Reduction by Adenosine of the Isoproterenol-Induced Increase in Cyclic Adenosine 3',5'-Monophosphate Formation and Glycogen Phosphorylase Activity in Rat Heart Muscle

JAMES G. DOBSON, JR.

SUMMARY  The effect of adenosine on the increase in cardiac cyclic adenosine 3',5'-monophosphate (cyclic AMP) concentration and glycogen phosphorylase activity produced by isoproterenol was investigated. Slices of rat ventricular myocardium 0.5 mm thick weighing 15-20 mg were cut, washed, and incubated at 37°C in physiological saline gassed with either O2 (oxygenated) or N2 (anoxic). The concentration of cyclic AMP declined as the time of incubation increased in both oxygenated and anoxic muscle. In oxygenated muscle, isoproterenol, 10 μM, produced a 2.2-fold increase in cyclic AMP concentration and phosphorylase activity. Adenosine at 1 μM caused a 35% and 75% reduction in the isoproterenol-produced increase in cyclic AMP concentration and phosphorylase activity, respectively, without affecting basal levels. Reduction of the isoproterenol-elicited increase in cyclic AMP occurred within 2 minutes. Adenosine alone only at a high concentration of 1 mM increased cyclic AMP by 38% in oxygenated muscle. Adenine and inosine did not mimic the effect of adenosine on the isoproterenol-induced augmentation of cyclic AMP. Addition of adenosine deaminase to the physiological saline prevented the effects of adenosine but did not affect basal cyclic AMP. In anoxic tissue, isoproterenol failed to produce an increase in cyclic AMP and phosphorylase. Addition of adenosine deaminase to anoxic tissue resulted in an isoproterenol-produced increase in cyclic AMP, indicating that adenosine may inhibit an isoproterenol-induced increase in cyclic AMP during anoxia. These results suggest that adenosine attenuates the catecholamine-induced increase in cyclic AMP concentration and phosphorylase activity in oxygenated cardiac muscle, whereas, in the anoxic myocardium, adenosine may be responsible for preventing an increase in cyclic AMP upon β-adrenergic stimulation. Thus, adenosine may antagonize catecholamine elicited glycogenolysis.

ADENOSINE, a vascular smooth muscle relaxant, normally is present in the heart and has been postulated to be an important metabolite involved in the metabolic regulation of coronary blood flow. The production of adenosine is markedly augmented by myocardial hypoxia and ischemic anoxia. Adenosine has been reported to stimulate the accumulation of myocardial cyclic adenosine 3',5'-monophosphate (cyclic AMP). This effect, as well as the effect of adenosine on vascular smooth muscle, have been postulated to involve interaction of the nucleoside with an extracellular receptor. The positive chronotropic and inotropic effects of isoproterenol on isolated heart preparations have been reported to be reduced by adenosine.

ADENOSINE has been shown to stimulate cyclic AMP accumulation in other tissues such as brain, platelets, and cultured cells. However, in isolated fat cells, adenosine inhibits cyclic AMP accumulation and glycerol production. The nucleoside also appears to attenuate the catecholamine-produced increase in cyclic AMP and lipolysis in adipocytes. It is well known that the β-adrenergic catecholamines are capable of producing an increase in cyclic AMP, glycogenolysis, and contractility in the heart. Cyclic AMP appears to be involved in the activation of glycogen phosphorylase and may be important in the inotropic response.

The purpose of this investigation was to study the effects of adenosine on cyclic AMP formation and phosphorylase activation in response to catecholamine stimulation in oxygenated and anoxic heart muscle.

