A1-Adenosine Receptor Inhibition of Adenylate Cyclase In Failing and Nonfailing Human Ventricular Myocardium

Ray E. Hershberger, MD; Arthur M. Feldman, MD, PhD; and Michael R. Bristow, MD, PhD

**Background.** Receptors that couple via the stimulatory G protein, Gs, to adenylate cyclase and to a positive inotropic response have been extensively investigated in failing human heart. In contrast, much less is known about receptors, such as the A1-adenosine receptor, that couple to adenylate cyclase via the inhibitory G protein, Gi, to give a negative inotropic response. Activation of such Gi-coupled receptors might worsen heart failure. Furthermore, a Gi is increased in failing human ventricular myocardium, which may enhance inhibitory receptor coupling to adenylate cyclase.

**Methods and Results.** A1-Adenosine receptor inhibition of adenylate cyclase was examined in crude particulate preparations derived from 12 nonfailing and 12 failing human left ventricles. Experimental conditions were designed for maximal inhibitory responses. Dose–response curves were performed with the selective A1-adenosine receptor agonist R-phenylisopropyladenosine (R-PIA). No differences in nonfailing versus failing heart were observed for basal adenylate cyclase activity (49.0±4.1 versus 45.7±2.6 pmol cyclic AMP/min/mg), maximal R-PIA-mediated inhibition (31.1±2.6 versus 30.2±1.6 pmol cyclic AMP/min/mg), ED50 (R-PIA×10^{-7} M, 1.28±0.10 versus 1.36±0.08), or slope (1.06±0.06 versus 1.03±0.10), respectively. Furthermore, fluoride, forskolin, and manganese adenylate cyclase activation were not different in failing heart, which is consistent with no change in the catalytic unit of adenylate cyclase. The inhibitory G protein aGi, as quantitated by pertussis toxin–catalyzed ADP-ribosylation, was increased in failing heart (105.7±5.8 versus 132.7±3.4 optical density units, p<0.003). Basal adenylate cyclase activity was reduced in failing heart (7.8±0.8 versus 4.5±0.4 pmol cyclic AMP/min/mg, p<0.005) with assay conditions designed to assess G protein effects.

**Conclusions.** The A1-adenosine receptor pathway exerts a major inhibitory effect on human myocardial adenylate cyclase activity. Although aGi was increased in failing heart, A1-adenosine receptor inhibition of adenylate cyclase was not altered in preparations of failing versus nonfailing human ventricular myocardium. (*Circulation* 1991;83:1343–1351)

Much effort has gone into the examination of receptors coupled to adenylate cyclase and to a positive inotropic response in human ventricular myocardium, especially in the setting of human heart failure.1–5 Such receptors are coupled by the stimulatory guanine nucleotide–bind-

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human atrial and ventricular myocardial preparations. However, A1-adenosine receptor-coupled functional responses have not been examined in failing versus nonfailing human heart.

Despite these numerous studies, the roles of adenosine and adenosine receptors are not well understood in cardiac muscle physiology. Based on the degree of negative inotropic effect produced by A1-adenosine receptor activation and the amount of adenosine present in cardiac tissue, it is conceivable that the A1-adenosine receptor pathway may maintain some basal or tonic negative inotropic influence in working myocardium. Although the kinetics of adenosine production and degradation are not known in failing human heart, cardiac adenosine release has been observed to be increased threefold in a canine model of heart failure. Whether such changes imply increased cardiac A1-adenosine receptor pathway activation is unknown.

The present study is based on the hypothesis that cardiac adenosine may play some role in inotropic control of failing human ventricular myocardium, particularly as the G protein to which the A1-adenosine receptor is coupled, Gi, is increased in activity in the failing human heart. Recent examinations of G proteins in failing human heart have demonstrated a 30–40% increase in a 40,000-MW pertussis toxin–sensitive substrate subsequently shown to be αGi21 with decreased adenylate cyclase activity in failing human heart consistent with increased inhibitory G protein (Gi) activity. Therefore, it is possible that a Gi-coupled receptor pathway such as the A1-adenosine receptor may have augmented signal transduction related to the increase in αGi.

