Characterization of A1 Adenosine Receptors in Atrial and Ventricular Myocardium From Diseased Human Hearts

Michael Böhm, Burkert Pieske, Martin Ungerer, and Erland Erdmann

The purpose of the present study was to characterize adenosine receptors in human atrial and ventricular myocardium. In isolated electrically driven preparations, adenosine produced "direct" negative inotropic effects in atrial myocardium (AT). In ventricular myocardium (VE), it only had negative inotropic properties when force of contraction had been stimulated with isoprenaline ("indirect" effect), but it has no inotropic effect alone. The adenosine receptor antagonist 8-phenyltheophylline antagonized the "direct" and "indirect" effects; these findings indicated that both effects were mediated by adenosine receptors. In cardiac membranes from human AT and VE, adenosine receptors were characterized with [3H]-8-cyclopentyl-1,3-dipropylxanthine (DPCPX) binding. The effects of agonists R-(-)-N*-phenylisopropyladenosine (R-PIA), S-(+)-N*-phenylisopropyladenosine (S-PIA), and S'-(N-ethylcarboxamido)adenosine (NECA) and the effects of guanine nucleotides [Gpp(NH)p] were studied also. The antagonist affinities as judged from the apparent affinity, $K_i$, of [3H]DPCPX were similar in AT (2.2 nmol/l; 95% confidence limits, 1.4-3.7) and VE (1.8 nmol/l; 95% confidence limits, 1.0-3.0). The number of adenosine receptors was 1.7 times greater in AT (26.9±2.3 fmol/mg protein; n=5) than in VE (16.2±2.3 fmol/mg protein; n=5). High and low affinity states of adenosine receptors evaluated with the influence of Gpp(NH)p on agonist competition with R-PIA were similar in AT or VE. The rank orders of potency for agonists (R-PIA>S-PIA>NECA) and antagonists (DPCPX>8-phenyltheophylline>theophylline) were characteristic for the A1 receptor subtype. It is concluded that A1 adenosine receptors exist in the human myocardium. Since binding properties were similar in AT and VE, the same A1 adenosine receptor probably couples to different effectors in a similar guanine nucleotide-dependent way. [3H]DPCPX is the first radiolabeled antagonist ligand that allows detection of A1 adenosine receptors and their coupling in the human myocardium. (Circulation Research 1989;65:1201-1211)
protein G\textsubscript{1}. Since adenosine receptors couple via a pertussis toxin-sensitive G-protein to the adenylate cyclase,\textsuperscript{18} effects on force of contraction could be augmented in heart failure and could play an important role in the diminished cAMP formation\textsuperscript{22} and reduced positive inotropic effects of \textbeta-adrenoceptor agonists.\textsuperscript{23} However, data on the characterization or the coupling of adenosine receptors in the human heart are lacking completely.

The present study was designed to characterize human myocardial adenosine receptors. Therefore, "direct" and "indirect" effects of adenosine and the adenosine receptor agonist \textit{R}(-)-N\textsuperscript{6}-phenylisopropyladenosine (R-PIA) were studied in atrial and ventricular preparations from the human myocardium. Radioligand binding experiments were performed using [\textit{3H}]-8-cyclopentyl-1,3-dipropylxanthine (DPCPX), a novel 1,3-dipropyl-8-phenylxanthine derivative that has increased potency and selectivity at A\textsubscript{1} adenosine receptors\textsuperscript{24-25} and, hence, allows detection in tissues with a low density of adenosine receptors. Coupling of adenosine receptors to G-proteins was studied using guanine nucleotides on agonist-binding properties. Herein, we report that human myocardial adenosine receptors mediate "direct" and "indirect" effects on human atrial and ventricular tissue. The rank order of potency for agonists and antagonists in both tissues was compatible with the A\textsubscript{1} adenosine receptor subtype.\textsuperscript{26} The effect of guanine nucleotides was similar in atrial and ventricular myocardium. Therefore, it must be assumed that the same adenosine receptors couple by similar guanine nucleotide-dependent mechanisms to different targets beyond receptor occupation in atrial and ventricular human myocardium, that is, cardiac potassium channels and/or adenylate cyclase.

