The Mechanism by Which Adenosine and Cholinergic Agents Reduce Contractility in Rat Myocardium
Correlation with Cyclic Adenosine Monophosphate and Receptor Densities

Joel Linden, Cathy E. Hollen, and Amrat Patel
From the Cardiovascular Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma

SUMMARY. The adenosine analogue phenylisopropyladenosine decreased the basal and isoproterenol-stimulated contractile state of isolated rat left atria. The ED50 levels for both responses were similar, suggesting that direct and antiadrenergic effects may be mediated by the same receptor. Phenylisopropyladenosine decreased the cyclic adenosine monophosphate content of isolated atria and inhibited isoproterenol-stimulated adenylate cyclase activity in membranes prepared from atria and ventricles, but not as much as did methacholine. A maximally effective concentration of phenylisopropyladenosine or methacholine greatly reduced atrial contractility measured in the presence of either isoproterenol (1 μM) or Ro7-2956 (a phosphodiesterase inhibitor, 1 μM); however, in the presence of isoproterenol plus Ro7-2956, the contractile effects of phenylisopropyladenosine and methacholine were greatly attenuated. From the contractile data and cyclic adenosine monophosphate analyses, we conclude that direct and antiadrenergic contractile effects of both phenylisopropyladenosine and methacholine result primarily from their effects on cyclic adenosine monophosphate metabolism. The densities of adenosine, muscarinic, and β-adrenergic receptors in rat atrial membranes were found to be 30, 551, and 24 fmol/mg protein, respectively, based on equilibrium-binding assays conducted with 125I-aminobenzyl-adenosine, [3H]quinuclidinyl benzilate, and 125I-labeled pindolol. The greater effectiveness of methacholine than phenylisopropyladenosine as a negative inotropic agent and an inhibitor of adenylate cyclase in atria may be related to the relative densities of muscarinic and adenosine receptors. (Circ Res 56: 728-735, 1985)

N^6-SUBSTITUTED adenosine derivatives retaining an unsubstituted ribose ring bind to alkylxanthine-sensitive "ribose-site" (R-site) extracellular receptors and bind poorly to internal sites which require an unsubstituted purine for binding to "purine-sites" (P-sites (Londos and Wolff, 1977)). There are two types of extracellular R-site adenosine receptors designated R, (A,)- which decrease adenylate cyclase activity, and R, (A) which stimulate adenylate cyclase activity (Londos et al., 1980). In heart, adenosine reduces catecholamine-stimulated cyclic adenosine monophosphate (cAMP) accumulation and acts on an extracellular receptor which can be blocked by alkylxanthines—attributes which are characteristic of mediation by R, receptors (Schrader et al., 1977; Belardinelli et al., 1982a). In mammalian ventricular preparations, adenosine alone scarcely attenuates basal tension development, but adenosine inhibits the positive inotropic effect exerted by β-adrenergic agents (Endoh and Yamashita, 1980). In contrast, in mammalian atria, adenosine produces a direct effect, i.e., it reduces basal contractility in the absence of catecholamines (Rockoff and Dobson, 1980; Evans et al., 1982).

The response of cardiac muscle to cholinergic muscarinic agents is strikingly similar to the response to adenosine. Direct effects observed in the absence of catecholamines are much more prominent in mammalian atria than ventricles, whereas large antiadrenergic responses are demonstrable in both atria and ventricles (Levy, 1971; Schwegler et al., 1976). Dobson (1983a) has asserted that the results of Degubareff and Sleator (1965) and Grossman and Furchgott (1964) suggest that antiadrenergic and direct effects of adenosine are different. However, the production of endogenous adenosine (Schrader et al., 1977) may affect the apparent potency of added adenosine. In this study, we have used adenosine deaminase to deaminate endogenous adenosine, and we have examined the contractile effects of the adenosine analogue N^6-(R-phenyl-isopropyl)-adenosine (PIA) which is resistant to deamination (Westerman and Stock, 1970). We have found that the potency of PIA for direct and antiadrenergic contractile effects on rat left atria is the same, and most or all of the contractile effects of PIA appear to be mediated by a reduction in cyclic adenosine monophosphate (cAMP). The greater effectiveness of methacholine than PIA as a negative inotropic agent and as an inhibitor of ade-
nlylate cyclase have been related to the greater density of muscarinic than adenosine receptors.