The purpose of the present study was to examine A1-adenosine receptor–mediated inhibition of adenylate cyclase in preparations of human ventricular myocardium and to compare these adenylate cyclase responses in failing and nonfailing human heart.

Methods

Tissue Procurement

Human cardiac tissue was obtained from the Utah Transplantation Affiliated Hospitals (UTAH) Cardiac Transplant Program. Twelve failing hearts were explanted from patients undergoing orthotopic heart transplant operations, and 10 nonfailing hearts were received from kidney donors whose hearts were not used for transplantation because of late developing recipient exclusions, recipient blood type or size mismatch, or age (n=2). In all cases, repeated attempts were made to place these hearts for organ donation using the United Network for Organ Sharing.

Failing hearts were from 10 men and two women (mean age, 39 years) with idiopathic dilated cardiomyopathy and chronic end-stage congestive heart failure. Mean cardiac index was 1.9±0.14 l/min/m2; mean pulmonary wedge pressure was 24.9±1.5 mm Hg; and mean ejection fraction was 17.3±1.9%. All patients were taking vasodilators, digoxin, and diuretics. Nonfailing hearts were from eight men and two women (average age, 30 years). The 10 donor hearts had normal cardiac function by history and echocardiography. Two additional nonfailing left ventricles were obtained from patients with primary pulmonary hypertension who were undergoing heart and lung transplant with well-preserved left ventricular function by clinical, echocardiographic, and biochemical indexes.

Immediately after cardectomy, hearts were placed into iced saline slush and then transported to the laboratory in ice-cold physiological salt solution. All 12 failing hearts and nine of 12 nonfailing hearts or left ventricles were procured locally, with less than 30 minutes between explantation to arrival in the laboratory. The three distantly procured donor hearts had cold ischemic times of 1, 1½, and 2 hours, with adenylate cyclase measurements that did not differ from those of locally procured donor hearts or left ventricles.

Myocardial Membrane Preparations

A preparation suitable for measuring hormone-stimulated adenylate cyclase was prepared as previously described. Briefly, 2 g of ventricular myocardium was trimmed free of endocardium and epicardium and placed into a sucrose (250 mM), Tris (10 mM), and EGTA (1 mM) buffer (pH 7.5). Myocardium was homogenized three times for 3 seconds each time with a polytron at full speed. The homogenate was centrifuged at 1,200g for 20 minutes, and the pellet was resuspended with a loosely fitting motorized pestle. After two additional centrifugations, the final pellet was resuspended in 12 ml sucrose-Tris-EGTA buffer and filtered through gauze into a glass homogenizer. After 20 passes with a closely fitting nylon pestle, the preparation (final protein concentration, 6–10 mg/ml) was stored at −80°C.

A crude membrane preparation suitable for measuring β-receptors was made as previously described. Briefly, ventricular myocardium was homogenized, after which contractile proteins were extracted in 0.5 M KCl. The homogenate was centrifuged at 50,000g and resuspended three times, with the final resuspension in high sucrose-Tris buffer. The preparation was frozen at −80°C until use.

The time between preparation of tissue and use for biochemical determinations ranged from 3 to 15 months, with preparation storage time matched between groups. We have been unable to demonstrate degradation of adenylate cyclase activity or receptor densities with storage times of more than 24 months under these conditions (unpublished data).

Biochemical Determinations

Adenylate cyclase assays were performed as previously described. For routine assay, a reaction mixture was made consisting of 100 mM Tris (pH 7.30 at 30°C), 10 mM phosphocreatine, and 0.1 mM Mg-ATP with 0.5 mM or 2.5 mM in excess MgCl2, 10 μM GTP, 1 mM cAMP, 1.75 units creatine kinase per
Assay tube, 0.3 units adenosine deaminase per assay tube, 1 μM propranolol, and a trace label of 3H cAMP (10,000–12,000 cpm per tube). Agonists of choice as well as reaction mixture and 0.05–0.125 mg of myocardial preparation were added to tubes, all of which were preincubated at 30°C for 10 minutes, after which a trace label of α-32P-ATP was added (800,000–1 million cpm per tube) in a final volume of 250 μL. After an additional 20-minute incubation at 30°C, 750 μL of 1% sodium dodecyl sulfate was added to stop the reaction, and reaction products were separated according to the two-column method of Salomon et al. Column recovery was routinely 70–90%. Protein concentrations were determined after the method of Lowry et al.

