DEMONSTRATION OF ADENYL CYCLASE ACTIVITY IN CANINE CARDIAC SARCOPLASMIC RETICULUM*

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SUMMARY

A microsomal fraction from canine cardiac muscle that actively accumulates calcium has been found to exhibit adenyl cyclase activity. The enzyme responds in a characteristic manner to fluoride and norepinephrine. Electron microscopic studies of the microsomal fraction demonstrate that it contains intact membrane vesicles; the lack of any glutamic dehydrogenase activity suggests that no contaminating mitochondrial fragments are present. In view of its active calcium accumulating ability, this fraction is believed to represent fragments of sarcoplasmic reticulum. This report represents the first demonstration of adenyl cyclase in cardiac sarcoplasmic reticulum.

INTRODUCTION

The interaction of cyclic 3', 5'-AMP with calcium ion has been observed in several systems (Nayler, 1967; Rasmussen and Tennenhouse, 1968; Sutherland and Robison, 1966). Not only has calcium ion been found necessary for some of the reactions catalyzed by cyclic 3', 5'-AMP, but it has been suggested that calcium is actually the secondary effector and that, in some instances, cyclic 3', 5'-AMP may act mainly to augment calcium transport across membranes (Rasmussen and Tennenhouse, 1968).

Sutherland et al (1962) have investigated the distribution of adenyl cyclase (the enzyme responsible for the conversion of ATP into cyclic 3', 5'-AMP and pyrophosphate) in the cardiac cell and found it to be chiefly in the low speed precipitate fraction containing nuclei and external cell membranes and to be membrane bound. These authors failed to find significant activity in the microsomal fraction that contains the fragments of sarcoplasmic reticulum.

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The adenyl cyclase system is believed by some investigators to be of prime importance in mediating the positive inotropic effects of the catecholamines and certain other hormones (Nayler, 1967; Sutherland and Robison, 1966). In addition, current information relating to excitation-contraction coupling suggests that the sarcoplasmic reticulum may play a central role in determining the intensity of mechanical contraction by regulating the calcium concentration at the actomyosin junction in systole (Entman et al, 1969). We therefore investigated a calcium accumulating microsomal fraction of canine myocardium, believed to represent the sarcoplasmic reticulum, for adenyl cyclase activity. This study demonstrates adenyl cyclase of high specific activity with characteristic augmentation of fluoride (Rall and Sutherland, 1958) and norepinephrine (Murad et al, 1962).

METHODS

The microsomal fraction from freshly killed dog heart was prepared as previously described (Entman et al, 1969; Katz and Repke, 1967) by differential centrifugation utilizing a 20 to 35% sucrose gradient as the final purification step. The resulting fraction was found to contain membranous vesicles of .5 to 1.5 microns in diameter and no other structures. No detectable glutamic dehydrogenase (an enzyme found in both membrane and soluble mitochondrial subfractions) was found in this fraction, demonstrating an absence of mitochondrial contamination (Sottacasa et al, 1967). All microsomal preparations used exhibited the calcium accumulating and exchange properties previously described (Entman et al, 1969; Katz and Repke, 1967) and had characteristic ATPase activities (Entman et al, 1969). They maintained these activities throughout the period that adenyl cyclase was investigated.

Adenyl cyclase was assayed by a recently developed method (Krishna and Brodie, 1968). The microsomal fraction, containing 0.02-0.025 mg protein in a total volume of 0.06 ml, was incubated at 37° C for 3 minutes with ATP, 1.6 mM; $AT^{32}P$, 2.5 to 3.5 x 10^6 cpm; theophylline, 8 mM; $MgCl_2$, 2mM; tris-Cl, 21 mM, (pH 7.7); human serum albumin, 0.8 mg/ml; and concentrations of fluoride

and norepinephrine as stated in the text. The incubations were started by adding the vesicles which had been kept at 1° C, to the other components which were at 23° C. Fluoride or norepinephrine were added to the vesicles just before the incubations were initiated. After the appropriate time, the incubations were stopped by adding 0.1 ml of a solution containing 4 μmoles of ATP, 1.25 μmoles of cyclic 3', 5'-AMP and 0.15 μc of ^{3}H-cyclic 3', 5'-AMP and boiled for 3 minutes. The ^{3}H-cyclic 3', 5'-AMP served to determine the recovery of cyclic 3', 5'-AMP during the procedure; recovery ranged from 30 to 35%. After boiling, 0.4 ml of water was added, the precipitate removed by centrifugation and the supernate applied to 0.5 x 2.0 cm Dowex-50 column. The column was washed with water and the eluate, between 3.0 to 6.0 ml, was collected and precipitated with 0.17 m $ZnSO_4$ and 0.15 M Ba $(OH)_2$ and the cyclic 3', 5'-AMP-^{32}P and ^{3}H-cyclic 3', 5'-AMP counted in a liquid scintillation spectrometer. The presence of cyclic 3', 5'-AMP was confirmed by thin-layer chromatography (Levey and Epstein, 1968). Phosphodiesterase activity was measured as previously described (Levey and Epstein, 1968). Protein was determined by the Biuret method.