**Methods**

Ro7-2956 and l-pindolol were the kind gifts of Dr. P.F. Sorter of Hoffman-LaRoche and Drs. H. Weidmann and H. Friedly of Sandoz, respectively. PIA and adenosine deaminase were purchased from Boehringer Mannheim; 2,5'-Dideoxyadenosine (didexyadenosine) was purchased from P-L Biochemicals; l-Isoproterenol hydrochloride (isoproterenol), atropine sulfate, phenylmethylsulfonic fluoride, and benzamidine were purchased from Sigma. The radioligands 125I-l-aminobenzyladenosine and 125I-pindolol were prepared as described by Linden et al. (1984) and Barovsny and Brooker (1980), respectively. [3H]quinuclidinyl benzilate ([3H]QNB) was purchased from New England Nuclear.

**Contractility Studies**

Left atria were obtained from pentobarbital-anesthetized 200- to 300-g male Sprague-Dawley rats and were stretched to a resting tension of 1 g between platinum hooks in buffer composed of (mM): NaCl, 120; KCl, 5; MgSO4, 1.2; NaH2PO4, 1.2; dextrose, 10; NaHCO3, 25; CaCl2, 1.5; pH 7.4, 30°C. The buffer was gassed with 95% nitrogen and homogenized with a Polytron tissue disruptor in 50 volumes of ice-cold medium containing 10% HED, diluted by 20% with glycerol, and frozen in aliquots at —70°C. For binding assays, 50 µl of the membrane suspension were incubated with 50 µl of radioligands in Tris buffer (50 mM Tris-Cl, 15 mM MgCl2, pH 7.3, at 21°C) and in some cases, 5 U/ml adenosine deaminase. After 2 hours of incubation, the membranes were filtered over glass fiber filters (Gelman type A/E). Nonspecific binding to muscarinic and adenosine receptors was assessed by the addition of 1 µM atropine and 10 µM PIA, respectively.

**Adenylate Cyclase Assays**

Membranes prepared as described above were thawed and incubated for 20 minutes at room temperature with 10 U/ml adenosine deaminase, and, in some cases, 0.025% sodium dodecyl sulfate, to uncover latent enzyme activity. The membranes were diluted 10-fold with ice-cold 10 mM Tris-Cl (pH 7.9 at 4°C). Fifty microliters of membrane suspensions containing 10–20 µg of protein were added to 50 µl of an enzyme cocktail prepared in 100 mM Tris-Cl (pH 7.5 at 37°C) to make 100 µl of a solution containing 0.5 mM Ro7-2956, 100 µM guanidine triphosphate (GTP), 5 mM phosphoenolpyruvate, 20 U/ml pyruvate kinase, 100 µM adenosine triphosphate (ATP), 2.5 mM MgCl2, 100 µM EDTA, 5 U/ml adenosine deaminase, and various drugs. The enzyme was incubated at 37°C for 10 minutes, and the assay was terminated by the addition of 250 µl of 0.1 M ZnSO4 and 250 µl of 0.1 M Na2CO3. The assay was linear with time for at least 15 minutes at protein concentrations up to 100 µg. After centrifugation at 1,500 g for 10 minutes, aliquots of the supernatants were acetylated and assayed for cAMP by automated radioimmunoassay (Brooker et al., 1979).

