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STUDIES ON THE RELATIONSHIP BETWEEN ADENYLATE CYCLASE ACTIVITY AND CALCIUM TRANSPORT BY CARDIAC SARCOTUBULAR MEMBRANES*

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SUMMARY

The relationship between changes in adenylate cyclase activity and calcium transporting ability of the dog heart sarcotubular membrane was examined under different experimental conditions. Treatment of the sarcotubular membranes with phospholipase C or trypsin decreased adenylate cyclase activity more than calcium uptake. In low concentrations both norepinephrine (10^{-7}–10^{-4} M) and NaF (1–5 mM) increased adenylate cyclase activity without any appreciable changes in calcium uptake; whereas in high concentrations, these agents decreased calcium uptake by the sarcotubular membranes. Different concentrations of glucagon and prostaglandins E_1 and $F_{2\alpha}$ increased adenylate cyclase activity without changing calcium uptake. The calcium binding ability of the sarcotubular membranes did not alter when examined in the presence of norepinephrine, glucagon and prostaglandins E_1 and $F_{2\alpha}$. Increasing the concentration of calcium in the incubation medium increased calcium binding by the sarcotubular membranes without appreciably affecting the adenylate cyclase activity. Adenosine 3′,5′-monophosphate (cyclic AMP) neither influenced the calcium binding constant nor the number of calcium binding sites. Although we have failed to show a direct relation between changes in adenylate cyclase and calcium transport under the experimental conditions employed in this study, the results suggest that adenylate cyclase–cyclic AMP and calcium transport systems of the heart sarcotubular membranes are independently controlled.

INTRODUCTION

The presence of adenylate cyclase in the heart sarcoplasmic reticulum, which is known to bind and accumulate calcium, was claimed by Entman *et al.*[1] and confirmed by others[2,3]. Although the skeletal muscle sarcoplasmic reticulum was also shown to contain adenylate cyclase activity[4], the possibility of contaminating plasma membranes was not excluded in these early studies. Recently it was demonstrated that the fraction of the sarcotubular membranes, obtained by sucrose density gradient, possessing the highest calcium accumulating ability also showed the highest adeny-

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late cyclase activity[5]. In the present study we wish to examine whether or not a relationship exists between the changes in adenylate cyclase activity and calcium transporting ability of the sarcotubular membranes. For this purpose, both of these parameters were monitored in the presence of various concentrations of NaF and different hormones such as norepinephine, glucagon and prostaglandins E_1 and $F_{2\alpha}$, as well as after treatment of the sarcotubular membranes with phospholipase C and trypsin. The adenylate cyclase activity and calcium binding by these membranes were also studied in the presence of different concentrations of calcium. The actions of adenosine 3′,5′-monophosphate (cyclic AMP) and its dibutyryl derivative on calcium binding kinetics were also investigated.

METHODS

The procedure for the preparation of dog heart sarcotubular membranes and for the determination of calcium transport and adenylate cyclase activity were the same as those described earlier from this laboratory[5,6]. Calcium binding was measured in the absence of oxalate whereas calcium uptake was determined in the presence of 5 mM potassium oxalate at 37 °C. Unless mentioned in the text, the concentration of total calcium in the incubation medium was 0.1 mM. Protein concentration was determined by the method of Lowry *et al.*[7]. The concentration of proteins in the incubation medium was 70–100 μg/ml for calcium binding experiments and 40–50 μg/ml for calcium uptake experiments.

In one series of experiments the sarcotubular membranes (2 mg/ml) were treated with phospholipase C for 10 min in medium containing 50 mM Tris–HCl (pH 7.5), 20 mM KCl and 0.2 mM $CaCl_2$. At the end of the incubation period, ethyleneglycol-bis-(α-amino ethyl ether)-*N*,*N*′-tetraacetic acid (2 mM final concentration) was added and the membranes were washed after separation by centrifugation at $40000 \times g$ for 45 min. In another set of experiments the sarcotubular membranes were incubated with trypsin for 10 min in medium containing 50 mM Tris–HCl (pH 7.5) and 20 mM KCl. The reaction was stopped by adding 3-fold excess of trypsin inhibitors and the membranes were washed after separation by centrifugation at $40000 \times g$ for 45 min. These phospholipase C-treated and trypsin-treated preparations were assayed for adenylate cyclase and calcium transporting activities.