RESULTS

The presence of adenyl cyclase of high specific activity is demonstrated in Table I. The enzyme reaction was linear with time up to 10 minutes in

TABLE I

	Picomoles Cyclic 3',5'-AMP Accumulated/mg Protein/10 min
Control	241.0 ± 16
Fluoride (8×10^{-3} M)	650 ± 15*
Norepinephrine (5×10^{-5} M)	364 ± 23+

The effect of fluoride and norepinephrine on cardiac adenyl cyclase. Each value represents the mean ± SE of 4-7 samples.
* $p < 0.01$
+ $p < 0.05$

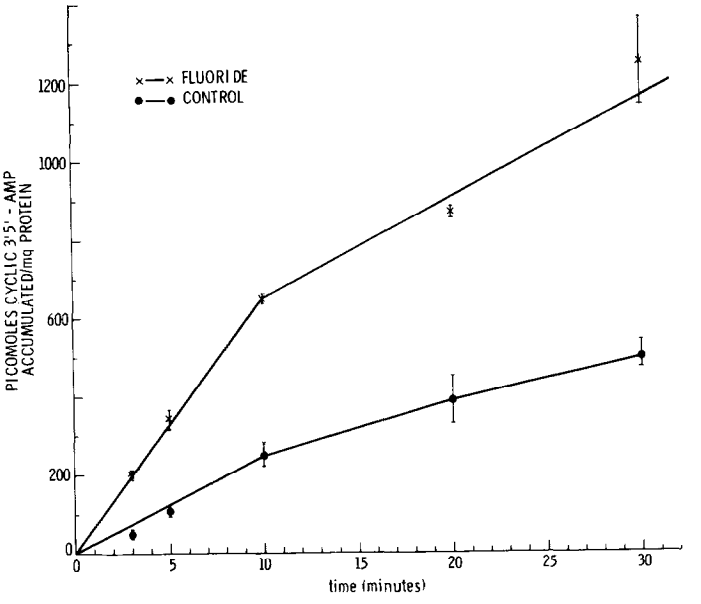

Fig. 1. Fluoride-activation of adenyl cyclase as a function of time. Incubation conditions are stated in the text. Fluoride was present at 8 mM. Each value represents the mean ± SE of 3 samples.

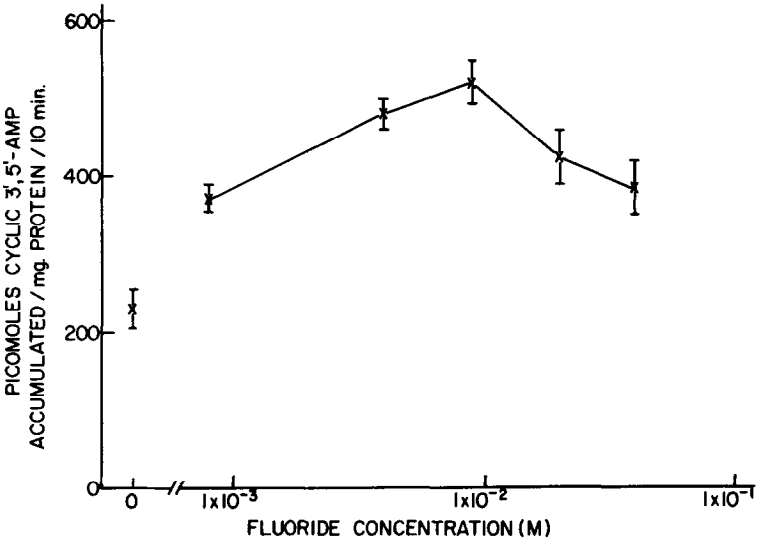

Fig. 2. Activation of adenyl cyclase as a function of fluoride concentration. Incubation conditions are stated in the text. Each value represents the mean ± SE of 4 samples.

control reactions as well as with fluoride stimulation (Fig. 1). Figure 2 demonstrates a dose response curve to fluoride activation showing increasing activation over fluoride concentration from 0.8 to 8.0 mM with half maximal activity at 1.0 mM. Concentrations in excess of 8 mM produced a decline in cyclic 3', 5'-AMP^{32} accumulation.

There was no detectable phosphodiesterase activity in the preparations used for these experiments.

DISCUSSION

The microsomal fraction described is thought to represent the fragmented sarcoplasmic reticulum because of its sedimentation characteristics (sedimentation at 100,000 g from a 13,000 g supernatant) and the fact that it rapidly accumulates large amounts of calcium ion (Entman et al, 1969; Katz and Repke, 1967). The demonstration of adenyl cyclase activity in this fraction is of considerable physiologic interest. Since adenyl cyclase has been found to enhance calcium transport in other systems (Nayler, 1967; Rasmussen and Tennenhouse, 1968), the enzyme might serve a similar function in the sarcoplasmic reticulum.

In all studies of microsomal activity the possibility of external membrane contamination must be evaluated. We feel that the results described in this report are not a consequence of contamination for the following reasons:

a) The microsomal fraction exhibited active calcium accumulation in specific activity similar to that reported in the past for cardiac microsomal fractions (Entman et al, 1969; Katz and Repke, 1967).

b) The enzyme specific activity was similar to that reported in the slower sedimenting fraction of dog heart homogenate (Sutherland et al, 1962), whereas it would be "diluted" if the activity were merely contamination.

c) The samples were not "overfragmentized" as evidenced by their excellent capacity to accumulate calcium (calcium metabolism has been shown to be very sensitive to too vigorous homogenization [Katz and Repke, 1967]).

d) Finally, there was no mitochondrial contamination as might be

expected if cellular organelles were being fragmented into particles of microsomal size.

Our use of a very sensitive assay for adenyl cyclase has enabled us to measure adenyl cyclase activity in this relatively pure microsomal preparation despite very low protein concentration. The lack of such a sensitive assay may account for the previous failure to detect enzyme activity in cardiac microsomes (Sutherland et al, 1962). In this regard, Rabinowitz has demonstrated the existence of adenyl cyclase in a 54,000 g fraction from skeletal muscle containing a relatively high protein concentration (Rabinowitz et al, 1965).

The demonstration of adenyl cyclase activity in a fraction thought to represent sarcoplasmic reticulum suggests that the enhancement of myocardial contractility by hormones known to activate adenyl cyclase may occur, at least in part, as a result of activation of enzyme localized to the area of the sarcoplasmic reticulum.

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