Materials and Methods

\textbf{Myocardial Tissue}

All experiments were performed on cell membrane preparations from human atrial and ventricular myocardium or on isolated electrically stimulated human right atrial or left ventricular papillary muscle strips. Tissue was obtained during either mitral valve replacement or heart transplantation. Patients had heart failure clinically classified as New York Heart Association (NYHA) II–III on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before operation (\textit{n}=10; three patients with predominant mitral valve stenosis, the other seven with combined mitral valve disease). Patients suffering from heart failure class NYHA IV (\textit{n}=7; all cardiac transplant recipients) were studied for comparison. The underlying disease was dilated cardiomyopathy (\textit{n}=5). One patient was transplanted because of cardiomyopathy due to longstanding decompensated aortic stenosis. All patients gave written informed consent before surgery. Medical therapy consisted of diuretics, nitrates, and cardiac glycosides in patients with NYHA II–III and NYHA IV heart failure. Catecholamines were not applied. All patients with NYHA IV heart failure also received enalapril. All patients gave informed written consent before the operation.

\textbf{Force of Contraction}

The experiments were performed on electrically driven (1 Hz) papillary muscle strips. Papillary muscle strips of uniform size (diameter<1.0 mm; length, 3–6 mm) were dissected in aerated bathing solution (see composition below) at room temperature. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75-ml glass tissue chambers for recording isometric contractions. The bathing solution was a modified Tyrode's solution containing (mmol/l) NaCl 119.8, KCl 5.4, CaCl\textsubscript{2} 1.8, MgCl\textsubscript{2} 1.05, NaH\textsubscript{2}PO\textsubscript{4} 0.42, NaHCO\textsubscript{3} 22.6, Na\textsubscript{2}EDTA 0.05, ascorbic acid 0.28, and glucose 5.0. It was continuously gassed with 95% O\textsubscript{2}-5% CO\textsubscript{2} and maintained at 35° C; the pH was 7.4. The force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Helco scriptor (Hellige, Freiburg, FRG) or a recorder (Gould Instruments, Cleveland, Ohio). Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 5 mN) was kept constant throughout the experiment. The preparations were electrically paced at 1 Hz with rectangular pulses of 5 msec (stimulator, model SD 9, Grass Instruments, Quincy, Massachusetts); the voltage was about 20% above threshold. All preparations were allowed to equilibrate in drug-free bathing solution until complete mechanical stabilization.

All compounds were freshly dissolved in prewarmed and preaerated bathing solution. Stock solutions of 8-phenyltheophylline (8-Phe-Theo) were prepared in 20% NaOH and 80% methanol. At 30 \textmu mol/l, 8-Phe-Theo had no effect on force of contraction, but higher concentrations produced negative inotropic effects. Concentration-dependent mechanical effects were obtained by cumulative application of adenosine or R-PIA; in experiments with 8-Phe-Theo, the preparations were preexposed to the antagonist for 30 minutes.

