Incorporation of the Terminal Phosphate of ATP into Membranal Protein of Rabbit Cardiac Sarcoplasmic Reticulum

CORRELATION WITH ACTIVE CALCIUM TRANSPORT AND STUDY OF THE EFFECTS OF CYCLIC AMP

By Donald H. Namm, Edward L. Woods, and Joan L. Zucker

ABSTRACT

The transfer of the terminal phosphate of ATP to a material from the sarcoplasmic reticulum of cardiac muscle which can be precipitated by trichloroacetic acid was studied, and the relationship of this biochemical event to active Ca\(^{2+}\) transport was examined. A component of the phosphoryl transfer reaction was stimulated specifically by Ca\(^{2+}\). Both the phosphorylation of the reticulum and the active sequestering of Ca\(^{2+}\) were proportional to the Ca\(^{2+}\) concentration between 10\(^{-7}\) and 10\(^{-5}\) M. The time course of both phenomena was similar. These and other observations relating Ca\(^{2+}\)-dependent phosphorylation of the membrane of the sarcoplasmic reticulum with the active sequestering of Ca\(^{2+}\) suggest that the phosphoryl transfer reaction may represent the formation of a carrier system which facilitates the inward flux of Ca\(^{2+}\) against a concentration gradient. Cyclic AMP, theophylline, or the combination of these two agents did not influence the rate or extent of the phosphorylation of the sarcoplasmic reticulum. These results have led us to question the hypothesis that the cardiac sarcoplasmic reticulum is the site of action of cyclic AMP in its role as a mediator of cardiac inotropic effects.

KEY WORDS

- cyclic AMP
- protein phosphorylation
- sarcoplasmic reticulum
- calcium uptake
- protein kinase
- cardiac contractility
- phosphoryl transfer

The sarcoplasmic reticulum of cardiac and skeletal muscle is important in the regulation of calcium ion concentration within these muscle cells (1, 2), because it can concentrate Ca\(^{2+}\) within its tubular structure and thus influence the contraction-relaxation events of the cells. Recently, a considerable effort has been directed towards the elucidation of the mechanism by which Ca\(^{2+}\) is transported across the membrane of the sarcoplasmic reticulum. Hasselbach and Makinose (3) reported that the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate was associated with the transport of Ca\(^{2+}\) by the sarcoplasmic reticulum of skeletal muscle. These same authors (4) and Ebashi and Lipman (5) later reported that fragments of the muscle sarcoplasmic reticulum were capable of catalyzing the exchange of phosphate from ATP to ADP. This observation led both groups to speculate that an energy-rich phosphate bond was formed in the membrane of the sarcoplasmic reticulum and that the phosphorylated site could function as a carrier substance for the transfer of Ca\(^{2+}\) from one side of this membrane to the other. This speculation has more recently received experimental support through the reports of Yamamoto and Tonomura (6, 7), Martonosi (8), Makinose (9), Inesi et al. (10), and Panet et al. (11). These workers demonstrated that the terminal phosphate of ATP was incorporated...
into a material of the skeletal muscle sarcoplasmic reticulum that was precipitated by trichloroacetic acid. The properties of this membrane-phosphate complex and the kinetics of its formation and degradation were consistent with the concept that this complex is involved in ATP-supported Ca\(^{2+}\) transport in the skeletal muscle sarcoplasmic reticulum (6, 7, 11).

The sarcoplasmic reticulum of cardiac muscle has not been studied to determine whether the mechanism for Ca\(^{2+}\) transport in this muscle type is analogous to that proposed for skeletal muscle. Our interest in examining this phenomenon in cardiac muscle arose as a result of the hypothesis that cyclic 3', 5'-adenosine monophosphate (cyclic AMP) might mediate changes in myocardial contractility. Since many of the effects of cyclic AMP (phosphorylase activation, lipolysis) are thought to result from the stimulatory effect of this nucleotide on reactions involving phosphorl transfer from ATP to a protein, it was of interest to us to ascertain whether cyclic AMP affected the phosphorylation of a protein associated with the sarcoplasmic reticulum of the heart. The possibility that cyclic AMP acts on the biochemical mechanism associated with Ca\(^{2+}\) transport was suggested by the demonstration that the cyclic nucleotide stimulated Ca\(^{2+}\) uptake into sarcoplasmic reticulum fragments isolated from the dog heart (12, 13). To test the effect of cyclic AMP on the biochemical mechanism of Ca\(^{2+}\) transport in the sarcoplasmic reticulum of the heart, it was necessary to establish whether the phosphoryl transfer observed by others in the skeletal muscle system did indeed take place in cardiac sarcoplasmic reticulum fragments and to demonstrate the relationship between this phenomenon and the uptake of Ca\(^{2+}\). Thus, this report includes a study which characterized the phosphoryl transfer reaction in cardiac sarcoplasmic reticulum and a study of the effects of cyclic AMP on this reaction.