To examine changing magnesium or substrate concentrations, substrate was held constant at 0.1 mM Mg·ATP or 0.5 mM Mg, respectively. For substrate kinetic studies, Mg·ATP concentrations ranging from 0.025 to 0.4 mM with 0.5 mM MgCl₂ in excess were used. For each concentration of substrate, water (basal determination), 0.3 μM and 0.1 mM isoproterenol, and 0.1 and 10 μM R-phenylisopropyl-adenosine (R-PIA) were added in duplicate tubes. Magnesium kinetics were performed with magnesium ranging from 0.25 to 5.0 mM. All kinetic data were converted to “s-multiplied” double-reciprocal linear plots (Hane’s plots). Substrate dissociation constant (Kd) and Vmax and magnesium Kᵢ and Vmax were then calculated as previously described.

For experiments to examine β-receptor effects on adenylyl cyclase activity, propranolol was omitted and Mg was 0.5 mM in excess, with adenosine deaminase either present or absent.

**β-Receptor Measurements**

Saturation binding assays to measure β-receptor density and affinity were conducted with 125I-cyanopindolol (125IICYP), as previously described. Specific binding routinely exceeded 90%. Protein concentrations of receptor-binding preparations were determined by the Peterson modification of the Lowry method.

**ADP-Ribosylation**

Pertussis toxin-sensitive G proteins were estimated in the crude particulate (adenylyl cyclase) preparations as previously described.

**Data and Statistical Analyses**

Adenylyl cyclase data points were means of duplicates, except for time course studies that were means of triplicates, and expressed as picomoles of cAMP produced per minute per milligram of added membrane protein. As previously described, dose-response curves were modeled with nonlinear least-squares computer-assisted curve fitting to a four-parameter logistic equation of DeLean et al. to find the best fit of a sigmoidal curve, including minimum, maximum, ED₅₀, and slope.

β-Receptor density (Bₘₐₓ) and Kᵢ were found by nonlinear modeling, as previously described.

For comparing differences between groups, a nonpaired Student’s t-test was used, with a probability value of less than 0.05 in the two-tailed distribution considered significant. Dose–response curves were compared using an analysis of covariance technique as previously described. In comparison of data in more than two groups, analysis of variance and the Bonferroni t-test with multiple comparisons were used.

**Results**

**Assay Characterization**

**Adenosine deaminase.** Increasing concentrations of adenosine deaminase progressively increased adenylyl cyclase activity with a maximal effect at 0.3 units adenosine deaminase per tube (1.2 units/ml). Adenylyl cyclase basal activity was increased approximately 80% with 0.3 units of adenosine deaminase (6.9±1.1 versus 12.6±2.1 pmol cAMP/min/mg, n=3). With the addition of the A₁-adenosine receptor agonist R-PIA, adenylyl cyclase activity was similar to basal activity in the absence of adenosine deaminase. Furthermore, the addition of R-PIA without adenosine deaminase present produced virtually no change in adenylyl cyclase activity (data not shown).

**Time course and protein concentration effects.** Time course effects were evaluated in three experiments and demonstrated linear activation of adenylyl cyclase with 0.3 units of adenosine deaminase per tube after the first 5 minutes for the duration of the experiment. Adenylyl cyclase activation was linear with protein concentration ranging from 0.1 to 0.8 mg/ml (0.03–0.20 mg per tube) with adenosine deaminase alone and in the presence of R-PIA in four experiments. Approximately 0.4 mg/ml (0.10 mg protein per tube) was used for standard assay conditions.

**Guanine nucleotide effects.** Adenylyl cyclase activity increased with increasing GTP and was maximal with 2–10 μM GTP; R-PIA-inhibited adenylyl cyclase inhibition was also maximal in this range. R-PIA–mediated adenylyl cyclase inhibition was not observed in the absence of GTP. For standard assay conditions, 10 μM GTP was used.