RESULTS

The dog heart sarcotubular membranes were treated with different amounts of phospholipase C or trypsin and calcium uptake and adenylate cyclase activity were determined after washing these preparations. The results are shown in Figs 1 and 2. Although phospholipase C and trypsin treatments decreased both calcium uptake and adenylate cyclase activity of the sarcotubular membranes, the depression in calcium uptake was more than that in adenylate cyclase activity.

The effect of different concentrations of two well known activators of adenylate cyclase, NaF and norepinephrine was studied on calcium uptake and adenylate cyclase activity of the heart sarcotubular membranes and the results are shown in Figs 3 and 4. It was found that low concentrations of NaF (1–5 mM) and nor-

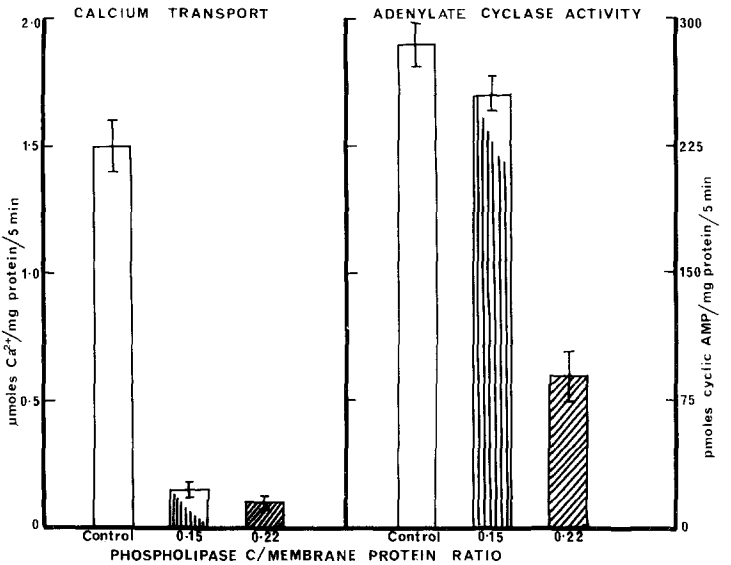

Fig. 1. Calcium uptake and adenylate cyclase activity of the phospholipase C -treated dog heart sarcotubular membranes. The membranes were incubated in the absence (control) or presence of indicated amounts of phospholipase C according to the procedure described in Methods, washed and assayed for activities. Each value is a mean ± S.E. of 6 experiments.

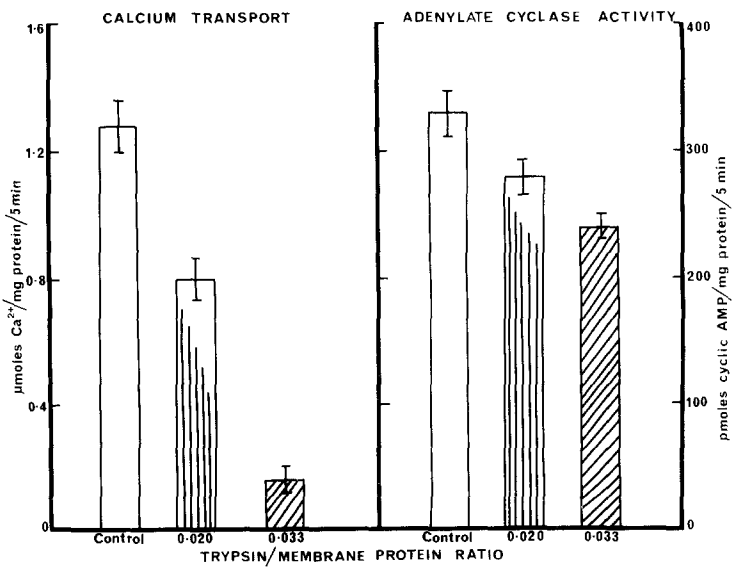

Fig. 2. Calcium uptake and adenylate cyclase activity of the trypsin-treated dog heart sarcotubular membranes. The membranes were incubated in the absence (control) or presence of indicated amounts of trypsin according to the procedure described in Methods, washed and assayed for activities. Each value is mean ± S.E. of six experiments.