**Methods**

**PREPARATION OF CARDIAC SARCOPLASMIC RETICULUM**

New Zealand white rabbits (3–4 kg) were killed by a blow on the head, and their hearts were rapidly removed and washed in isotonic saline at 0°C. Fat, atria, and large blood vessels were removed by dissection, and the ventricles were homogenized in a Waring blender in seven volumes of a solution containing 0.3 M sucrose and 0.02 M Tris-HCl at pH 7.0. Homogenization and all subsequent isolation procedures were carried out at 2–4°C. The heart homogenate was centrifuged at 1,000 g for 10 minutes in a Sorvall RC-2B centrifuge. The supernatant fluid was centrifuged again at 29,000 g for 30 minutes. The supernatant fluid from the centrifugation at 29,000 g was centrifuged at 100,000 g for 90 minutes in a Beckman L3-50 centrifuge. The sediment from this final centrifugation was suspended in the homogenizing medium and gently hand-homogenized with a Teflon pestle. The suspension contained 12–25 mg protein/ml and was always used on the day that it was prepared. Cytochrome c oxidase activity was measured in the various subcellular fractions obtained by differential centrifugation and served as a marker for mitochondria and mitochondrial contaminants. The activity of this enzyme was measured by the method described by Brody et al. (14). Protein was assayed by the method of Lowry et al. (15).

The transfer of the terminal phosphate of ATP to cardiac sarcoplasmic reticulum was followed by incubating the sarcoplasmic reticulum fraction (0.75–1.0 mg/ml) in a solution containing 0.3 M sucrose and 20 mM Tris-HCl. Ethyleneglycol bis(β-aminoethyl)ether)-N, N′-tetraacetic acid (EGTA) and CaCl\(_2\) were added in a ratio that yielded the desired concentration of free Ca\(^{2+}\) assuming a binding constant of 5 × 10\(^{5}\) M\(^{-1}\) at pH 7.0 (2). Magnesium acetate was present at a concentration equimolar to that of ATP unless otherwise noted. \(^{32}\)P-ATP (labeled at the terminal phosphate) was added to initiate the reaction, and the reaction was terminated by the addition of 5 mM trichloroacetic acid (TCA) containing 1 mM ATP and 1 mM Pi (at 0°C). The TCA-insoluble precipitate was collected by centrifugation and the supernatant fluid aspirated. The sediment was washed twice again in the TCA-ATP-P\(_i\) solution and then twice in 2% TCA (0°C). The washed TCA-insoluble material was dissolved in concentrated formic acid, and a sample of this solution was counted for \(^{32}\)P in a Packard liquid scintillation counter. Studies of calcium uptake were carried out using the same reaction conditions as those described for the phosphorylation studies with the following exceptions: unlabeled ATP was used, \(^{40}\)CaCl\(_2\) was used, and the uptake reaction was terminated by filtering the reaction medium through 0.45 μM Millipore filters. Oxalate was not added to our uptake medium. The filtrate was counted for...
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45Ca, and the difference between the radioactivity in a filtered zero-time control and that in the sample filtrate was calculated as uptake.

MATERIALS

32P as inorganic orthophosphate was obtained in dilute HCl from Amersham/Searle and was incorporated into the terminal phosphate of ATP by a modification (16) of the method of Glynn and Chappell (17). 44CaCl2 was obtained from New England Nuclear Corporation. ATP was obtained from P-L Biochemicals; enzymes used in the synthesis of 32P-ATP were obtained from Boehringer-Mannheim. Cyclic AMP was purchased from Schwarz/Mann. 14C-8-Adenosine triphosphate was purchased from Amersham/Searle.