**Magnesium and substrate effects.** Increasing magnesium concentration substantially augmented basal adenylyl cyclase activity. When taken as a fraction of basal activity, maximal R-PIA–mediated inhibition of adenylyl cyclase activity occurred with from 2 to 3 mM MgCl₂; therefore, 2.5 mM MgCl₂ was used for A₁-adenosine receptor experiments. In contrast, the greatest ratio of isoproterenol-stimulated adenylyl cyclase activity to basal activity was present with 0.5 mM MgCl₂, which was used for β-receptor experiments. This ratio diminished as magnesium increased.

Examination of substrate kinetics demonstrated that Vmax increased for isoproterenol but not for R-PIA (Table 1). Examination of magnesium kinetics demonstrated that the magnesium Kᵢ was substantially decreased for isoproterenol but only minimally...
TABLE 1. Substrate and Mg\textsuperscript{2+} Kinetic Data for Adenylate Cyclase Activity in Membrane Fractions Derived From Nonfailing and Failing Human Left Ventricular Myocardium

<table>
<thead>
<tr>
<th>Mg ATP substrate</th>
<th>(K_m) (mM)</th>
<th>(V_{max}) (pmol/min/mg)</th>
<th>(K_m) (mM)</th>
<th>(V_{max}) (pmol/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonfailing (n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H\textsubscript{2}O blank</td>
<td>0.028±0.004</td>
<td>17.3±0.70</td>
<td>2.61±0.15</td>
<td>86.1±1.16</td>
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<tr>
<td>R-PIA 0.1 (\mu)M</td>
<td>0.034±0.002</td>
<td>16.8±0.85</td>
<td>2.32±0.06</td>
<td>70.8±1.54*</td>
</tr>
<tr>
<td>R-PIA 10 (\mu)M</td>
<td>0.055±0.008*</td>
<td>17.0±1.3</td>
<td>1.99±0.09*</td>
<td>53.0±1.72*</td>
</tr>
<tr>
<td>ISO 0.3 (\mu)M</td>
<td>0.037±0.003</td>
<td>38.5±5.1</td>
<td>1.14±0.10*</td>
<td>89.3±3.95</td>
</tr>
<tr>
<td>ISO 0.1 mM</td>
<td>0.052±0.008*</td>
<td>77.1±10.3*</td>
<td>0.51±0.06*</td>
<td>100.9±8.5</td>
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<tr>
<td>Failing (n=3)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H\textsubscript{2}O blank</td>
<td>0.018±0.003</td>
<td>19.3±2.4</td>
<td>2.64±0.33</td>
<td>98.1±13.1</td>
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<td>R-PIA 0.1 (\mu)M</td>
<td>0.033±0.010</td>
<td>18.5±1.91</td>
<td>2.40±0.26</td>
<td>77.8±15.5</td>
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<tr>
<td>R-PIA 10 (\mu)M</td>
<td>0.058±0.012*</td>
<td>17.6±1.5</td>
<td>2.30±0.25</td>
<td>58.6±10.1</td>
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<tr>
<td>ISO 0.3 (\mu)M</td>
<td>0.035±0.006</td>
<td>40.3±4.31*</td>
<td>1.12±0.17*</td>
<td>96.6±10.9</td>
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<tr>
<td>ISO 0.1 mM</td>
<td>0.061±0.008*</td>
<td>91.3±7.0*</td>
<td>0.56±0.05*</td>
<td>116.9±11.0</td>
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<tr>
<td>Failing and nonfailing (n=6)</td>
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</tr>
<tr>
<td>H\textsubscript{2}O blank</td>
<td>0.022±0.003</td>
<td>18.3±1.20</td>
<td>2.62±0.16</td>
<td>92.1±6.48</td>
</tr>
<tr>
<td>R-PIA 0.1 (\mu)M</td>
<td>0.034±0.011</td>
<td>17.7±1.01</td>
<td>2.36±0.12</td>
<td>74.3±7.12</td>
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<tr>
<td>R-PIA 10 (\mu)M</td>
<td>0.056±0.007*</td>
<td>17.3±0.88</td>
<td>2.15±0.14*</td>
<td>55.8±4.76*</td>
</tr>
<tr>
<td>ISO 0.3 (\mu)M</td>
<td>0.036±0.003</td>
<td>39.6±2.99*</td>
<td>1.13±0.09*</td>
<td>92.9±5.43</td>
</tr>
<tr>
<td>ISO 0.1 mM</td>
<td>0.056±0.006*</td>
<td>84.2±6.43*</td>
<td>0.53±0.04*</td>
<td>108.9±7.2</td>
</tr>
</tbody>
</table>