Methods

Cardiac Muscle Preparation

Sprague-Dawley rats (220-320 g) were obtained from Charles River and maintained on nonmediated Purina rat chow in rooms with a lighting
sequence of 12 hours light and 12 hours dark. The rats were anesthetized (ip) with sodium pentobarbital, 40 mg/kg. The hearts were excised rapidly and placed immediately in 50 ml of physiological saline solution (PSS, see below) at 0°C. Slices 0.5 mm thick containing right and left ventricular myocardium were made with a Stadie-Riggs tissue slicer. The slices obtained from one heart were washed in 500 ml of fresh PSS at 0°C for 30 minutes. During the wash the slices were made to swirl in the PSS by gassing vigorously with 95% O₂-5% CO₂. The ventricular slices were then cut into thirds with a sharp razor blade on a firm surface (methacrylate) covered with PSS at 0°C. Three pieces from different ventricular slices were transferred to 25 ml Erlenmeyer flasks containing 10 ml of fresh PSS. The flasks contained 40-60 mg (wet weight) of tissue and were held at 0°C for 5-30 minutes until incubation was initiated.

The flasks were transferred to a shaking water bath (115 oscillations/min) and the tissue incubated at 37°C under various conditions as described in Results. Each flask was gassed individually with either 95% O₂-5% CO₂ (oxygenated) or 95% N₂-5% CO₂ (anoxic) during the incubation. After incubation, the myocardial tissue pieces from each flask were separated from the incubation medium by pouring the contents of the flask into a funnel containing Whatman no. 1 filter paper. The pieces of tissue were either frozen immediately with polished aluminum clamps precooled in liquid nitrogen or immediately homogenized without prior freezing as described in the Analytical Procedures.

The PSS was prepared fresh daily and contained, in millimoles per liter: NaCl, 118.4; KCl, 4.69; CaCl₂, 2H₂O, 2.52; NaHCO₃, 25.0; MgSO₄·7H₂O, 1.18; KH₂PO₄, 1.18; and glucose, 10.0. When gassed above, the homogenate was centrifuged at 3000 g for 1 minute at 0°C. The homogenate was centrifuged at 3000 g for 15 minutes at 0°C. The ice that formed on the surface of the muscle pieces was scraped off with a scalpel. Generally, the entire muscle sample from a given Erlenmeyer flask was weighed and transferred to a Duall homogenizing tube (size 20, Kontes Glass) at −25°C.

**Analytical Procedures**

**Tissue Preparation for Biochemical Determinations**

Frozen pieces of myocardium were stored at −80°C in screw cap vials and prepared for analysis in a cold chamber at −25°C. The ice that formed on the surface of the muscle pieces was scraped off with a scalpel. Generally, the entire muscle sample from a given Erlenmeyer flask was weighed and transferred to a Duall homogenizing tube (size 20, Kontes Glass) at −25°C.

**Cyclic AMP**

Two hundred microliters of ice-cold 10% (wt/vol) trichloroacetic acid were added to the homogenization tube, and tissue plus acid were homogenized for 1 minute at 0°C with a motor-driven ground glass pestle rotating at 100–300 rpm. The homogenate was centrifuged at 12,000 g for 20 minutes at 0°C and the supernatant fluid extracted four times with 4 volumes of H₂O-saturated diethyl ether. The extract was placed in a boiling water bath until the odor of ether was no longer detectable. The concentration of cyclic AMP was measured in the extracts by a method involving the activation of skeletal muscle protein kinase as previously described. The concentration of cyclic AMP is expressed as pmol/mg protein. In some instances, the pieces of ventricular myocardium were not frozen but rather were homogenized as described above immediately after the tissue pieces were collected on filter paper following the incubation. Approximately 5–8 seconds elapsed between the termination of incubation and the initiation of homogenization. Using this technique, the myocardial concentration of cyclic AMP was not different from the concentration of the cyclic nucleotide observed when the tissue was frozen.

**Phosphorylase**

A 5-μg portion of the frozen muscle was transferred to a Duall homogenization tube at −25°C. An ice-cold solution (50–100 volumes) containing KF, 20 mM; ethylenediaminetetraacetate (EDTA), 4 mM; β-glycerophosphate, 20 mM; and β-mercaptoethanol, 20 mM (pH 6.8) was added rapidly and the mixture homogenized at 0° C as described above. The homogenate was centrifuged at 3000 g for 10 minutes at 0°C. Glycogen phosphorylase activity was measured by the production of glucose-1-phosphate in the absence and presence of 2 mM AMP as previously described. One unit of phosphorylase is defined as the amount of enzyme that produces 1 μmol/min of glucose-1-phosphate from glycogen at 30°C. The results are expressed as the ratio of phosphorylase activity without AMP (a) to phosphorylase activity assayed with AMP (a + b).

