Positive Inotropic Effects of Dibutyryl Cyclic Adenosine 3',5'-Monophosphate

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ABSTRACT

The positive inotropic effects of catecholamines have been postulated to result from an increase in the intracellular level of cyclic AMP (adenosine 3',5'-monophosphate) produced by activation of adenyl cyclase. Although lack of an inotropic effect by exogenously administered cyclic AMP has cast doubt on this hypothesis, cardiac cells are not readily permeable to cyclic AMP. The N°,2'-O-dibutyryl derivative of cyclic AMP is thought to enter cells more readily and is resistant to enzymatic degradation by phosphodiesterase. We examined the effects of cyclic AMP and its dibutyryl derivative on the contractile performance of isolated cat right ventricular papillary muscles. Cyclic AMP (1 × 10⁻⁴ to 5 × 10⁻⁵M) had no effect on papillary muscle function. However, dibutyryl cyclic AMP caused a concentration-dependent increase in isometric tension and rate of tension development, the threshold concentration being 5 × 10⁻⁴M. The increments in tension (4.5 ± 0.4 g/mm²) and rate of tension development (58.4 ± 5.4 g/mm²/sec) at peak concentration (3 × 10⁻⁴M) were similar to those found at peak norepinephrine concentration (10⁻⁵M). Dibutyryl cyclic AMP (10⁻³M) also caused a marked shift of the force-velocity curve upward and to the right. Although 10⁻⁶M propranolol depressed the inotropic effects of norepinephrine, it did not alter the contractile response to dibutyryl cyclic AMP. These findings are consistent with the hypothesis that the positive inotropic effects of catecholamines are mediated by cyclic AMP.

ADDITIONAL KEY WORDS adenyl cyclase papillary muscle 5'-AMP contractility isometric tension force-velocity relations propranolol norepinephrine cyclic AMP cat

The positive inotropic effects of catecholamines have been postulated to result from an increase in the intracellular level of cyclic AMP (adenosine 3',5'-monophosphate) produced by activation of adenyl cyclase. This hypothesis is based on the following observations: (a) myocardial adenyl cyclase in broken cell preparations is activated by catecholamines (1); (b) tissue levels of cyclic AMP in intact heart muscle increase in response to catecholamine stimulation (2-5); and (c) the inotropic response to catecholamines is enhanced by theophylline (6). Theophylline inhibits phosphodiesterase, the enzyme responsible for metabolic degradation of cyclic AMP to 5'-AMP. One observation at variance with the above hypothesis has been the failure to reproduce the inotropic effects of the catecholamines by the administration of exogenous cyclic AMP (2, 6-8). However, it has been shown that cyclic AMP does not enter cardiac cells at a rate sufficient to achieve an effective intracellular concentration (2). This finding may explain the inability of cyclic AMP to alter myocardial contractility.

Derivatives of cyclic AMP have been synthesized by incorporating lipid-soluble fatty acid residues, a modification that facilitates passage of these agents across cellular membranes and increases their resistance to enzymatic hydrolysis by phosphodiesterase (9, 10). The N°,2'-O-dibutyryl derivative of cyclic AMP has recently been studied in a variety of tissues in which it either mimicked or...
exceeded the effects of the parent compound (8). The structural formula of this derivative is shown in Figure 1 along with that of cyclic AMP. In several tissues this more lipid-soluble derivative exerted physiological effects under conditions in which cyclic AMP was inactive (11-15). However, attempts to produce positive inotropic effects with dibutyryl cyclic AMP have been unsuccessful (2, 8).

The present study was performed to define the direct effects, if any, of cyclic AMP and its dibutyryl derivative on the contractile properties of isolated right ventricular papillary muscles of cats, a preparation that allows precise quantification of myocardial mechanics under controlled experimental conditions.