*\(p<0.05\) by analysis of variance compared with H\textsubscript{2}O blank.
All values are given as mean±SEM.
R-PIA, R-phenylisopropyl-adenosine; ISO, isoproterenol.

decreased for R-PIA. In contrast, the magnesium \(V_{max}\) was not changed for isoproterenol but significantly diminished by R-PIA (Table 1).

Summary of adenylate cyclase assay conditions.
Three assay conditions have been used in the present study to examine different components of the receptor–G protein–adenylate cyclase system. The initial conditions described in earlier reports\textsuperscript{1,3,29} were chosen for the maximal \(G_s\)-coupled receptor–stimulated response because they give the greatest degree of stimulation relative to basal activity. In the present study, this condition is called the “stimulatory receptor (\(G_s\)) condition” (Table 2). However, the optimal condition to observe \(G_i\)-coupled receptor inhibition of adenylate cyclase differs from this and is called the “inhibitory receptor (\(G_i\)) condition” (Table 2). We also previously described optimal conditions to examine G protein effects on adenylate cyclase activity,\textsuperscript{18} herein called the “G protein condition” (Table 2), in which the reaction mixture has no added guanine nucleotides.

**A\textsubscript{1}-Adenosine Receptor Characterization:**
R-Phenylisopropyl-Adenosine and N-Ethyl Adenosine-5'-Uronamide Effects on Adenylate Cyclase Activity

The adenosine receptor has been categorized into \(A_1\)- and \(A_2\)-subtypes.\textsuperscript{30,31} In an effort to detect any stimulatory \(A_2\)-adenosine receptors,\textsuperscript{31} the effects of increasing concentration of N-ethyl adenosine-5'-uronamide (NECA), a high-affinity \(A_2\)-adenosine receptor agonist,\textsuperscript{30,31} were examined (Figure 1) with stimulatory \(G_i\) receptor conditions. Adenylate cyclase activity was inhibited with NECA, and the dose–response curve was shifted to the right of an R-PIA dose–response curve by approximately sevenfold, in agreement with the affinity of NECA for the \(A_2\)-adenosine receptor.\textsuperscript{30}

**A\textsubscript{1}-Adenosine- and \(\beta\)-Receptor–Mediated Adenylate Cyclase Responses in Failing and Nonfailing Human Heart**

**A\textsubscript{1}-Adenosine receptor–mediated adenylate cyclase inhibition.** The effects of R-PIA on adenylate cyclase

### Table 2. Summary of Adenylate Cyclase Assay Conditions

<table>
<thead>
<tr>
<th></th>
<th>Stimulatory receptor ((G_s)) assay condition</th>
<th>Inhibitory receptor ((G_i)) assay condition</th>
<th>G protein ((G_i)) assay condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTP ((\mu)M)</td>
<td>10</td>
<td>10</td>
<td>...</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+} ATP (mM)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Mg\textsuperscript{2+} Cl\textsuperscript{2-} (mM)</td>
<td>0.5</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Adenosine deaminase (units/tube)</td>
<td>...</td>
<td>0.3</td>
<td>...</td>
</tr>
<tr>
<td>l-Propranolol ((\mu)M)</td>
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<td>1</td>
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</tbody>
</table>
inhibition were examined in particulate preparations derived from 12 failing and 12 nonfailing hearts with the Gi receptor adenylate cyclase assay condition (Table 2). No differences were present between preparations derived from failing and nonfailing heart for R-PIA-mediated inhibition of adenylate cyclase activity or for ED₅₀'s or slopes (Figure 2). With identical conditions except for 0.5 instead of 2.5 mM Mg²⁺, again no differences were present between preparations from nonfailing and failing heart (Table 3).