**ATP**

Forty-sixty milligrams of frozen tissue were transferred to a 16-mm × 150-mm test tube at −25°C. Immediately upon the addition of 0.8 ml of 0.6 N perchloric acid, the mixture was homogenized with a Polytron (PT-10) at a speed setting of 6 for 1 minute at 0°C. The homogenate was centrifuged at 3000 g for 15 minutes and the supernatant fluid was neutralized with approximately 0.2 ml of 3.2 M KHCO₃. After most of the evolved CO₂ had dissipated, the KClO₄ precipitate was removed by centrifugation. The concentration of ATP in the extract was determined enzymatically by a direct fluorometric method as previously described. The recovery of ATP through the extraction procedure was 60–70%.

**Protein**

Small samples of whole homogenates were diluted and the content of protein determined by the method of Lowry et al. Bovine serum albumin was used as a standard.
Materials

Racemic isoproterenol hydrochloride (Sigma) was prepared in 0.2% (wt/vol) sodium metabisulfite. All salts, dextrose, β-mercaptoethanol, EDTA, and solvents were certified grade from Fisher Scientific. Nucleotides, nucleosides, bases, β-glycerophosphate, sugar phosphates, and 8-azaguanine were obtained from Boehringer-Mannheim or Sigma. Adenosine deaminase (from calf intestine) and all enzymes used for phosphorylase and ATP assays and the synthesis of [γ-32P]ATP (a substrate for the cyclic AMP assay) were from Boehringer-Mannheim. Carrier-free (32P) inorganic phosphate was obtained from New England Nuclear. Crystalized bovine albumin was obtained from Pentex Biochemicals.

Statistical Methods

Analysis of variance was performed for paired and unpaired observations. A probability of <0.05 was accepted as indicating a significant difference.

Results

Effect of Incubation Time, Adenosine and Isoproterenol on cyclic AMP

The concentration of cyclic AMP in ventricular muscle decreased during a 60-minute incubation period (Fig. 1). In oxygenated ventricular muscle, cyclic AMP decreased from 3.4 to 1.6 pmol/mg protein in 60 minutes (Fig. 1A). In muscle made anoxic, cyclic AMP decreased from 3.7 to 1.3 pmol/mg protein during the 60-minute incubation period (Fig. 1B). Cyclic AMP decreased significantly within 10 minutes in anoxic muscle, whereas, in oxygenated muscle, the decrease in the cyclic nucleotide became significant only after 30 minutes of incubation (Fig. 1, A and B). When present during the incubation for 60 minutes, 1 mM adenosine increased cyclic AMP from 1.6 to 2.2 pmol/mg protein in oxygenated ventricular muscle but did not affect the concentration of the cyclic nucleotide in anoxic muscle (Fig. 1, A and B). Adenosine (0.01-1 mM) did not affect the concentration of adenosine 5′-triphosphate (ATP, substrate for cyclic AMP). The concentration of ATP was 5.56 ± 0.61 nmol/mg protein at zero time and decreased significantly by the end of the 60-minute incubation to 2.64 ± 0.26 and 1.69 ± 0.20 nmol/mg protein, respectively, in oxygenated and anoxic muscle. The differences in ATP concentrations between oxygenated and anoxic muscle at the end of the 60-minute incubation period were significantly different from each other and the respective controls. The protein content of both oxygenated and anoxic muscle ranged from 68.4 to 72.2 µg/mg tissue and did not vary during the incubation period, nor was it affected by adenosine or isoproterenol.