Methods

Fifty-four right ventricular papillary muscles were removed from adult cats anesthetized with sodium pentobarbital (25 mg/kg, ip). Cross-sectional area of these muscles ranged from 0.23 to 1.43 mm² with an average of 0.80 mm². The muscles were placed in 15-ml baths containing a modified Krebs bicarbonate solution (16) aerated with 95% O₂-5% CO₂. Temperature was maintained constant at 37° ± 0.5°C for all experiments. The lower nontendinous end of the papillary muscle was held in a spring-loaded lucite clip placed at the end of a fine steel rod which passed through the bottom of the bath. In one bath, adapted for recording only isometric contractions, the steel rod was attached to a rigid brass connector and the tendinous end of the papillary muscle secured directly to a force transducer by a short length of 4-0 braided silk suture. Another bath was arranged for recording either isometric or isotonic contractions by attaching the steel rod directly to a force transducer below the bath, while the upper portion of the muscle was connected to an isotonic lever. Displacement of the isotonic lever was measured with a photodiode system linear over a 2-mm range (17). Either the velocity of shortening, dL/dt, or rate of isometric tension development, dT/dt, was obtained by electronic differentiation with an RC circuit and displayed, along with muscle shortening or isometric tension, on an oscillographic recorder at a paper speed of 100 mm/sec. Electrical field stimulation at a frequency of 12/min was performed via thin platinum electrodes placed parallel to the entire length of the muscle, using pulses of 5-msec duration at a voltage 10% above threshold.

All muscles were allowed to stabilize for at least 90 minutes prior to study. Isometric experiments were performed at the apex of the length-active tension curve, Lmax. Concentration-response relations were determined under isometric conditions by the cumulative addition of increasing concentrations of the test agent, allowing adequate time between additions for the attainment of a maximal response. The isometric contractile response to 3',5'-cyclic AMP¹ (1 X 10⁻⁴ to 5 X 10⁻³M) was studied in five muscles. In six muscles concentration-response curves to N⁶-2'-O-dibutyryl 3',5'-cyclic AMP as the monosodium salt² were obtained at concentrations ranging from 1 X 10⁻⁴ to 3 X 10⁻²M. This preparation was found to contain no measurable calcium. In seven other muscles the effect of only one concentration of dibutyryl cyclic AMP, 1 X 10⁻³ or 3 X 10⁻²M, was examined to better define the time course of the response. Force-velocity curves were obtained in six papillary muscles before and during exposure to dibutyryl cyclic AMP (10⁻³M) using a preload of 0.4 g (0.63 ± 0.05 g/mm²). Concentration-response curves to dibutyryl cyclic AMP were also determined in seven muscles following 60-minute exposure to 10⁻⁶M dl-propranolol³. This concentration of propranolol did not have a significant direct effect on papillary muscle function. For comparative purposes, concentration-response curves for L-norepinephrine⁴ (10⁻¹⁰ to 10⁻⁴M) were determined in 12 muscles; in 7 other muscles the response to norepinephrine was studied in the presence of 10⁻⁶M propranolol. The effects of 5'-AMP² (1 X 10⁻⁴ to 3 X 10⁻³M) on isometric contractile function were determined in five muscles, and finally, five muscles were studied during exposure to sodium butyrate at

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¹Calbiochem, Los Angeles, California. ²Calbiochem, Los Angeles, California. ³Ayerst Laboratories Inc., New York, New York. ⁴Sigma Chemical Co., St. Louis, Missouri.
Concentration-response curves to dibutyryl cyclic AMP in papillary muscles performed in the presence and absence of beta-receptor blockade with $10^{-6}$ M propranolol. The average increment in isometric tension, A, and increment in rate of tension development, B, are plotted as a function of dibutyryl cyclic AMP concentration. Each point on the control curves represents the mean value of 5 to 10 muscles. Each point on the curves obtained in the presence of propranolol represents the mean value of 7 muscles. Vertical lines = SE.

concentrations ranging from $10^{-5}$ to $10^{-2}$ M. Results are expressed as the mean ± SE. Statistical analysis of the results was performed using Student's t-test for paired and unpaired data where applicable.

**Results**

**Effects of Cyclic AMP.**—Cyclic AMP in concentrations ranging from $1 \times 10^{-4}$ to $5 \times 10^{-3}$ M had no effect on the contractile behavior of the five papillary muscles studied, even when the muscles were exposed to the compound for periods up to 60 minutes.