**Results**

**Comparison of Direct and Antiadrenergic Effects of PIA**

The direct effects of PIA on rat left atrial contractility were assessed after the addition of pindolol

![Diagram](image-url) **FIGURE 1.** Effect of PIA on rat left atrial contractility. Computer-monitored twitch tension (maximum–resting) of an isolated rat left atrium is plotted as a function of time. The arrows indicate the time of addition of 0.1 µM pindolol, 0.2 U/ml adenosine deaminase, and various cumulative concentrations of PIA. The ED50 of PIA was calculated from the x-axis intercept of the Hill plot shown in the inset; %1 = percent of maximum PIA-induced contractile response.
TABLE 1
Summary of the Direct and Antiadrenergic Effects of PIA on Rat Left Atrial Contractility

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PIA ED50 (nm)*</th>
<th>Twitch tension (g)†</th>
<th>Efficacy‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>12.5 ± 2.3</td>
<td>0.52 ± 0.08</td>
<td>73.6 ± 3.8</td>
</tr>
<tr>
<td>Isoproterenol (1 μM)</td>
<td>9.9 ± 0.9</td>
<td>1.37 ± 0.16</td>
<td>87.2 ± 1.9§</td>
</tr>
<tr>
<td>Isoproterenol (10 nm)</td>
<td>12.0 ± 1.1</td>
<td>0.98 ± 0.10</td>
<td>84.1 ± 1.1§</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM; n = 9.

* ED50 values were derived from the x-axis intercepts of Hill plots as illustrated in Figure 1. Each atrium was treated with 6–8 cumulative doses of PIA ranging between 10^{-8} and 10^{-5} M at 10-minute intervals after a 15-minute pretreatment with the indicated drugs. None of the groups differed significantly from the others, based on the F-ratio after analysis of variance.
† Twitch tension measured just before addition of the first dose of PIA.
‡ Efficacy = [(developed tension before PIA - developed tension after 10 μM PIA)/developed tension before PIA].
§ Greater than no treatment by the Dunnett test (P < 0.05).

Effect of Drugs which Modify cAMP Content on the Atrial Contractile Response to PIA and Methacholine

To assess the role of cAMP in mediating the contractile effects of PIA, the contractile response of atria to the most effective dose of PIA (1 μM) was measured in the presence of various other drugs which increase the tissue content of cAMP. PIA produced a large negative inotropic effect when added to atria alone, or when added after either 1 mM Ro7-2956 (86.5 ± 2.26%, n = 3) or isoproterenol (Table 2). However, the inotropic response to the purine was greatly attenuated in the presence of both Ro7-2956 and isoproterenol (Table 2). PIA produced a substantial decrease in the tissue content of cAMP in the presence of Ro7-2956 plus isoproterenol, but even in the presence of the purine, tissue

TABLE 2
Direct and Antiadrenergic Effects of PIA and Methacholine on Rat Left Atrial Contractility and cAMP Content

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cAMP (pmol/mg protein, n = 10)</th>
<th>cAMP (% decrease, n = 10)</th>
<th>Contractility (% decrease, n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>7.5 ± 0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIA (1 μM)</td>
<td>5.8 ± 0.62*</td>
<td>33 ± 8.3</td>
<td>72 ± 6.2</td>
</tr>
<tr>
<td>MCh (0.3 μM)</td>
<td>5.9 ± 0.72*</td>
<td>31 ± 9.6</td>
<td>64 ± 4.1</td>
</tr>
<tr>
<td>MCh (10 μM)</td>
<td>4.6 ± 0.75*</td>
<td>39 ± 10</td>
<td>86 ± 1.4‡</td>
</tr>
<tr>
<td>ISO (1 μM)</td>
<td>16.7 ± 3.05</td>
<td>43 ± 2.7</td>
<td>89 ± 1.2</td>
</tr>
<tr>
<td>ISO + PIA</td>
<td>9.5 ± 0.45*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISO + 0.3 μM MCh</td>
<td>9.3 ± 0.36*</td>
<td>44 ± 2.2</td>
<td>83 ± 3.1</td>
</tr>
<tr>
<td>ISO + 10 μM MCh</td>
<td>7.0 ± 0.19*</td>
<td>58 ± 1.1</td>
<td>95 ± 0.3</td>
</tr>
<tr>
<td>ISO + Ro (1 mM)</td>
<td>39.6 ± 3.9</td>
<td>39 ± 3.5</td>
<td>14 ± 1.1</td>
</tr>
<tr>
<td>ISO + Ro + PIA</td>
<td>24.0 ± 1.4*</td>
<td>39 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>ISO + Ro + 0.3 μM MCh</td>
<td>24.1 ± 1.5*</td>
<td>39 ± 3.8</td>
<td>9 ± 0.8</td>
</tr>
<tr>
<td>ISO + Ro + 10 μM MCh</td>
<td>15.1 ± 0.8‡</td>
<td>62 ± 2.0</td>
<td>64 ± 4.8‡</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. Statistical significance was assessed by the Tukey test at P < 0.05 (MCh = methacholine, ISO = isoproterenol, Ro = Ro7-2956).
* Significant effect by PIA or MCh.
† Less than PIA and 0.3 μM MCh.
‡ Greater than PIA and 0.3 μM MCh.
levels of cAMP remained substantially higher (3.2-fold) than basal levels. These data strongly suggest that the contractile effects of PIA are mediated primarily by a reduction of cAMP.