Adenosine and isoproterenol increased the concentration of cyclic AMP in oxygenated ventricular muscle (Fig. 2). Adenosine only at a high concentration of 1 mM produced a significant increase in ventricular muscle cyclic AMP concentration. Isoproterenol at 0.1 µM increased ventricular muscle cyclic AMP from 2.1 to 3.1 pmol/mg protein, and at 1-10 µM, produced a 2-fold increase in cyclic AMP concentration. The ventricular muscle cyclic AMP increased from 2.1 to 4.3-4.5 pmol/mg protein within 2 minutes in the presence of 1-10 µM isoproterenol. After a 5-minute exposure of the muscle to the isoproterenol, the increase in cyclic AMP became maximal at 4.6 pmol/mg protein, and remained elevated for 20 minutes.

Effect of Adenosine on Isoproterenol-Produced Increase in Cyclic AMP and Phosphorylase Activity.

Adenosine reduced the magnitude of the isoproterenol-produced increase in ventricular muscle cyclic AMP concentration in oxygenated ventricu-
Isoproterenol at 10 μM increased muscle cyclic AMP from 1.8 to 4.7 pmol/mg protein in the absence of adenosine. In the presence of 1 μM adenosine, isoproterenol increased cyclic AMP from 1.8 to only 3.4 pmol/mg protein. Although ventricular muscle cyclic AMP was not influenced as the concentration of adenosine increased from 0 to 100 μM, the isoproterenol-produced increase in cyclic AMP was progressively reduced. Concentrations of adenosine above 10 μM caused no further decrease in the formation of cyclic AMP in response to 10 μM isoproterenol, but at a concentration of 1 mM did elevate muscle cyclic AMP concentration in the absence of isoproterenol. The possibility that the extracts obtained from ventricular muscle exposed to isoproterenol plus adenosine interfered in some way with the assay for cyclic AMP, was eliminated by (1) detection in these extracts of all exogenously added cyclic AMP and (2) detection of all of the endogenous cyclic AMP in samples of combined muscle extracts from ventricular slices treated with adenosine, isoproterenol, and adenosine plus isoproterenol.

Adenosine caused a reduction of the isoproterenol-produced increase in cyclic AMP in oxygenated ventricular muscle in 2 minutes (Fig. 4). Isoproterenol alone at 10 μM increased ventricular muscle cyclic AMP from 3.4 to 5.6 pmol/mg within 2 minutes; however, in the presence of 10 μM adenosine (Fig. 4), the adenosine effect on the increase in cyclic AMP concentration produced by isoproterenol in rat heart ventricular muscle. The incubation of muscles in PSS gassed with 95% O₂-5% CO₂ was initiated at zero time. The PSS contained 10 μM isoproterenol (○), 10 μM isoproterenol plus 10 μM adenosine (□) or neither isoproterenol nor adenosine (■). Each point represents the mean of four experiments. Asterisks denote significant difference from the value without isoproterenol at the same time. Daggers denote significant difference from the isoproterenol value at zero adenosine. Bars represent ± 1 SE.
osine, the catecholamine increased the concentration of the cyclic nucleotide to only 4.5 pmol/mg protein.

Adenosine reduced the isoproterenol-produced activation of phosphorylase in oxygenated ventricular muscle (Fig. 5). Adenosine at 0-100 \( \mu M \) had no affect on the phosphorylase activity ratio of ventricular muscle in the absence of isoproterenol. Isoproterenol at 10 \( \mu M \) caused the phosphorylase activity ratio to increase from 0.06 to 0.13. Isoproterenol in the presence of 0.1 \( \mu M \) adenosine increased the activity ratio to only 0.10. Concentrations of adenosine above 1 \( \mu M \) essentially inhibited the isoproterenol-induced increase in the phosphorylase activity ratio. Total phosphorylase activity \((a + b)\) was not influenced by adenosine, isoproterenol, or adenosine plus isoproterenol and ranged from 0.10 to 0.12 U/mg protein.