RESULTS

ISOLATION OF FRAGMENTS OF THE SARCOPLASMIC RETICULUM

Since mitochondria of heart muscle can actively sequester Ca2+ (18) and in view of their role in the metabolism of high-energy phosphates, it was imperative to remove, as extensively as possible, all traces of mitochondrial contamination from the sarcoplasmic reticulum fraction. By our fractionation procedure, the sediment obtained by centrifugation at 100,000 g was essentially devoid of activity of cytochrome c oxidase, an enzyme considered to be primarily localized within mitochondria. Expressing the cytochrome c oxidase activity of the heart homogenate as 100%, the distribution of its activity was as follows: 1,000-g sediment 55.5%, 29,000-g sediment 43.8%, 100,000-g sediment 0.35%. Less than 0.2% of the total activity was unaccounted for in our analysis of the various sediments, and presumably it resided in the supernatant fractions.

PHOSPHORYLATION OF THE SARCOPLASMIC RETICULUM

Preliminary studies revealed that it was necessary to employ a minimum of about 1 mg of sarcoplasmic reticulum protein to detect phosphate incorporation. Using this amount of sarcoplasmic reticulum in a reaction at 30 or 37°C caused rapid splitting of ATP and depletion of this substrate. Thus, we chose, as have others in studies of skeletal muscle sarcoplasmic reticulum phosphorylation (10, 11), to follow the reaction at 0°C since at this temperature the activity of adenosinetriphosphatase was low enough so that sampling for the intermediate could be accomplished before the ATP was extensively degraded.

Phosphate incorporation into the TCA-precipitable material of the sarcoplasmic reticulum fraction was followed for 10 minutes. When the concentration of free Ca2+ was reduced below 10–6 M by the presence of 5 mM EGTA, there was a time-dependent incorporation of 32P from ATP into the sarcoplasmic reticulum. This labeling could not be reduced by extensive washing with the TCA solution containing P1 and ATP and, thus, represents a tight binding of the phosphate to the sarcoplasmic reticulum. We have termed this incorporation the Ca2+-independent phosphorylation (Fig. 1). At an ATP concentration of 0.1 mM, this component of sarcoplasmic reticulum phosphorylation reached a maximum of about 0.2 μmoles/g protein in about 10 minutes. When Ca2+ was

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**Figure 1**

**Time course of phosphate incorporation into cardiac sarcoplasmic reticulum.** Ca2+-independent (○) and total (●) phosphate incorporation were measured using 32P-ATP at 1.1 × 106 counts/min μmole−1 at a final concentration of 0.1 mM. Ca2+-dependent (●) incorporation was calculated from these observations as described in the text. Points represent means of triplicate observations from one sarcoplasmic reticulum preparation.

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present at a concentration of the free ion of $10^{-6}$M, the pattern of phosphorylation was altered considerably. We have termed this the total phosphorylation since it is composed of both the Ca$^{2+}$-independent and Ca$^{2+}$-dependent components (Fig. 1). The Ca$^{2+}$-dependent component was calculated as the difference between total and Ca$^{2+}$-independent components. The Ca$^{2+}$-dependent phosphorylation of the sarcoplasmic reticulum material occurred very rapidly, peaking at about 30 seconds, and was apparently degraded at an exponential rate over the remaining 9.5 minutes. Like the Ca$^{2+}$-independent phosphorylation, the Ca$^{2+}$-dependent phosphorylation reached a maximum of about 0.2 µmoles/g when the initial ATP concentration was 0.1 mM. In most of our subsequent experiments we chose to measure Ca$^{2+}$-dependent phosphorylation 30 seconds after initiation of the reaction since this was the time of peak effect and of maximal difference between the two components.

Experiments were carried out to characterize the requirement of the phosphoryl transfer reaction for Mg$^{2+}$. In the absence of Mg$^{2+}$, Ca$^{2+}$-independent phosphorylation was detected at 30 seconds; the extent of this phosphorylation was somewhat decreased by the addition of Mg$^{2+}$ (Fig. 2). When Ca$^{2+}$ was present at $10^{-6}$M, phosphate incorporation was augmented with no added Mg$^{2+}$. When Mg$^{2+}$ was added, phosphate incorporation was further increased with a maximum occurring at about $5 \times 10^{-5}$M Mg$^{2+}$.