The effects of two doses of methacholine on rat atrial contractility and cAMP content are also summarized in Table 2. The ED_{50} of the cholinergic agent as a negative inotropic agent was 82 ± 11 nM (n = 4, not shown). A moderate dose of methacholine, 0.3 μM, produced effects similar to those of 1 μM PIA (Table 2). Large negative inotropic responses measured in the presence of Ro7-2956 alone or isoproterenol alone were greatly attenuated in the combined presence of Ro7-2956 and isoproterenol. However, unlike PIA the most effective dose of methacholine (10 μM) did produce a substantial negative inotropic response in the presence of both Ro7-2956 and isoproterenol, possibly because it was more effective than PIA at reducing the tissue content of cAMP.

Effects of PIA and Methacholine on Adenylate Cyclase Activity

The effects of PIA and methacholine on basal and isoproterenol-stimulated adenylate cyclase activity were assessed in rat ventricle and atrial membranes. If membranes were pretreated with a low concentration of sodium dodecyl sulfate, latent adenylate cyclase activity was unmasked, and the response of the enzyme to drugs was enhanced. Therefore, subsequent assays employed membranes which were pretreated with the detergent. PIA and methacholine produced small effects on the basal activity of adenylate cyclase; the isoproterenol-stimulated enzyme was inhibited to a greater extent (Table 3). Similar statistically significant effects of PIA and methacholine were observed with two additional batches of ventricular membranes. Since the effects of PIA and methacholine on atrial adenylate cyclase activity were quite small, experiments to assess the effects of these drugs on the enzyme in the presence of isoproterenol were repeated with four additional preparations of atrial membranes. PIA and methacholine reduced isoproterenol-stimulated adenylate cyclase activity by an average of 9.05 ± 1.41% and 17.8 ± 2.47%, respectively. Paired analysis of the mean data from these five experiments indicated that the PIA response differed significantly from the control and methacholine responses at P < 0.01.

The inhibitory effects of adenosine R-site agonists on adipocyte membranes have been reported to be amplified by sodium (Londos et al., 1981). Therefore, the adenylate cyclase activity of heart membranes was measured in response to the most effective concentrations of PIA, methacholine, and isoproterenol as a function of sodium concentration. Results obtained with a typical batch of membranes are illustrated in Figure 2. Similar results were obtained with three additional batches of membranes. PIA failed to decrease the basal activity of adenylate cyclase in the absence of sodium, although the isoproterenol-stimulated enzyme was inhibited by 7–10% in the absence of sodium. At NaCl concentra-

![Graph](https://via.placeholder.com/150)

**FIGURE 2.** Effects of PIA and NaCl on rat ventricular adenylate cyclase activity. Adenylate cyclase activity was measured using the cocktail described in Methods, supplemented with various concentrations of NaCl. Most effective concentrations of PIA (10 μM), methacholine (100 μM), and isoproterenol (10 μM) were used. Data represent the mean ± SEM, n = 5; standard errors smaller than the symbols are not shown.
DIDEOXYADENOSINE (M)

FIGURE 3. Effects of IBMX on the dose-response curves of PIA and dideoxyadenosine for inhibition of adenylate cyclase in ventricular membranes. Enzyme activity was measured in the presence of 100 mM NaCl. 100% = 427 pmol cAMP formed/mg protein per 10 min. Data represent the mean ± SEM, n = 4; standard errors smaller than the symbols are not shown.

