rehomogenized, and assayed for adenyl cyclase activity. Preparations of particulate adenyl cyclase were derived from the same hearts by a method previously described (3). Adenyl cyclase was assayed by a recently developed method (4,5). The fractions for assay, containing 0.05 to 0.12 mg protein in a total volume of 0.06 ml, were incubated at 37° C for 5 minutes with ATP, 1.6 mM, [^{32}P] ATP, 2.5 to 3.5 x 10^{6} cpm, theophylline, 8 mM; $MgCl_2$, 2 mM; tris-Cl, 21 mM, (pH 7.7); human serum albumin, 0.8 mg/ml. When present, sodium fluoride was at 8 x 10^{-3} M, norepinephrine at 5 x 10^{-5} M, glucagon at 1 x 10^{-5} M, and L-thyroxine at 2 x 10^{-6} M. The incubations were started by adding the enzyme at 1° C, to the other components which were at 23° C. After 5 minutes the incubations were stopped and the cyclic 3',5'-[^{32}P] AMP produced was determined as described previously (6). Protein was determined by the method of Lowry et al (7).

RESULTS

Adenyl cyclase activity in left ventricular cat heart homogenates is associated with the particulate fractions and is precipitable by centrifuga-

Table 1

Expt	Lubrol	Picomoles Cyclic 3',5'-AMP Accumulated/5 min/mg Protein				
		12,000 x g Precipitate		12,000 x g Supernatant		% Solubilized
		Control	NaF	Control	NaF	
1	-	91 ± 10	394 ± 31	-	-	
	+	<1	<1	143 ± 10	358 ± 23	90%
2	-	251 ± 10	585 ± 50	<1	70 ± 10	
	+	<1	<1	218 ± 7	582 ± 18	90%
3	-	95 ± 10	414 ± 16	20 ± 10	60 ± 10	
	+	24 ± 5	44 ± 2	178 ± 6	420 ± 13	91%
4	-	195 ± 6	564 ± 15	25 ± 13	75 ± 21	
	+	<1	<1	295 ± 5	755 ± 16	100%
5	-	193 ± 17	620 ± 10	-	-	
	+	-	-	170 ± 6	565 ± 10	93%

The effect of Lubrol on precipitation of myocardial adenyl cyclase. Each value represents the mean ± SE of 3-6 samples. Sodium fluoride was present at 8×10^{-3} M.

tion at 12,000 x g for 10 minutes. Table 1 demonstrates that following the addition of the detergent Lubrol-PX to the homogenizing mixture adenyl cyclase activity is located in the 12,000 x g supernatant and virtually none in the precipitate. In left ventricular homogenates from 5 cats prepared in the presence of Lubrol, 90-100 per cent of the fluoride-stimulatable adenyl cyclase activity was found in the soluble fraction (Table 1). Furthermore, the adenyl cyclase activity in the 12,000 x g supernatant was not precipitable at ultracentrifuge speeds of 105,000 x g and 250,000 x g for 2 hours. (Table 2). Although no significant adenyl cyclase activity was detected in the precipitates the centrifugations were associated with approximately a 40-50 per cent loss in fluoride-stimulatable activity in the supernatants (Table 2). The 12,000 x g supernatant was also filtered through a 0.22 μ

Table 2

Expt	Picomoles Cyclic 3',5'-AMP Accumulated/5 min/mg Protein: Control	NaF (8×10^{-3} M)
1		
12,000 x g Supernatant	-	-
105,000 x g Supernatant	25 ± 3	205 ± 29
105,000 x g Precipitate	<1	<1
2		
12,000 x g Supernatant	178 ± 6	420 ± 13
105,000 x g Supernatant	46 ± 17	210 ± 29
105,000 x g Precipitate	<1	<1
3		
12,000 x g Supernatant	280 ± 18	532 ± 10
250,000 x g Supernatant	121 ± 13	368 ± 4
250,000 x g Precipitate	<5	<5

Ultracentrifugation for 2 hours of 12,000 x g supernatant from Lubrol-treated homogenates. Each value represents the mean ± SE of 3 samples.

Table 3

	Picomoles Cyclic 3',5'-AMP Accumulated/5 min/mg Protein: Control	NaF (8×10^{-3} M)
12,000 x g Supernatant	280 ± 18	532 ± 10
Millipore Filtrate	262 ± 6	609 ± 7

Effect of millipore filtration on adenyl cyclase activity in 12,000 x g supernatant. Each value represents the mean ± SE of 3 samples.

millipore filter (Table 3). Total adenyl cyclase activity was obtained in the filtrate. Electromicroscopic appearance of the 105,000 x g and 250,000 x g supernatants was compared to that of the 12,000 x g supernatant. In

contrast to the 12,000 x g supernatant which contained an abundance of particulate material, areas containing particulate material in the 105,000 x g and 250,000 x g supernatants were scattered and sparse (Figure 1).