Using a ratio of the concentrations of ATP and Mg$^{2+}$ of one to one in our reaction medium, we investigated the extent of phosphorylation at different initial ATP concentrations. At concentrations of ATP above 0.1 mM the Ca$^{2+}$-independent component of phosphorylation of the sarcoplasmic reticulum increased markedly, exceeding that of the Ca$^{2+}$-dependent component, and thus made quantification of the latter difficult (Fig. 3). Maximal Ca$^{2+}$-dependent phosphorylation occurred at $10^{-4}$M ATP (Fig. 3, insert).

The specificity of the Ca$^{2+}$-induced phosphoryl transfer was determined by substituting other cations for Ca$^{2+}$ in the reaction medium. Other than Ca$^{2+}$, none of the cations

\[ \text{Effects of Mg}^{2+} \text{ on phosphate incorporation into the cardiac sarcoplasmic reticulum. The line marked NO CALCIUM indicates experiments in which 5 mM EGTA was present.} \]

\[ \text{Effects of ATP concentration on the Ca}^{2+} \text{-independent component of sarcoplasmic reticulum phosphorylation.} \]

(○) = phosphorylation in presence of $10^4$M Ca$^{2+}$; (*) = phosphorylation in presence of 3 mM EGTA. Insert: Ordinate and abscissa are same as large graph. (●) = calculated Ca$^{2+}$-dependent phosphorylation. Sampling was done at 30 seconds after start of reaction.
tested significantly stimulated phosphate incorporation into the sarcoplasmic reticulum (Table 1).

Once the conditions which optimized Ca²⁺-dependent phosphorylation of the sarcoplasmic reticulum were established, we attempted to determine if a direct phosphoryl transfer reaction was involved or if the ³²P incorporation occurred through other mechanisms. One possible alternative mechanism was that unhydrolyzed ATP was bound to the sarcoplasmic reticulum thus allowing ³²P incorporation without phosphoryl transfer. We tested this possibility by substituting ¹⁴C-8-adenosine triphosphate for the ³²P-ATP in our reaction medium. No ¹⁴C labeling of the washed TCA-insoluble precipitate was detected in either the presence or the absence of Ca²⁺. Next we carried out studies to determine whether the ³²P incorporated into the sarcoplasmic reticulum in our experiments came from inorganic ³²P in the medium after hydrolysis from ³²P-ATP by the action of adenosinetriphosphatase. Studies were carried out using unlabeled ATP and adding ³²P-orthophosphate to our usual reaction medium. Both ATP and P₁ were present at 10⁻⁴ M and the specific activity of the P₁ exceeded that of the ³²P-ATP used in previous experiments. In these experiments there was a small incorporation of ³²P. The magnitude of this incorporation from P₁ never exceeded 0.02 µmoles/g protein, which was less than 10% of that seen when ³²P-ATP was used as substrate at a tenth the Ca²⁺ concentration.

**TABLE 1**

<table>
<thead>
<tr>
<th>Cation added</th>
<th>Phosphate incorporation (µmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.047 ± 0.024</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.198 ± 0.034</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.042 ± 0.027</td>
</tr>
<tr>
<td>K⁺</td>
<td>0.059 ± 0.020</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>0.077 ± 0.021</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.041 ± 0.021</td>
</tr>
</tbody>
</table>

All ions were added as chloride salts at a concentration of 10⁻⁴ M. [ATP] = [Mg²⁺] = 10⁻⁴ M. The control reaction medium contained 5 mEq EGTA. Reaction time was 30 seconds. Specific activity of ATP was 1.1 X 10⁸ counts/min µmole⁻¹.