Tions above 100 mM, 1 μM PIA reduced both basal and isoproterenol-stimulated enzyme activity 10–20%. Methacholine was more effective than PIA, decreasing enzyme activity 20–30%.

To determine whether the inhibitory effect of PIA on basal adenylate cyclase activity is receptor mediated, we used a known inhibitor of adenosine receptors, the methylxanthine IBMX (Schwabe and Trost, 1980). IBMX shifted the dose-response curve of PIA to the right, indicative of a competitive inhibitor (Fig. 3). In contrast, inhibition of adenylate cyclase produced by the "P-site" agonist dideoxyadenosine was not affected by IBMX. These data confirm that inhibition of basal ventricular adenylate cyclase activity is mediated by an R-receptor.

Quantification of Adenosine, Cholinergic-Muscarinic, and β-Adrenergic Receptors in Atrial and Ventricular Membranes

The density of various receptors was assessed in membranes prepared from atria and ventricles. The greatest discrepancy between atria and ventricles was in the density of muscarinic-cholinergic receptors assessed by [3H]QNB binding (Fig. 4). The results of equilibrium-binding assays using 125I-aminobenzyladenosine, [3H]QNB, and 125I-pindolol to label adenosine, muscarinic, and β-adrenergic receptors, respectively, are summarized in Table 4. There was a large excess of cholinergic-muscarinic receptors compared to adenosine or β-adrenergic receptors, particularly in atria.

<p>| TABLE 4 | Equilibrium Binding of Various Radioligands to Membranes Derived from Rat Atria and Ventricles |
|-----------------|-------------------|-----------------|------------------|-----------------------|</p>
<table>
<thead>
<tr>
<th>Radioligand (receptor)</th>
<th>Rmax (fmol/mg protein)</th>
<th>Ratio of Rmax (atria/ventricle)</th>
<th>Kd (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricle</td>
<td>125I-Aminobenzyladenosine</td>
<td>23</td>
<td>1.3</td>
</tr>
<tr>
<td>Atria</td>
<td>Adenosine</td>
<td>30</td>
<td>3.0</td>
</tr>
<tr>
<td>Ventricle</td>
<td>[3H]QNB (Muscarinic)</td>
<td>88</td>
<td>6.3</td>
</tr>
<tr>
<td>Atria</td>
<td></td>
<td>551</td>
<td>0.18</td>
</tr>
<tr>
<td>Ventricle</td>
<td>125I-Pindolol (β-Adrenergic)</td>
<td>10</td>
<td>2.4</td>
</tr>
<tr>
<td>Atria</td>
<td></td>
<td>24</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Six to 10 radioligand concentrations were used in each binding assay. The same preparation of membranes was used for all assays. Similar results were obtained in a second experiment using different membrane preparations.
Discussion

The results of this study indicate that the potency of the adenosine analogue PIA as a negative inotropic agent in rat left atria is the same, regardless of whether direct or antiadrenergic responses are measured. By "direct," we are referring to the tissue response in the absence of other drugs and in the presence of pindolol, a β-adrenergic receptor antagonist, which was added to counter the effects of any endogenous catecholamine released by sympathetic nerves contained in the isolated atria. By "antiadrenergic," we are referring to the response elicited by PIA after the prior addition of the catecholamine isoproterenol. These studies were conducted in the presence of adenosine deaminase in order to deaminate any endogenous adenosine released by the atria to inosine, which is inactive. As mentioned in the introduction, it has been suggested that direct and antiadrenergic effects of adenosine may be mediated by different receptors. However, these responses are probably influenced by the release of endogenous adenosine, as well as by adenosine uptake and degradation. This study, which indicates that the potency of PIA is equivalent for direct and antiadrenergic contractile effects, suggests that both responses may be mediated by the same receptor.