**Effect of Adenosine and Isoproterenol on Cyclic AMP and Phosphorylase Activity during Anoxia**

Isoproterenol at 10 \( \mu M \) did not produce an increase in ventricular muscle cyclic AMP concentration or phosphorylase activity ratio when the muscle slices were incubated under anoxic conditions for 60 minutes (Table 3). Adenosine at 1 nm and 4 \( \mu g/ml \) adenosine deaminase had no affect on anoxic muscle cyclic AMP or phosphorylase activity ratio. Isoproterenol in the presence of adenosine deaminase increased anoxic ventricular muscle cyclic AMP from 1.7 to 2.5 pmol/mg protein, but did not increase the phosphorylase activity ratio. Incubation of ventricular muscle under anoxic conditions for only 10 minutes also prevented an isoproterenol elicited increase in muscle cyclic AMP. When 4 \( \mu g/ml \) adenosine deaminase was present in the PSS during this anoxic period, isoproterenol increased significantly ventricular cyclic AMP from 2.5 ± 0.2 to 3.4 ± 0.3 pmol/mg protein.

**Discussion**

**Incubation Time, Adenosine, and Isoproterenol**

The rat heart ventricular muscle preparation was particularly advantageous in these studies because usually 12 experiments could be performed from
the muscle obtained from one heart and the incubation conditions of the muscle controlled precisely. The decrease in cyclic AMP of ventricular muscle that occurred with incubation was observed on the initiation of the studies (Fig. 1). It was necessary to characterize this decrease in oxygenated and anoxic muscle so that the basal concentration of the cyclic nucleotide would be known for subsequent experiments. The ventricular muscle ATP concentration decreased in a manner similar to that for cyclic AMP. However, the protein content of the muscles remained constant throughout the incubation period, indicating that cyclic AMP and ATP concentrations fell without a loss of tissue protein.

Adenosine at a high concentration of 1 mM produced an increase in cyclic AMP concentration in oxygenated muscle; however, it did not affect the concentration of the cyclic nucleotide in anoxic muscle (Figs. 1 and 2). Adenosine did not affect the phosphorylase activity ratio in either oxygenated (Fig. 5 and Table 2) or anoxic muscle (Table 3). Huang and Drummond\(^7\) have reported that 1.0 to 100 \(\mu M\) adenosine stimulated a dose-dependent accumulation of cyclic AMP in chopped slices of guinea pig ventricle. The discrepancy between the results reported here and those of Huang and Drummond\(^7\) could be due to preparation and species differences. Since the basal adenosine concentration of the oxygenated rat heart\(^2\) is approximately 7.5 times greater than the concentration of the nucleoside in the oxygenated guinea pig heart,\(^5\) it is possible that the rat myocardium is less sensitive to adenosine-elicited cyclic AMP accumulation.

Isoproterenol at 0.01 to 10 \(\mu M\) produced a dose-dependent increase in cyclic AMP concentration in rat ventricular muscle (Fig. 2). These results are similar to those reported previously for isolated papillary muscles.\(^19\)

### Table 2: Effect of Adenosine, Adenosine Deaminase, and 8-Azaguanine on Cyclic AMP and Phosphorylase Activity in Rat Ventricular Muscle

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclic AMP (pmol/mg protein)</th>
<th>Phosphorylase activity ratio (−AMP+/AMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.10 ± 0.17</td>
<td>0.060 ± 0.004</td>
</tr>
<tr>
<td>+</td>
<td>5.15 ± 0.17*</td>
<td>0.123 ± 0.008*</td>
</tr>
<tr>
<td><strong>Adenosine (10⁻⁶ M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.98 ± 0.12</td>
<td>0.062 ± 0.007</td>
</tr>
<tr>
<td>+</td>
<td>3.78 ± 0.20†</td>
<td>0.063 ± 0.006†</td>
</tr>
<tr>
<td><strong>Adenosine deaminase (4 (\mu g/ml))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.06 ± 0.13</td>
<td>0.055 ± 0.004</td>
</tr>
<tr>
<td>+</td>
<td>5.01 ± 0.37*</td>
<td>0.092 ± 0.005†</td>
</tr>
<tr>
<td><strong>8-Azaguanine (4 (\times 10^{-4} M))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.89 ± 0.08</td>
<td>0.058 ± 0.005</td>
</tr>
<tr>
<td>+</td>
<td>4.74 ± 0.18*</td>
<td>0.090 ± 0.005†</td>
</tr>
<tr>
<td><strong>Adenosine (10⁻₅ M) plus adenosine deaminase (4 (\mu g/ml))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.02 ± 0.18</td>
<td>0.058 ± 0.005</td>
</tr>
<tr>
<td>+</td>
<td>4.62 ± 0.26*</td>
<td>0.090 ± 0.005†</td>
</tr>
</tbody>
</table>