**RELATIONSHIP BETWEEN THE CALCIUM-DEPENDENT PHOSPHORYL TRANSFER REACTION AND CALCIUM UPTAKE BY THE SARCOPLASMIC RETICULUM**

The time courses of Ca²⁺-dependent phosphate incorporation into cardiac sarcoplasmic reticulum and ATP-dependent Ca²⁺-binding are shown in Figure 4. ATP-dependent binding was calculated as the net difference between the amount of Ca²⁺ bound in the presence of ATP (0.1 mM) and that bound when no ATP was added to the reagent. The ATP-independent binding (not shown) was rapid, reaching a maximum of 4.4 µmoles Ca²⁺ bound/g at 30 seconds and was unchanged for at least 5 minutes. As seen in previous experiments, phosphate incorporation was rapid, i.e., demonstrable at 30 seconds. Likewise, most of the Ca²⁺ that was sequestered by cardiac sarcoplasmic reticulum...
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was bound within that time period. The degradation of the membrane-phosphate complex was not associated with any marked loss of Ca2+ from the sarcoplasmic reticulum. We analyzed the relationship between the concentration of Ca2+ in the medium and both Ca2+ uptake and phosphoryl transfer. Both phenomena were similarly dependent on the Ca2+ concentration from 10^-7 to 10^-5 M (Table 2). Half-maximum effects for both phenomena occurred at Ca2+ concentrations of 2-4 x 10^-6 M. There appears to be a proportionality between the two phenomena which is approximately linear between 0.005 and 0.025 μmoles phosphate incorporated/g protein.

EFFECTS OF CYCLIC AMP AND THEOPHYLLINE ON THE CALCIUM-DEPENDENT PHOSPHORYLATION OF CARDIAC SARCOPLASMIC RETICULUM

We tested the effects of cyclic AMP alone and in the presence of an inhibitor of its degradation (theophylline) on the transfer of the terminal phosphate of ATP to the sarcoplasmic reticulum fragments. Theophylline alone had no effect on phosphate incorporation in either the presence or the absence of Ca2+ (Fig. 5). Cyclic AMP at 10^-5 M, a concentration 100 times that which produces maximal stimulation of protein kinases (16), also was without effect on the extent of sarcoplasmic reticulum phosphorylation. Finally, the combination of cyclic AMP and theophylline was also ineffective in altering the degree of phosphate incorporation. Studies were carried out sampling the sarcoplasmic reticulum at earlier (10, 15, and 20 seconds) time periods to determine whether the nucleotide affected the rate of phosphorylation. Again, no effect of the nucleotide was detectable. Likewise, preincubation of the sarcoplasmic reticulum with 10^-4 M cyclic AMP for up to 10 minutes did not affect the phosphoryl transfer reaction.

Discussion

Specific cationic stimulation of the formation of a membranal protein-phosphate complex has been demonstrated in association with the ATP-supported transport of Na+ and K+ by kidney membranes (19) and of Ca2+ by sarcoplasmic reticulum of skeletal muscle (6-11). Extensive kinetic analysis of the formation and degradation of the skeletal muscle membranal protein-phosphate complex has suggested that the phosphoryl transfer reaction results in the formation of an energetic carrier molecule which is capable of translocating Ca2+ from the outer to the inner surface of the sarcoplasmic reticulum membrane (6-11, 20). Our in vitro experiments indicate that a similar reaction mechanism may be operative in association with Ca2+ transport across the membrane of the sarcoplasmic reticulum of heart muscle. We demonstrated a specific Ca2+ stimulation of

<table>
<thead>
<tr>
<th>PCa</th>
<th>Original radioactivity (counts/min ml^-1)</th>
<th>ATP (counts/min ml^-1)</th>
<th>ATP-dependent calcium binding (μmoles/g)</th>
<th>Calcium-dependent phosphorylation (μmoles/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4</td>
<td>5,042</td>
<td>3,939</td>
<td>1.4</td>
<td>0.006</td>
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<tr>
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<td>20,060</td>
<td>18,690</td>
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<td>53,411</td>
<td>51,971</td>
<td>6.3</td>
<td>0.019</td>
</tr>
<tr>
<td>6.0</td>
<td>84,144</td>
<td>82,403</td>
<td>10.5</td>
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</tr>
<tr>
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<td>134,319</td>
<td>17.8</td>
<td>0.049</td>
</tr>
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<td>188,640</td>
<td>24.0</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>217,504</td>
<td>215,043</td>
<td>28.6</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Experimental conditions: [Mg2+] = [ATP] = 0.1 mm, EGTA 0.5 mm, 4CaCl2 specific activity 544,000 counts/min μmole^-1. Uptake and phosphorylation studies were terminated at 30 seconds. All values represent means of triplicate observations using one preparation of the sarcoplasmic reticulum. PCa = - log [Ca2+].