Our results also suggest that effects of PIA and methacholine on rat atrial contractility are mediated predominantly or entirely by inhibition of adenylyl cyclase activity. If the atrial concentration of cAMP was elevated to a very high level by the combined addition of isoproterenol and Ro7-2956, the ability of PIA or a low dose (0.3 μM) of methacholine to reduce contractility was greatly attenuated. The adenylyl cyclase inhibitors still lowered tissue levels of cAMP, but the cyclic nucleotide concentration remained well above the basal level. Possibly, this combination of drugs causes saturation of cAMP-dependent protein kinase by the cyclic nucleotide even after the addition of PIA or methacholine. At the most effective concentrations, methacholine was more effective than PIA at reducing the cAMP content of atria. A large dose of methacholine (10 μM) reduced the atrial content of cAMP to near the basal level and produced a substantial negative inotropic response, even in the presence of isoproterenol and Ro7-2956. However, we have shown previously that in the presence of both isoproterenol (0.1 μM) and IBMX (300 μM), even a high concentration of methacholine (20 μM) produced only a small negative inotropic effect (Linden and Brooker, 1979).

The results of this study indicate that PIA reduces rat atrial contractility, cAMP content and adenylyl cyclase activity. Numerous other studies confirm that adenosine agonists produce negative inotropic effects in atria and ventricles through an extracellular receptor blocked by methylxanthines (see the introductory paragraphs). However, Seidelberger et al. (1984) have suggested that adenosine effects are not important under physiological conditions in anesthetized dogs. In contrast, adenosine does influence myocardial function in humans, and has been used successfully to treat paroxysmal supraventricular tachycardia, suggesting a physiologically important role by adenosine in man (DiMarco et al., 1983; Belhassen and Pelleg, 1984).

As in this study, Leung et al. (1983) demonstrated an adenosine receptor-mediated decrease in adenylyl cyclase activity in membranes derived from guinea pig ventricles, but Schütz and Tuisl (1981) did not. We have found that receptor-mediated effects of adenosine on myocardial adenylyl cyclase are small, about half the size of cholinergic effects, require high GTP (100 μM), are reduced or abolished by KCl extraction of heart membranes, and require the addition of adenosine deaminase to deaminate endogenous adenosine even in the absence of added ATP. Schütz and Tuisl (1981) used 10 μM GTP, extracted membranes with KCl, and did not add adenosine deaminase, whereas Leung et al. (1983) used 100 μM GTP, did not extract membranes with KCl, and did add adenosine deaminase. There are also conflicting data on the effects of adenosine on the cAMP content of intact hearts. Most investigators have found that adenosine agonists decrease the myocardial content of cAMP. cAMP was reduced by phenylisopropyladensin (in this study using rat atria. The cyclic nucleotide was also lowered by adenosine agonists in enzymatically dispersed embryonic chick heart cells and intact embryonic chick ventricles (Belardinelli et al., 1982b; Linden et al., 1984), in enzymatically dispersed rat heart cells (Hazecki and Uj, 1981), in perfused guinea pig hearts (Schrader et al., 1977; Belardinelli et al., 1982a), and perfused rat hearts (Dobson, 1978; Dobson, 1983b). In contrast, Schmitz et al. (1981) and Böhm et al. (1984) failed to detect statistically significant effects of adenosine on the cAMP content of guinea pig hearts. Possibly, these negative data result from the partial occupancy of adenosine receptors by endogenously released adenosine, since the addition of adenosine deaminase in some cases has been shown to increase myocardial contractility (Dobson, 1983a) and cAMP content (Hazecki and Uj, 1984).