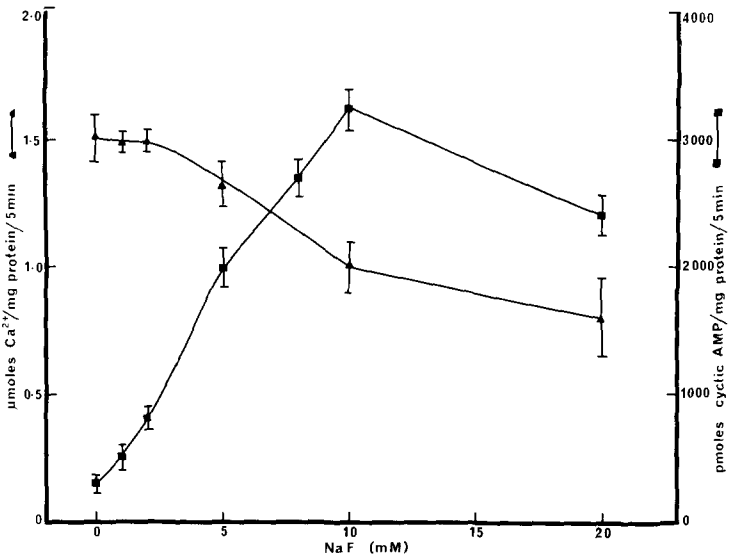

Fig. 3. Effect of different concentrations of NaF on calcium uptake and adenylate cyclase activity of dog heart sarcotubular membranes. Each value is a mean ± S.E. of four experiments.

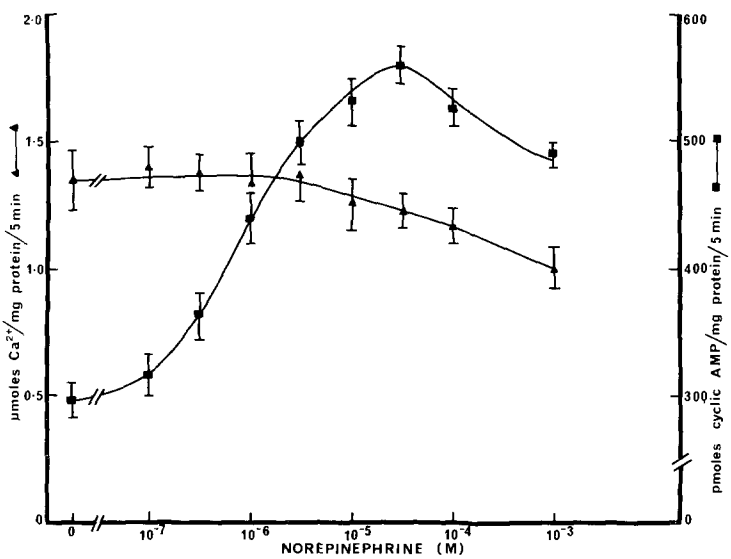

Fig. 4. Effect of different concentrations of norepinephrine on calcium uptake and adenylate cyclase activity of dog heart sarcotubular membranes. Each value is a mean ± S.E. of five experiments.

epinephrine (10^{-7}–10^{-4} M) markedly increased adenylate cyclase activity without appreciably changing calcium uptake by the sarcotubular membranes. High concentrations of NaF (10–20 mM) and norepinephrine (10^{-3} M), which increased adenylate cyclase activity, produced a significant ($P<0.05$) decrease in calcium uptake.

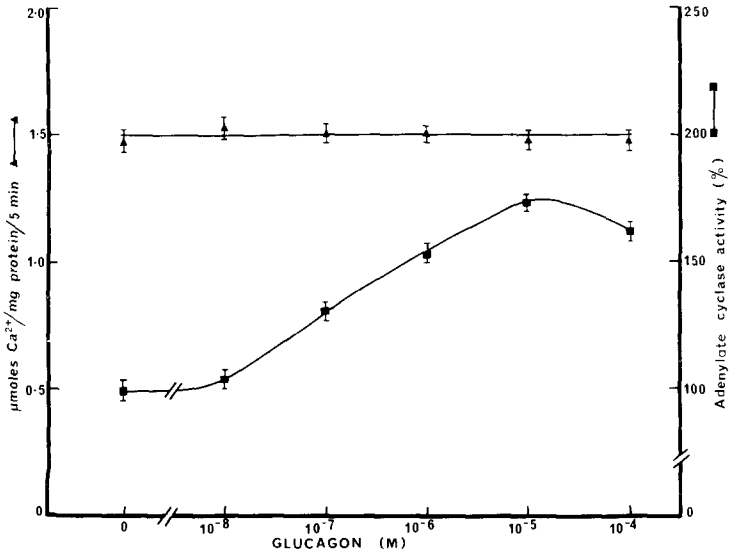

Fig. 5. Effect of different concentrations of glucagon on calcium uptake and adenylate cyclase activity of dog heart sarcotubular membranes. Each value is a mean ± S.E. of six experiments.