**Effects of Dibutyryl Cyclic AMP.**—Dibutyryl cyclic AMP concentrations of $5 \times 10^{-4}$ M or greater resulted in a concentration-dependent increase in isometric tension and rate of tension development (Fig. 2) and a decrease in time to peak tension in all muscles studied. Figure 3 shows a representative tracing of the augmentation of active tension and rate of tension development resulting from $3 \times 10^{-4}$ M dibutyryl cyclic AMP. The typical time course of action is also well shown. An increase in myocardial contractility is detectable after 2 minutes, and a peak effect is attained after 30 to 40 minutes. Once established, the effect is prolonged, gradually decreasing over a 60-minute period but not returning to previous base-line contractile levels during this time interval.

The force-velocity relation was shifted upward and to the right in all six muscles studied at $10^{-3}$ M dibutyryl cyclic AMP. Representative force-velocity curves depicting a typical response in one muscle are shown in Figure 4. The maximal velocity of muscle shortening measured at a load of 0.4 g (max V) in these six muscles increased from a mean control value of $1.31 ± 0.12$ to $2.35 ± 0.29$ muscle lengths/sec ($P < 0.01$) during exposure to $10^{-3}$ M dibutyryl cyclic AMP.

**Comparison of the Effects of Dibutyryl Cyclic AMP and Norepinephrine.**—The peak attainable isometric responses to dibutyryl
cyclic AMP (3 x 10^-3 M) and norepinephrine (10^-5 M) are compared in Table 1. Control contractile levels were similar in the two muscle groups. Each agent caused a significant increase in active tension and rate of tension development, and a significant decrease in time to peak tension. However, no significant differences were found between the peak effects achieved by the two compounds.

Effects of Propranolol on Responses to Dibutyryl Cyclic AMP and Norepinephrine.
—Concentration-response curves demonstrating the effects of dibutyryl cyclic AMP on isometric contractile function in the presence and absence of beta-receptor blockade produced by 10^-6 M propranolol are shown in Figure 2. Propranolol did not alter the increment in tension or increment in rate of tension development produced by dibutyryl cyclic AMP. The contractile response to
TABLE 1

Comparison of the Peak Effects of Dibutyryl Cyclic AMP and Norepinephrine on Isometric Contractile Function

<table>
<thead>
<tr>
<th></th>
<th>Active tension (g/mm²)</th>
<th>Rate of tension development (g/mm²/sec)</th>
<th>Time to peak tension (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5 muscles)</td>
<td>5.0 ± 1.0</td>
<td>38.3 ± 7.0</td>
<td>208 ± 19</td>
</tr>
<tr>
<td>Dibutyryl cyclic AMP (3 x 10⁻⁵M)</td>
<td>9.5 ± 1.1</td>
<td>96.7 ± 11.1</td>
<td>170 ± 12</td>
</tr>
<tr>
<td>Difference</td>
<td>+4.5 ± 0.4</td>
<td>+58.4 ± 5.4</td>
<td>-38 ± 13</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Control (10 muscles)</td>
<td>5.0 ± 0.4</td>
<td>34.7 ± 3.6</td>
<td>236 ± 12</td>
</tr>
<tr>
<td>Norepinephrine (1 x 10⁻⁵M)</td>
<td>0.3 ± 0.3</td>
<td>82.0 ± 5.0</td>
<td>196 ± 10</td>
</tr>
<tr>
<td>Difference</td>
<td>+4.3 ± 0.3</td>
<td>+47.3 ± 3.2</td>
<td>-40 ± 8.1</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Stimulation frequency 12/min, temperature 37°C.

Concentrations of dibutyryl cyclic AMP greater than 3 x 10⁻⁵M could not be consistently obtained because of the development of rapid spontaneous papillary muscle contractions.