β-Receptor adenylate cyclase activation and β-receptor density. β-Receptor activation of adenylate cyclase was examined with assay conditions as previously reported (Gₛ receptor assay condition).¹²⁹ Isoproterenol-mediated adenylate cyclase activity was decreased in preparations derived from failing human ventricular myocardium (Figure 2).

Previous examinations from our laboratory¹²⁹ of β-receptor–activated adenylate cyclase have not used adenosine deaminase; therefore, these earlier studies¹²⁹ were performed with full activation of the A₁-adenosine receptor pathway. We evaluated the effects of adenosine deaminase on isoproterenol-stimulated adenylate cyclase activity. Adenosine deaminase increased basal adenylate cyclase activity and remained additive to the isoproterenol-activated adenylate cyclase activity (Figure 3).

β-Receptor density was decreased in failing (n=12) compared with nonfailing (n=12) left ventricles (45.7±2.85 versus 88.5±7.14 fmol/mg, p<0.001), with no change in receptor affinity (17.9±4.9 versus 13.9±2.8 pM, p=NS) as measured by ¹²⁵Icyp saturation binding curves.²

Examination of Postreceptor (G Protein, Catalytic Adenylate Cyclase) Adenylate Cyclase Activation and Pertussis Toxin–Catalyzed ADP-Ribosylation

Basal adenylate cyclase activity was measured under previously described conditions designed for Gₛ-
couples G protein effects, and \(G_\tau\)-coupled receptors (Table 2). With conditions designed to measure G protein effects, basal adenylate cyclase activity was decreased in preparations of failing heart (Table 4).

Fluoride- (10 mM), forskolin- (0.1 mM), and manganese- (1 mM) stimulated adenylate cyclase activities were not different in failing versus nonfailing heart with \(G_\tau\) receptor conditions (Table 4).

Pertussis toxin-catalyzed ADP-ribosylation was performed in preparations derived from six nonfailing and six failing left ventricles. Pertussis toxin-sensitive autoradiographic bands of 40,000 MW were increased in failing heart (Table 4). These 40,000-MW bands colocalized with a pertussis toxin–sensitive substrate from human erythrocyte and have been shown to comigrate with immunochemically detectable \(\alpha G_\tau\).21

**Discussion**

The present study is the first to examine the coupling of an adenylate cyclase–associated inhibitory receptor pathway in preparations of failing versus nonfailing human ventricular myocardium. In human ventricular myocardium, the \(A_1\)-adenosine receptor pathway is powerfully coupled to inhibition of adenylate cyclase activity, with 34% and 36% inhibition rates of basal activity in failing and nonfailing heart, respectively.

In agreement with one recent report,11 results from the present study suggest that adenosine receptors in human ventricular myocardium appear to be a homogenous population consistent with an \(A_1\)-adenosine receptor subtype known to inhibit adenylate cyclase.30,31 A dose–response curve performed with NECA, an \(A_1\)-adenosine receptor agonist, yielded only adenylate cyclase inhibition and a right-shifted dose–response curve consistent with NECA effects at an \(A_1\)-adenosine receptor.30,31

Experimental conditions were optimized to observe maximal \(A_1\)-adenosine receptor–mediated adenylate cyclase inhibition in particulate preparations.