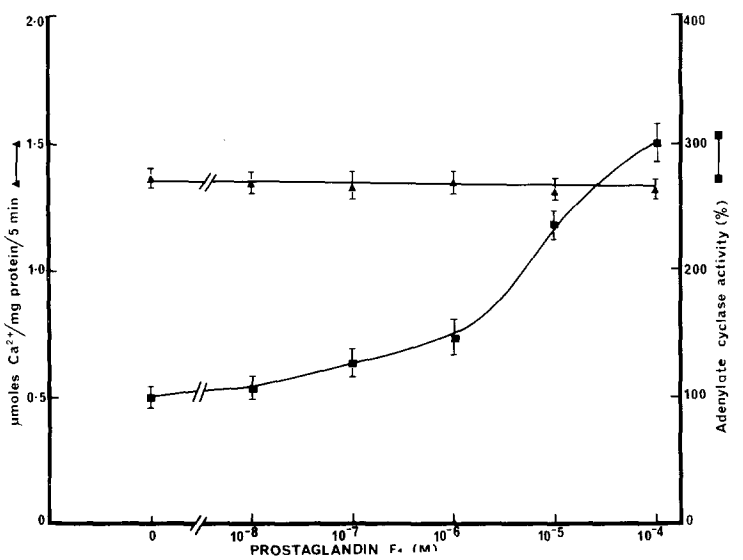

Fig. 6. Effect of different concentrations of prostaglandin E_1 on calcium uptake and adenylate cyclase activity of dog heart sarcotubular membranes. Each value is a mean ± S.E. of six experiments.

Adenylate cyclase and calcium uptake by the heart sarcotubular membranes were also determined in the presence of various concentrations of different hormones such as glucagon, prostaglandin E_1 and prostaglandin $F_{2\alpha}$ and the results are shown in Figs 5–7. Glucagon, prostaglandin E_1 and prostaglandin $F_{2\alpha}$ (10^{-7}–10^{-4} M) increased the adenylate cyclase activity but had no effect on calcium uptake. Nor-

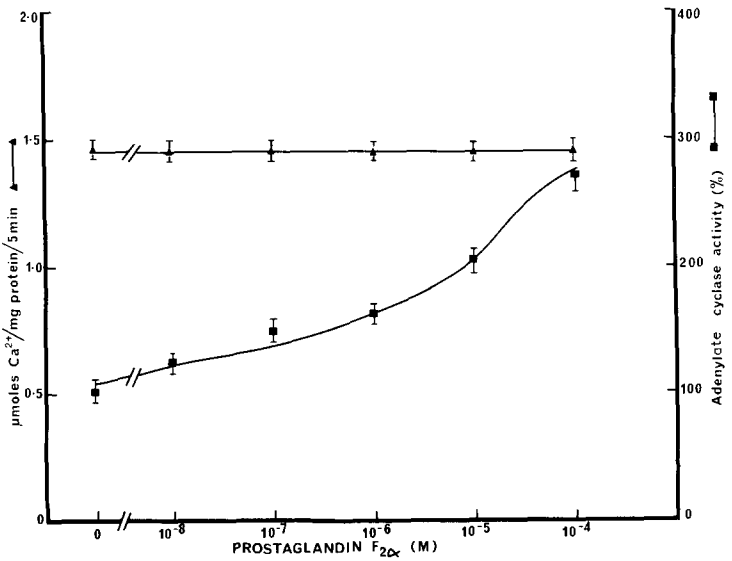

Fig. 7. Effect of different concentrations of prostaglandin $F_{2\alpha}$ on calcium uptake and adenylate cyclase of dog heart sarcotubular membranes. Each value is a mean ± S.E. of six experiments.

epinephrine (10^{-5} M), glucagon (10^{-5} M), prostaglandin E_1 (10^{-4} M) and prostaglandin $F_{2\alpha}$ (10^{-4} M) did not alter calcium binding by sarcotubular membranes (Table I).

TABLE I

EFFECT OF DIFFERENT HORMONES ON CALCIUM BINDING BY DOG HEART SARCOTUBULAR MEMBRANES

Calcium binding was determined in the absence of potassium oxalate. Each value is a mean ± S.E. of six experiments.