Values represent the mean ± 1 SE of four experiments.
Rat ventricular muscle was incubated in PSS gassed with 95% O₂-5% CO₂ under the above conditions for 60 minutes. Isoproterenol was added for the last 10 minutes of the incubation period.
* Significantly different from the same condition minus isoproterenol.
† Significantly different from control plus isoproterenol.

### Table 3: Effect of Adenosine and Adenosine Deaminase on Cyclic AMP and Phosphorylase Activity in Anoxic Rat Ventricular Muscle

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cyclic AMP (pmol/mg protein)</th>
<th>Phosphorylase activity ratio (−AMP+/AMP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.67 ± 0.11</td>
<td>0.030 ± 0.003</td>
</tr>
<tr>
<td>+</td>
<td>1.94 ± 0.13</td>
<td>0.039 ± 0.003</td>
</tr>
<tr>
<td><strong>Adenosine (10⁻³ M)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.49 ± 0.21</td>
<td>0.032 ± 0.002</td>
</tr>
<tr>
<td>+</td>
<td>1.97 ± 0.12</td>
<td>0.035 ± 0.001</td>
</tr>
<tr>
<td><strong>Adenosine deaminase (4 (\mu g/ml))</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.64 ± 0.13</td>
<td>0.031 ± 0.003</td>
</tr>
<tr>
<td>+</td>
<td>2.50 ± 0.17†</td>
<td>0.033 ± 0.002</td>
</tr>
</tbody>
</table>

Values represent the mean ± 1 SE of six experiments.
Rat ventricular muscle was incubated in PSS gassed with 95% O₂-5% CO₂ for 60 minutes. Adenosine or adenosine deaminase was present during the entire incubation period as indicated. Isoproterenol was added for the last 10 minutes of the incubation period.
* Significantly different from all other values.
tion by 32-56% (Figs. 3 and 4). Although 1 mM adenosine caused an increase in basal cyclic AMP concentration, the nucleoside at this high concentration did not further attenuate the isoproterenol produced increase in the cyclic nucleotide. Adenosine reduction of the isoproterenol-produced increase in cyclic AMP concentration occurred with incubation of the ventricular muscle in the presence of the nucleoside for only 2 minutes. These results are similar to those reported by Schrader et al. in which 10 μM adenosine inhibited the isoproterenol-produced increase in isolated guinea pig heart cyclic AMP concentration. The present results are also similar to findings reported by Schwabe et al. and Fain in which 5-50 μM adenosine inhibited a nor- epinephrine-produced increase in isolated fat cell cyclic AMP accumulation.

An interesting observation was that adenosine reduced by 42-81% the isoproterenol-produced increase in the phosphorylase activity ratio in ventricular muscle without influencing the basal activity ratio (Fig. 5). At a given adenosine concentration from 0.1 to 100 μM, the reduction of the isoproterenol-produced increase in phosphorylase activity ratio was greater than the decrease in cyclic AMP formation (compare Figs. 3 and 5). The reason for this difference is not yet apparent. Nevertheless, the simplest explanation for adenosine reduction of the isoproterenol-induced activation of phosphorylase is that the nucleoside attenuated the formation of cyclic AMP.

**Adenine, Inosine, 8-Azaguanine, and Adenosine Deaminase**

Two metabolites of adenosine degradation, adenine and inosine, did not influence ventricular muscle cyclic AMP concentration and were without effect on the increase in cyclic AMP produced by isoproterenol (Table 1). The results suggest that the attenuation of β-adrenergic stimulation is specific for adenosine. Huang and Drummond used several analogues of adenosine and reported that the stimulation of cardiac cyclic AMP accumulation appeared to be specific for adenosine.