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phosphate incorporation into TCA-precipitable material of cardiac sarcoplasmic reticulum and showed that the phosphate was derived directly from ATP. The extent of phosphate incorporation was dependent on the external Ca$^{2+}$ concentration in a range thought to exist within muscle cells ($10^{-7}$ to $10^{-5}$M). The effect of Ca$^{2+}$ on this reaction was specific, and the time course of the phosphoryl transfer was comparable to that of the active sequestering of Ca$^{2+}$ by the sarcoplasmic reticulum. Further extensive kinetic analyses and correlation would be necessary to prove more definitively the relationship between the movement of the divalent ion and the phosphorylation reaction. Such extensive analyses have been done with skeletal muscle where the sarcoplasmic reticulum is considerably more stable after isolation and is more easily obtained in larger quantities than are the cardiac particles.

Many similarities between the phosphoryl transfer reaction in skeletal and cardiac sarcoplasmic reticulum are indicated by our results. First, Ca$^{2+}$, but not Mg$^{2+}$, appears to be absolutely required for substantial phosphate incorporation (8, 10, 11). Mg$^{2+}$ augments the Ca$^{2+}$ stimulatory effect and appears to have its maximal effect when employed in a concentration about equimolar to that of ATP. In both heart and skeletal muscle, the sarcoplasmic reticulum is phosphorylated rapidly with maximal incorporation occurring at 5–30 seconds. The phosphorylation is a function of the concentration of Ca$^{2+}$ between $10^{-7}$ and $10^{-5}$M in both heart and skeletal muscle sarcoplasmic reticulum fragments (7, 9, 11). Thus, it is probable that the transport systems for Ca$^{2+}$ in these membrane fragments are qualitatively similar. However, quantitative differences are obvious. Skeletal muscle sarcoplasmic reticulum is able to accept considerably more phosphate (ranging from 0.4 to 3 μmoles/g) than is cardiac sarcoplasmic reticulum (maximum of 0.2 μmoles/g). Assuming similar degrees of purity of the preparations, our results indicate that the number of transport sites on the cardiac sarcoplasmic...
reticulum membranes may be less than that on the skeletal muscle membranes. This observation may relate directly to the fact that cardiac sarcoplasmic reticulum is known to possess a Ca^{2+} transport system considerably inferior to that of muscle in terms of both ion flux and storage capacity (21).

Our experiments provided no indication that cyclic AMP was able to modify the phosphoryl transfer reaction in cardiac sarcoplasmic reticulum. The ineffectiveness of cyclic AMP cannot be due to its inability to cross membranes since the phosphorylation must take place on the outer surface of the sarcoplasmic reticulum, a site readily accessible to ATP in the medium. Thus, we can conclude that, if cyclic AMP does act to modify the Ca^{2+} pump of cardiac sarcoplasmic reticulum, it does so by means other than activation of sarcoplasmic reticulum phosphorylation by ATP. That cyclic AMP acts on the Ca^{2+} uptake process of cardiac sarcoplasmic reticulum is itself open to serious question. Shinbourne and White (13) were only able to show definitive effects of the nucleotide on Ca^{2+} uptake by cardiac sarcoplasmic reticulum at 10^{-3}M cyclic AMP and did not attempt to determine whether these effects of the nucleotide, at 10,000 times its physiological concentration, were specific. The report by Entman et al. (12) that cyclic AMP stimulates Ca^{2+} uptake by cardiac sarcoplasmic reticulum when employed at very low concentrations has been recently contradicted by the work of Sulakhe and Dhalla (22); the latter workers were unable to find any effects of cyclic AMP on ion movements in canine cardiac sarcoplasmic reticulum. We have also been unable to detect a specific effect of cyclic AMP on Ca^{2+} uptake into or efflux from dog or rabbit cardiac sarcoplasmic reticulum (unpublished observations). Until these disparities in the literature are resolved, the concept that the mediation of cardiac inotropic effects by cyclic AMP results from the effects of cyclic AMP on the Ca^{2+} pump of the sarcoplasmic reticulum should be considered with reservation.

**References**


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