**Figure 3.** Plots of isoproterenol dose–response curves performed with standard stimulatory G protein assay conditions not in the presence of adenosine deaminase with preparations derived from six nonfailing (○) and six failing (●) hearts. All results are given as nonfailing versus failing: minima, 10.6±1.4 versus 8.4±0.9 pmol cyclic AMP/min/mg; maxima, 47.4±2.1 versus 35.8±3.4 pmol cyclic AMP/min/mg (p<0.05); net (max minus min), 36.8±1.3 versus 27.4±2.6 pmol cyclic AMP/min/mg (p<0.05); \(ED_{50}\) (isoproterenol×10\(^{-7}\) M), 1.84±0.45 versus 3.03±0.73; slope, −0.59±0.03 versus −0.62±0.02. In the same matched experiments, isoproterenol dose–response curves were performed in the presence of adenosine deaminase for nonfailing (○) or failing (●) heart. All results are given as nonfailing versus failing: minima, 21.9±3.1 versus 16.2±1.7 pmol cyclic AMP/min/mg; maxima, 66.0±3.5 versus 48.8±4.8 pmol cyclic AMP/min/mg (p<0.05); net (max minus min), 44.1±1.6 versus 32.2±3.5 pmol cyclic AMP/min/mg; \(ED_{50}\) (isoproterenol×10\(^{-7}\) M), 1.70±0.43 versus 2.22±0.34; slope, −0.66±0.07 versus −0.69±0.05.
of human ventricular myocardium. It was necessary to remove adenosine present in our preparations with adenosine deaminase to observe inhibition by A$_1$-adenosine receptor agonists. Concentrations of Mg (2.5 mM) and guanine nucleotides (GTP 10 μM) were also optimized for maximal adenylate cyclase inhibition. Formal examination of magnesium kinetics for A$_1$-adenosine receptor–mediated adenylate cyclase inhibition suggested that magnesium $V_{\text{max}}$ was decreased with receptor activation.

Results from the present study indicate that the A$_1$-adenosine receptor pathway was not altered in failing human heart. In particular preparations derived from 12-end stage failing left ventricles exploited at the time of cardiac transplantation, adenylate cyclase inhibition mediated by the A$_1$-adenosine receptor agonist R-PIA was not different compared with that in control preparations from nonfailing left ventricles. The respective ED$_{50}$S and slopes of mean dose–response curves were also similar. In contrast, maximal isoproterenol-stimulated adenylate cyclase activity and total β-adrenergic receptor density were decreased in failing heart, in agreement with previous reports.\textsuperscript{1,2,30}

Perhaps the most interesting observation of the present study is the lack of differential inhibition of adenylate cyclase activity in failing versus nonfailing heart by the A$_1$-adenosine receptor pathway. In view of the recently described 30–40% increases in a 40,000-MW pertussis toxin–sensitive substrate termed αG$_{G_{0}}$ in failing human heart,\textsuperscript{18–20} subsequently demonstrated to be immunoreactive to antiserum for αG$_{G_{0}}$,\textsuperscript{21} it might be expected that A$_1$-adenosine receptor–mediated inhibition of adenylate cyclase would be accentuated in failing heart. In our earlier report,\textsuperscript{18} adenylate cyclase activity was decreased in failing human heart when guanine nucleotide was not present in the assay (termed "G protein assay condition" in the present study). In the present study with the same (no guanine nucleotide) assay condition, decreased adenylate cyclase activity was also observed in failing heart. In addition, a 40,000-MW pertussis toxin–sensitive substrate was increased by approximately 30%, also in agreement with our previous report.\textsuperscript{18} Therefore, both ADP-ribosylation and adenylate cyclase data support the contention that an inhibitory G protein had increased activity in failing human heart.

However, with assay conditions carefully designed to observe maximal receptor-mediated adenylate cyclase inhibition, in the present study termed "inhibitory receptor (G) assay condition," the A$_1$-adenosine receptor pathway in failing human heart was not different from that in nonfailing controls. With a modified assay condition (0.5 mM MgCl$_2$) similar to the G$_i$ condition, again no difference was observed. In summary, even though αG$_{i}$ was increased in failing human ventricular myocardium, the G$_i$-coupled A$_1$-adenosine receptor pathway did not show enhanced inhibition of adenylate cyclase under two different experimental conditions.

With increased G$_i$ activity identified by pertussis toxin–sensitive ADP-ribosylation, the most straightforward explanation for a lack of increased adenylate cyclase inhibition by the A$_1$-adenosine receptor pathway is that the species of increased G$_i$ may couple poorly to the A$_1$-adenosine receptor, and therefore enhanced A$_1$-adenosine responses will not be observed.\textsuperscript{32} An alternative explanation is that the decreased A$_1$-adenosine receptor density combined with increased G$_i$ in failing heart may have functionally canceled one another, a possibility that awaits