Further evidence that the effect of adenosine is specific was obtained by using adenosine deaminase. When adenosine was added to the incubation flask containing ventricular muscle plus adenosine deaminase, the effect of the nucleoside on the isoproterenol-produced increase in cyclic AMP and phosphorylase was prevented (Table 2). Schwabe et al. reported that adding adenosine deaminase to adipocytes prevented the adenosine inhibition of cyclic AMP accumulation. An adenosine deaminase inhibitor, 8-azaguanine, did not influence basal cyclic AMP nor did it affect the isoproterenol produced increase in the cyclic nucleotide (Table 2). These data suggest that the limited amount of adenosine produced by the well-oxygenated myocardium probably does not limit the isoproterenol-induced increase in cyclic AMP.

**Adenosine and Anoxia**

Adenosine itself did not produce an increase in the concentration of cyclic AMP in anoxic ventricular muscle (Fig. 1). Isoproterenol at 10 μM failed to elicit a significant increase in either cyclic AMP concentration or the phosphorylase activity ratio (Table 3). However, in the presence of adenosine deaminase, the catecholamine caused an increase in cyclic AMP but not phosphorylase activity. Anoxia is known to produce a 20-fold increase in rat myocardial adenosine concentration. Although the ATP concentration of anoxic ventricular muscle was very low, apparently there still was sufficient nucleotide available for cyclic AMP formation. Therefore, it is possible that the accumulation of adenosine in anoxic cardiac muscle prevents an isoproterenol-induced increase in cyclic AMP and that exogenous adenosine deaminase is capable of partially reversing this effect. It has been reported previously that isoproterenol produced an increase in cyclic AMP concentration in anoxic guinea pig papillary muscles. However, the muscles were exposed to the anoxic conditions for only 20 minutes, and their initial ATP concentration was 10 to 12 times greater (4.5-5.0 mM) than that found in the ventricular muscle preparation. Since isoproterenol failed to activate phosphorylase in the presence of adenosine deaminase, factor(s) other than adenosine, perhaps very low ATP levels, may be responsible for preventing a catecholamine-induced activation of phosphorylase in anoxia.

The mechanism by which adenosine causes the effects reported here are not known. However, Huang and Drummond, using agents which block both the adenosine uptake into cardiac cells and the adenosine-produced increase in cyclic AMP accumulation, suggest that the nucleoside interacts with a receptor on the external cell surface. It has been reported that adenosine is capable of inhibiting adenylyl cyclase of liver and adipocyte plasma membranes. Therefore, adenosine at low or physiological concentrations may interact with a cell membrane receptor and in turn attenuate the β-adrenergic-induced activation of adenylyl cyclase.

Whether the action of adenosine as a vasoactive factor, presumably involved in the metabolic regulation of coronary blood flow, is related to the attenuation by the nucleoside of the isoproterenol-produced increase in cyclic AMP concentration and phosphorylase activity cannot be determined from this study. However, the concentrations of adenosine required for the vasoactive and metabolic responses are similar. The adenosine hypothesis for the regulation of coronary blood flow is based on the fact that, in response to either an increased oxygen demand or a decreased oxygen supply, cardiac muscle is capable of increasing its production of adenosine. The interstitial concentration of adenosine would then increase and in turn would cause coronary resistance vessels to dilate and myo-
cardiac blood flow to increase to a level that would maintain myocardial oxygen balance. The catecholamine-produced increase in myocardial adenosine production is presumably elicited by the enhanced oxygen demand caused by the biogenicamines. Thus, adenosine may serve two important roles in the heart which are related in that an increase in myocardial adenosine not only functions as a vasodilating agent but as a factor that protects the heart against excessive \( \beta \)-adrenergic stimulation and an inordinate activation of glycogenolysis when there is either an increase in myocardial oxygen demand or a decrease in oxygen supply.

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**References**

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