Both acetylcholine (TenEick et al., 1976) and adenosine (Belardinelli and Isenberg, 1983; Jochem and Nawrath, 1983) have been reported to activate a potassium conductance in mammalian atria, but not ventricles. The latter investigators speculated that ventricular heart muscle may lack a receptor-controlled potassium conductance. These observations raise the possibility that cAMP mediates antiadrenergic effects observed in both atria and ventricles, whereas a cAMP-independent effect on potassium conductance may mediate direct contractile effects observed in atria. Alternatively, both direct and indirect effects of acetylcholine and adenosine may be mediated by cAMP. The results of this study favor the latter hypothesis. A mechanism by...
which cAMP may influence potassium conductance is suggested by the observation that cAMP enhances calcium pumping into the cardiac sarcoplasmic reticulum by increasing the phosphorylation of phospholamban (Kranias and Solaro, 1982). Since raising intracellular calcium activates a potassium conductance in nervous tissue (Meech, 1978) and Purkinje fibers (Isenberg, 1977), effects of acetylcholine and adenosine on potassium channel conductance may be secondary to reduced myocardial cAMP and increased diastolic levels of calcium.

The possibility that the contractile effects of adenosine and acetylcholine are mediated by inhibition of adenylyl cyclase is supported by studies on the effects of phosphodiesterase inhibitors on the sensitivity of mammalian ventricles to inhibitors of adenylyl cyclase. Direct effects of acetylcholine are minimal or absent in mammalian ventricles (Schwegler et al., 1976) and embryonic chick ventricles (Biegon et al., 1980), but acetylcholine inhibits the stimulatory effects of phosphodiesterase inhibitors in both mammalian ventricles (Meester and Hardman, 1967; Endoh and Honma, 1979) and embryonic chick ventricles (Biegon et al., 1980). Similarly, we have shown previously that although adenosine alone has no effects on slow Ca2+-dependent action potentials in embryonic chick ventricles, the stimulatory effects of Ro7-2956 in embryonic chick ventricles are inhibited by adenosine (Linden et al., 1982). Both cholinergic agents (Murad et al., 1962) and PIA (this report and Leung et al., 1983) can decrease the basal activity of adenylyl cyclase measured in ventricular membranes. These data suggest that the absence of direct ventricular contractile effects by adenylyl cyclase inhibitors may be a consequence of low basal levels of cAMP in a physiologically important pool in the ventricular myocardium, i.e., basal levels may be below the threshold for activation of cAMP-dependent protein kinase.

Both PIA and dideoxyadenosine were found to decrease adenylyl cyclase activity in rat ventricle membranes, but only the effect of PIA was blocked by a methylxanthine (IBMX). Dobson (1983b) reported that adenosine reduced isoproterenol-stimulated but not basal rat ventricle adenylyl cyclase activity. However, these responses were measured in the absence of adenosine deaminase and were not inhibited by theophylline. Therefore, they probably represent intracellular P-site rather than receptor-mediated R-site effects (Londos et al., 1978, 1981). P-site-mediated effects produced by dideoxyadenosine (this report) or by adenosine (Dobson, 1983b) are probably not related to the contractile effects of adenosine, which appear to be mediated by extracellular R-site receptors that can be blocked by alkylxanthines (Schrader et al., 1977; Belardinelli et al., 1982).

Methacholine was found to be more effective than PIA as a negative inotropic agent and as an inhibitor of adenylyl cyclase activity. Also, the density of muscarinic receptors was found to be greater than the density of adenosine receptors in both rat atria and ventricles. Adenosine and muscarinic receptors probably are coupled to adenylyl cyclase through a common guanine nucleotide-binding protein (Gilman, 1984). These data conform to a model in which the effectiveness of inhibitors of adenylyl cyclase depends on receptor density. The probability of an interaction between agonist-occupied receptors and inhibitory guanine nucleotide-binding proteins is enhanced as receptor density increases.

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Address for reprints: Dr. Joel Linden, Cardiovascular Research Program, Oklahoma Medical Research Foundation, 825 Northeast 13th Street, Oklahoma City, Oklahoma 73104.

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J Linden, C E Hollen and A Patel

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