The effectiveness of propranolol in blocking beta-receptor sites is demonstrated in Figure 5, which shows norepinephrine concentration-response curves performed in the presence and absence of 10⁻⁶M propranolol. As previously demonstrated by Blinks (18), this concentration of propranolol shifts the norepinephrine concentration-response curve to the right. Spontaneous contractions usually occurred at norepinephrine concentrations greater than 10⁻⁵M in the absence of propranolol, but did not occur in the presence of 10⁻⁶M propranolol.
propranolol until norepinephrine concentrations were greater than 10^{-4}M.

**Effects of 5'-AMP.**—5'-AMP, the metabolic end-product of cyclic AMP, exerted no effects on isometric contractile function in five muscles when administered in concentrations ranging from 1 \times 10^{-4} to 3 \times 10^{-3}M.

**Effects of Sodium Butyrate.**—To ensure that the inotropic response to dibutyryl cyclic AMP was not a nonspecific effect related to an increased butyrate concentration, five papillary muscles were studied at concentrations of sodium butyrate ranging from 10^{-5} to 10^{-2}M. The contractile behavior of the muscles was not altered by exposure to these concentrations of the butyrate salt.

**Discussion**

The possible role of cyclic AMP in mediating the positive inotropic response to catecholamines has been the subject of numerous investigations during the past few years. Sutherland and co-workers recently reviewed the subject (19) and defined four criteria they believed should be fulfilled before an alteration in the intracellular level of cyclic AMP is reasonably established as the mechanism by which effects of catecholamines are produced in the heart. First, adenyl cyclase in broken cell preparations should be stimulated by catecholamines and the increase in cyclic AMP levels should precede the physiological response. Second, tissue levels of cyclic AMP should increase in response to catecholamine stimulation, and the increase in cyclic AMP levels should precede the physiological response. Third, the catecholamine response should be potentiated by drugs that inhibit phosphodiesterase activity. Finally, the addition of exogenous cyclic AMP or a derivative should reproduce catecholamine effects.

The first three of these criteria have been fulfilled (1-6). However, attempts to elicit a positive inotropic response with exogenously administered cyclic AMP in the isolated rat heart (2), rabbit atrium (6), guinea pig atrium (7), or intact dog heart (8) have been unsuccessful. Levine and co-workers reported that the injection of large doses of cyclic AMP in unanesthetized man and dogs resulted in a prompt increase in heart rate and cardiac output (20, 21). The experimental protocol used in these investigations, however, did not completely eliminate the possibility that the observed changes could have been reflexly induced and therefore cannot be accepted as proof of a direct cardiac response to cyclic AMP. In the present study, we found that concentrations of cyclic AMP ranging from 1 \times 10^{-4} to 5 \times 10^{-3}M had no effect on the contractile properties of the isolated cat papillary muscle. Although this might imply that the augmentation of myocardial contractility produced by norepinephrine is unrelated to enhanced production of cyclic AMP, several studies have suggested that cyclic AMP does not readily cross cell membranes, a property that might critically limit the increase in intracellular concentrations that could be achieved by the exogenous administration of this compound.

The N^{6}-2'-O-dibutyryl derivative of cyclic AMP combines increased lipid solubility with resistance to phosphodiesterase degradation while maintaining the capacity to mimic or exceed the biologic effects of cyclic AMP (8). In the present investigation, this derivative markedly augmented the contractile state of isolated cat papillary muscles as manifested by an increased isometric tension and rate of tension development and by a shift upward and to the right of the force-velocity relation. The increase in myocardial contractility caused by dibutyryl cyclic AMP is concentration dependent over a narrow range, with the threshold response occurring at 5 \times 10^{-4}M and the peak attainable response at 3 \times 10^{-3}M. The peak isometric contractile response to dibutyryl cyclic AMP is remarkably similar to that achieved by norepinephrine. However, the time course of action of these two agents is considerably different. Dibutyryl cyclic AMP requires 30 to 40 minutes to exert its full effect while the norepinephrine response is maximal in 2 to 4 minutes. Furthermore, the lowest concentration of dibutyryl cyclic AMP (5 \times 10^{-4}M) required to elicit a positive inotropic response in papillary muscles appears high when compared to concentrations of norepinephrine (10^{-8}M) evoking a compar-
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able response. However, the concentrations of dibutyryl cyclic AMP resulting in a positive inotropic response are comparable to those required to stimulate lipolysis in isolated rat epididymal fat cells, \(1.1 \times 10^{-6}\)M (11); to increase TSH release from rat adenohypophyses, \(5.8 \times 10^{-8}\)M (12); to induce colloid droplet formation in dog thyroid slices, \(5 \times 10^{-4}\)M (13); to cause a release of amylase from rat parotid glands, \(1 \times 10^{-4}\)M (14); and to stimulate bone resorption from rat calvaria, \(3 \times 10^{-6}\)M (15). In each of these tissues, as in the heart, the response to cyclic AMP was negligible. The explanation for the differences in the cardiac response to dibutyryl cyclic AMP and norepinephrine is not known, but may be due to differences in the rate at which each agent reaches its active site.