Hormone	*Calcium binding (nmoles Ca^{2+}/mg protein per 5 min)*
Control	36.2 ± 2.1
Norepinephrine (10^{-5} M)	34.4 ± 1.5
Glucagon (10^{-5} M)	34.8 ± 1.9
Prostaglandin E_1 (10^{-4} M)	34.7 ± 1.8
Prostaglandin $F_{2\alpha}$ (10^{-4} M)	36.1 ± 1.7

Since increased adenylate cyclase activity results in the increased formation of cyclic AMP, the effects of cyclic AMP or its more permeable derivative, dibutyryl cyclic AMP, were tested on calcium binding by sarcotubular membranes. A double reciprocal plot of the data on calcium binding revealed that neither the calcium binding constant nor the number of calcium binding sites were influenced by the presence of cyclic AMP or dibutyryl cyclic AMP (Fig. 8). In another series of experi-

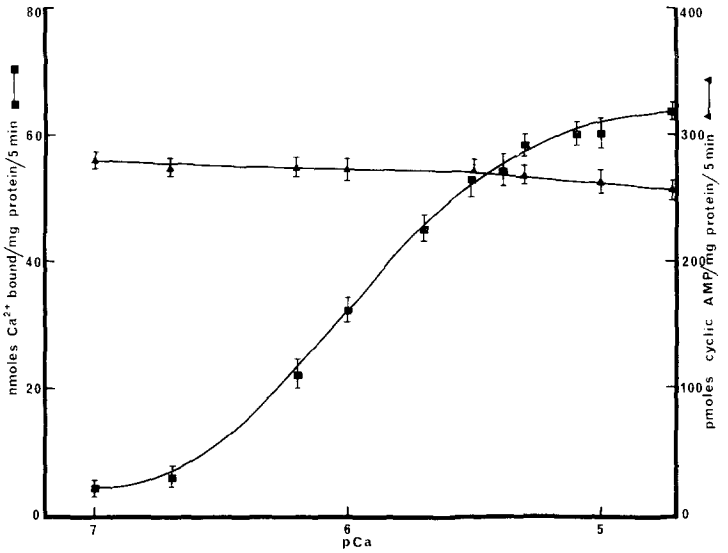

Fig. 8. Effect of cyclic AMP and dibutyryl cyclic AMP on calcium binding by dog heart sarcotubular membranes in the presence of different concentrations of calcium. The values are typical of three such experiments. The values for the number of calcium binding sites and calcium binding constants varied from 65–74 nmoles/mg protein and $0.69–0.80 \cdot 10^6$ M^{-1}, respectively.

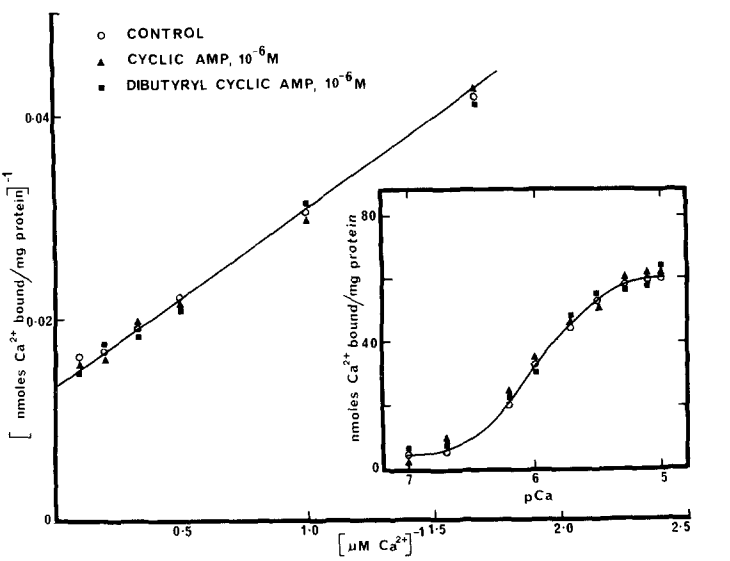

Fig. 9. Effect of different concentrations of calcium on calcium binding and adenylate cyclase activity of dog heart sarcotubular membranes. Each value is a mean ± S. E. of four experiments.

ments, the status of adenylate cyclase activity was examined at different levels of calcium binding by sarcotubular membranes. The results shown in Fig. 9 reveal that calcium binding by sarcotubular membranes increased by increasing the concentration of calcium in the incubation medium without any changes in adenylate cyclase activity.