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<th>Table 4. Summary of Postreceptor Adenylate Cyclase Activity Under Three Conditions and Pertussis Toxin–Catalyzed ADP-Ribosylation</th>
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<tr>
<td>Nonfailing LVs</td>
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<td>---------------------------------------------------------------</td>
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<tr>
<td><strong>Adenylate cyclase activity</strong></td>
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<tr>
<td>Inhibitory receptor condition (2.5 mM MgCl$_2$, 10 μM GTP, adenosine deaminase, I-propranolol)</td>
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<tr>
<td>Basal (pmol cAMP/min/mg)</td>
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<tr>
<td>10 mM NaF (pmol cAMP/min/mg)</td>
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<tr>
<td>0.1 mM forskolin (pmol cAMP/min/mg)</td>
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<tr>
<td>1 mM Mn$^{2+}$ (pmol cAMP/min/mg)</td>
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<tr>
<td><strong>Stimulatory receptor condition</strong></td>
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<tr>
<td>Basal (pmol cAMP/min/mg)</td>
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<tr>
<td>Basal with adenosine deaminase (pmol cAMP/min/mg)</td>
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<tr>
<td><strong>G protein condition</strong> (0 GTP, 0.5 mM MgCl$_2$, 1 μM I-propranolol)</td>
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<tr>
<td>Basal (pmol cAMP/min/mg)</td>
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<tr>
<td><strong>Pertussis toxin–catalyzed ADP-ribosylation</strong></td>
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<tr>
<td>Optical density (arbitrary units)</td>
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<td>LV, left ventricle.</td>
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</tbody>
</table>
adenosine receptor density measurements. Although radiolabeled ligands are available that have a high affinity and adequate activity in enriched membranes prepared in large quantities of bovine heart, we have not been able to reliably and reproducibly measure A1-adenosine receptor binding in crude membrane preparations available from human heart.

Another possibility is that the presence of 10 mM GTP might shift the ratio of $\alpha G_i \cdot \beta \cdot \gamma$ heterotrimer to dissociated $\alpha G_i$ in the cardiac membranes. If both the increased functional capacity of $\alpha G_i$ and the increased pertussis toxin-sensitive substrate in the failing human heart resulted from a greater proportion of $\alpha G_i$ being in the bound than in the dissociated state, then the addition of 10 $\mu$M exogenous GTP to membranes derived from both control and failing heart might shift the dissociation kinetics so that no difference could be seen between the two groups. Finally, the A1-adenosine receptor has demonstrated receptor reserve, which if present in the present study could make it difficult to identify alterations in receptor-coupled adenylate cyclase inhibition.

The present study raises one additional question about previous research with $G_i$-coupled receptors in myocardial preparations. Results from the present study indicate that unless adenosine deaminase is added to the adenylate cyclase reaction mixture, the A1-adenosine receptor is fully activated by adenosine present in the myocardial preparations. We previously reported that $\beta$-receptor-stimulated adenylate cyclase activity is subsensitive in failing heart. In these reports, adenosine deaminase was not used, and therefore the A1-adenosine receptor pathway was fully activated. To examine the A1-adenosine receptor effects on $\beta$-adrenergic receptor adenylate cyclase activation, isoproterenol dose–response curves were performed in the presence and absence of adenosine deaminase. The two sets of isoproterenol dose–response curves were similar, except that the increased basal adenylate cyclase activity noted in the presence of adenosine deaminase appeared to be additive to the isoproterenol responses obtained without adenosine deaminase (Figure 3). In addition, the mean isoproterenol dose–response curve performed in failing heart in the presence of adenosine deaminase exceeded the response in nonfailing heart without adenosine deaminase. This suggests that adenosine antagonism might increase some of the $\beta$-adrenergic receptor–mediated subsensitivity present in failing human heart.

In summary, adenylate cyclase activity in preparations of human ventricular myocardium is substantially inhibited by A1-adenosine receptor pathway activation. Although functional changes in adenylate cyclase activation are present, consistent with increased $G_i$ in failing human heart, no A1-adenosine receptor–coupled difference was observed in failing heart.

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KEY WORDS • R-phenylisopropyl-adenosine • heart failure • purinergic receptors • adenylate cyclase
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