The myocardial stimulation obtained with dibutyryl cyclic AMP in the present study contrasts with the findings of other investigators who examined the cardiovascular effects of this derivative. Henion et al. found in anesthetized dogs that the intravenous injection of dibutyryl cyclic AMP in a dose of 4 mg/kg body weight caused only small and variable changes in cardiovascular performance (8). Robison et al. reported that concentrations of dibutyryl cyclic AMP as high as \(6 \times 10^{-4}\)M exerted no effect on the performance of the isolated perfused rat heart (2). This concentration of dibutyryl cyclic AMP is within the concentration range eliciting a positive inotropic response in cat papillary muscle. The explanation for the observed differences in the cardiac response to dibutyryl cyclic AMP is unknown but may be related to species differences with respect to the ability of dibutyryl cyclic AMP to penetrate myocardial cell membranes.

Although it is possible that the enhancement of myocardial contractility produced by dibutyryl cyclic AMP occurs via a mechanism independent of its structural relation to cyclic AMP, it seems more likely that the differences observed between the inotropic properties of these two agents are related to differences in their capacities to enter the myocardial cell or to differences in their resistance to phosphodiesterase degradation. The observation that sodium butyrate exerts no inotropic effects is consistent with this interpretation. Moreover, since the myocardial effects of dibutyryl cyclic AMP are not inhibited by the beta-receptor blocking agent propranolol, it would appear that its cardiac actions are not caused by direct stimulation of the beta receptor or by release of endogenous norepinephrine stores (22, 23).

The mechanism by which cyclic AMP or its dibutyryl derivative enhances myocardial contractility is not entirely clear. Phosphorylase activation is not thought to be directly involved in the contractile response (2, 3, 24-29), although this action may exert an indirect effect by providing the substrates needed to replenish the energy stores used in muscle contraction. Alternatively, it has been postulated that cyclic AMP may exert its effects by changing intracellular calcium stores through regulation of the permeability of cellular membranes to calcium, or alteration of calcium binding to membranes (30-33). In this regard, recent studies have demonstrated that a microsomal fraction of canine myocardium thought to represent sarcoplasmic reticulum contains an adenyl cyclase system responsive to norepinephrine stimulation (34). Furthermore, it has been shown that calcium accumulation by this preparation is increased by norepinephrine and cyclic AMP (35). It is thus possible that catecholamines activate the adenyl cyclase present in association with sarcoplasmic reticulum and thereby increase cyclic AMP levels. This increase in cyclic AMP may in turn modulate the strength of myocardial contraction by increasing the amount of sarcotubular calcium released following membrane depolarization.

In conclusion, the demonstration that dibutyryl cyclic AMP causes a marked positive inotropic effect in the isolated cat papillary muscle provides further evidence that the positive inotropic response to catecholamines is mediated by an increase in the intracellular level of cyclic AMP.
References


Circulation Research, Vol. XXVI, January 1970
Positive Inotropic Effects of Dibutyryl Cyclic Adenosine 3',5'-Monophosphate
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Circ Res. 1970;26:35-43
doi: 10.1161/01.RES.26.1.35

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