DISCUSSION

The hypothesis that increased levels of cyclic AMP cause an augmentation of the sarcotubular calcium pool is interesting in the sense that it helps in explaining the positive inotropic effect of various interventions at the molecular level. It is based on the data that catecholamines and glucagon increased adenylate cyclase activity and calcium uptake by the heart sarcotubular membranes[8–10]. Furthermore, some investigators[8,11] have shown that cyclic AMP increases calcium transport across the heart sarcotubular vesicles. On the other hand, other workers[6,12] have failed to confirm the existence of such an effect of cyclic AMP. Likewise, both glucagon and catecholamines have also been reported to be ineffective in enhancing calcium uptake by the heart sarcotubular vesicles[6,12,13]. In the present study we have shown that cyclic AMP did not influence the calcium binding constant or the number of calcium binding sites in the sarcotubular membranes. Furthermore, interventions such as NaF (1–5 mM), norepinephrine (10^{-7}–10^{-4} M), glucagon and prostaglandins E_1 and $F_{2\alpha}$, which increase adenylate cyclase activity, did not increase calcium transport by the heart sarcotubular membranes. These results suggest that the proposed mechanism of hormonal action on calcium transport across sarcotubular vesicles through direct participation of cyclic AMP should be considered with some caution.

Recently cardiac sarcotubular membranes, possessing adenylate cyclase and calcium accumulating activities, have been shown to contain cyclic AMP-stimulated protein kinase[14]. It has been suggested that cyclic AMP-stimulated formation of a membrane phosphoprotein mediates the cyclic AMP-induced changes in calcium transport. Although the presence of protein kinase has also been reported by LaRaia[15] and has been confirmed in the preparation employed in this study (unpublished data), it is not possible for us to show augmentation of calcium transport on addition of exogenous cyclic AMP and dibutyryl cyclic AMP or due to the endogenously formed cyclic AMP in the presence of different hormones. The experiments described by Kirchberger *et al.*[16] reveal that the addition of exogenous protein kinase in high concentrations was necessary to show the effect of cyclic AMP on calcium uptake. In spite of our extensive efforts, we have not been successful in obtaining favourable experimental conditions to demonstrate the calcium transporting actions of cyclic AMP–protein kinase on the freshly prepared cardiac sarcotubular membranes (unpublished observations). Since Kirchberger *et al.*[16] failed to observe an action of cyclic AMP–protein kinase on calcium binding by sarcotubular vesicles, the significance of their reported effect on calcium uptake is subject to some serious question. In this regard it should be mentioned that Gertz *et al.*[17] have attributed the stimulatory effect of cyclic AMP to a non-specific protective effect of the nucleotide to retard the *in vitro* deterioration of cardiac sarcotubular calcium uptake. Furthermore, cyclic AMP has been shown to be without effect on phosphoryl transfer reaction which may represent the formation of a carrier system and facilitate the influx of calcium into the sarcotubular vesicles[18].

Under the experimental conditions employed in this study, we have been unable to find any relationship between changes in calcium transport and adenylate cyclase activity of the sarcotubular membranes. For example, we have observed that glucagon prostaglandins E_1 and $F_{2\alpha}$ norepinephrine (10^{-7}–10^{-4} M) and NaF

(1–5 mM) activated adenylate cyclase without any effect on calcium transport. High concentrations of both norepinephrine and NaF were found to inhibit calcium uptake significantly. Furthermore increasing the concentration of calcium in the incubation medium increased calcium binding without any effect on adenylate cyclase activity. Although both adenylate cyclase activity and calcium uptake ability declined on pretreating the sarcotubular membranes with phospholipase C or trypsin, the calcium uptake system was found to be more sensitive than adenylate cyclase. In the light of these observations it is difficult for us to support the view concerning direct involvement of adenylate cyclase–cyclic AMP system in the manifestation of the calcium transport process of the heart sarcotubular vesicles. On the other hand, it still remains possible that the adenylate cyclase system is involved in the calcium transport process, and that the present experimental conditions are such that it cannot be demonstrated. Likewise, our results concerning the actions of various hormones and NaF as well as different sensitivities of adenylate cyclase and calcium transport to phospholipase C or trypsin can be interpreted that calcium transport and adenylate cyclase systems are independently controlled.

ACKNOWLEDGEMENTS

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