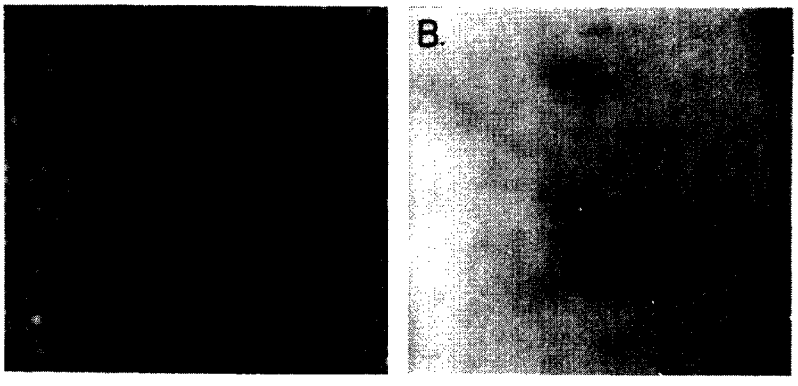

Figure 1. Electronmicroscopy of the 12,000 x g supernatant (A) and 105,000 x g supernatant (B). The particles denoted by the arrows (B) are approximately 500 A. x 30,000.

The soluble enzyme preparation was passed through a column of Sephadex G-200 at a rate of 0.05 ml per min. Adenyl cyclase activity emerged at 34 to 50 per cent of the column volume with a peak at approximately 40 per cent. Blue Dextran (M.W.~2,000,000), which is excluded by Sephadex G-200, appeared in the eluate between 26 to 38 per cent of the column volume with the peak at approximately 30 to 32 per cent. In contrast, enzyme activity on a Sephadex G-100 column emerged at 24 to 37 per cent with a peak at 30 to 32 per cent, a pattern identical to that of Blue Dextran on the same column. The data suggest a molecular weight of 100,000 to 200,000 for the solubilized enzyme.

The particulate myocardial adenyl cyclase is activated by norepinephrine, glucagon, and thyroid hormone (6,8,9). However, adenyl cyclase in the soluble fraction was not activated by these hormones.

DISCUSSION

These results demonstrate that the detergent, Lubrol-PX, has the capacity to solubilize approximately 90-100% of the membrane-bound adenyl cyclase in cat left ventricular myocardial homogenates. In the absence of Lubrol most of the adenyl cyclase activity is precipitated by centrifugation at 12,000 x g for 10 minutes, whereas in its presence virtually all the activity is located in the 12,000 x g supernatant and cannot be sedimented at centrifugal speeds as great as 105,000 x g and 250,000 x g for 2 hours.

Although the 105,000 x g and 250,000 x g precipitates are devoid of adenyl cyclase activity, the centrifugation is associated with approximately a 40-50 per cent loss in activity. Since the 12,000 x g supernatant shows no loss of activity if allowed to remain at 4° C for two hours, it is possible that a particulate factor is necessary for maintenance of total adenyl cyclase activity, which is precipitated at the higher speeds.

The concentration of Lubrol used in the homogenizing solution, 1×10^{-2} M, does not impair fluoride-stimulatable activity as compared to untreated heart homogenates or particulate preparations. This fact, in addition to the almost complete solubilization achieved, makes this detergent preferable to Triton as an agent for solubilizing adenyl cyclase. Triton causes a decrease in adenyl cyclase activity and its use achieves only approximately 20 per cent solubilization of the enzyme (2).

Lubrol has been reported to solubilize another membrane-bound enzyme, Na-K adenosinetriphosphatase (Na-K ATPase) from guinea pig brain microsomes (10,11). Approximately 40-60 per cent of the Na-K ATPase in the microsomes was solubilized after being allowed to stand for 15 minutes at 0° C in the presence of Lubrol.

It is of considerable interest that the solubilized adenyl cyclase was activated by sodium fluoride but not by hormones. The data may be consistent with the hypothesis that the receptors are a physical component of the cell membrane and solubilization abolishes the capacity of adenyl cyclase to

respond to its activating hormones. However, definite conclusions are not warranted until the enzyme is separated from the detergent since the detergent itself may be inhibiting hormone activation. Furthermore, it would be of interest to determine whether the enzyme remains in the soluble state in the absence of the detergent. Studies in this regard are now in progress.

ACKNOWLEDGEMENTS

I am grateful to Dr. Joan Blanchette-Mackie for preparation and interpretation of the electronmicrographs and to Mrs. Hope Cook for her expert technical assistance.

REFERENCES

1. SUTHERLAND, E. W., AND ROBISON, G. A. Pharmacol. Rev. 18: 145 (1966).
2. SUTHERLAND, E. W., RALL, T. W., AND MENON, T. J. Biol. Chem. 237: 1220 (1962).
3. LEVEY, G. S., AND EPSTEIN, S. E. Biochem. Biophys. Res. Commun. 33: 990 (1968).
4. KRISHNA, G., WEISS, B., AND BRODIE, B. B. J. Pharm. Exptl. Therap. 163: 379 (1968).
5. RODBELL, M. J. Biol. Chem. 242: 5744 (1967).
6. LEVEY, G. S., AND EPSTEIN, S. E. Circulation Res. 24: 151 (1969).
7. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. J. Biol. Chem. 193: 265 (1951).
8. MURAD, F., CHI, Y. M., RALL, T. W., AND SUTHERLAND, E. W. J. Biol. Chem. 237: 1228 (1962).
9. LEVEY, G. S., AND EPSTEIN, S. E. J. Clin. Invest. 48: 1663 (1969).
10. SWANSON, P. D., BRADFORD, H. F., AND MC ILWAIN, H. Biochem. J. 92: 235 (1964).
11. MEDZIHRADSKY, F., KLINE, M. H., AND HOKIN, L. E. Arch. Biochem. Biophys. 121: 311 (1967).