\textbf{Radioligand Binding Experiments}

\textbf{Membrane preparation.} Myocardial tissue was chilled in 30 ml ice-cold homogenization buffer containing 10 mmol/l Tris-HCl, 1 mmol/l EDTA, and 1 mmol/l dithiothreitol at pH 7.4. Connective tissue was trimmed away, and myocardial tissue was minced with scissors and homogenized with a motor-driven glass-Teflon potter for 1 minute. Afterwards, the crude membrane preparation was homogenized by hand for 1 minute with a glass-glass potter. The homogenate was spun at 10,000 rpm for 10 minutes. The supernatant was filtered through two layers of gauze and used in the experiments. The pellet was discarded. This homogenate was diluted with an equal volume of ice-cold 1 mmol/l...
potassium chloride and stored on ice for 10 minutes. The supernatant was centrifuged at 100,000g for 30 minutes. The pellet was resuspended in 50 vol incubation buffer containing 50 mmol/l Tris-HCl and 10 mmol/l MgCl₂ at pH 7.4 and homogenized for 1 minute with a glass-glass Potter. This suspension was recentrifuged at 100,000g for 45 minutes. The pellet was resuspended in incubation buffer (50 vol). In all experiments 0.2 µg/ml adenosine deaminase was present. Membranes were preincubated and stirred for 40 minutes at 4°C with the enzyme. Adenosine deaminase at this concentration was capable of abolishing the “direct” negative inotropic effect of 1,000 µmol/l adenosine in isolated atrial preparations. Myocardial A₁ receptors were measured using [³H]DPCPX as radiolabeled ligand (specific activity 110 Ci/mmol). Membranes were incubated with different concentrations of [³H]DPCPX (0.05–12.0 nmol/l). The assay was performed in a total volume of 250 µl incubation buffer. The incubation was carried out at 22°C for 120 minutes. These conditions allowed complete equilibration of the receptors with the radioligand. Moreover, some kinetic studies were performed at 37°C. The reaction was terminated by rapid vacuum filtration through GF/C filters (Whatman, Clifton, New Jersey); the filters were washed immediately three times with 6 ml ice-cold incubation buffer. Before filtration, filters were presoaked in incubation buffer with 0.1% 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) to reduce nonspecific binding. All experiments were performed in triplicate. Filters were dried at 90°C and placed in 10 ml scintillation fluid (Quickzint 501, Zinsser Analytics, Frankfurt, FRG), and radioactivity was determined in a liquid scintillation counter. Specific binding was defined as the difference of binding in the absence and the presence of 1 mmol/l theophylline or 10 µmol/l R-PIA. Nonspecific binding was similar with the antagonist 8-Phe-Theo. In atrial myocardium than in ventricular tissue. Adenosine and isoprenaline were more pronounced in atrial myocardium. The negative inotropic effects of the agonist R-PIA alone and in the presence of isoprenaline, adenosine and R-PIA alone produced negative inotropic effects. However, in ventricular tissue, no effect of either compound was observed. In the presence of isoprenaline, adenosine and R-PIA produced negative inotropic effects in atrial and ventricular myocardium. The negative inotropic effect of the purine derivatives in the presence of isoprenaline were more pronounced in atrial myocardium than in ventricular tissue. Adenosine and R-PIA alone produced negative inotropic effects in atrial tissue; in the ventricle, an increase of force of contraction and cellular cAMP levels with isoprenaline was a prerequisite for adenosine and R-PIA to elicit negative inotropic effects. Therefore, the effect of adenosine analogues are termed “direct” in atrial myocardium and “indirect” in ventricular myocardium.

Materials. Adenosine and CHAPS were from Sigma (Weisenhofen, FRG). R-PIA, 5'-[+]-N⁶-phenylisopropyladenosine (S-PIA), 5'-[N-ethylcarboxamido]adenosine (NECA), and guanylylimidodiphosphate (Gpp(NH)p) were purchased from Boehringer (Mannheim, FRG). Theophylline was from Serva (Heidelberg, FRG), and 8-Phe-Theo was from Calbiochem (La Jolla, California). Adenosine deaminase from calf intestine was from Boehringer. Unlabeled DPCPX was provided by Dr. Karl-Nober Klotz (Institute of Pharmacology, University of Heidelberg, FRG). All other compounds were of analytical or best grade commercially available. Only deionized and twice-distilled water was used throughout.

Statistics

The data shown are mean±SEM. Statistical significance was estimated with Student’s t test for unpaired observations and analysis of variance. A value of p<0.05 was considered significant. Kᵣ values and the drug concentration producing 50% of the maximal effect (EC₅₀) were determined graphically in each individual experiment. The Kᵣ and EC₅₀ values are given with 95% confidence limits. Binding data of agonist competition curves were analyzed by the computer-modeling method of De Lean et al.⁷ One-state and two-state fits were tested for improvement of the fit by an F test. The one-state or two-state model was judged to be appropriate when it proved to be significantly better (p<0.0001) than the preceding one. To further characterize A₁ adenosine receptors in atrial and ventricular myocardium and to assess interaction of the antagonist 8-Phe-Theo at these sites, the pA₂ (competitive antagonist affinity constant) values were calculated according to the equation: pA₂=log(DR)-logA, where DR is the agonist dose ratio (+/- antagonist) and A is the molar concentration of the antagonist.²⁹,³⁰

Results

Force of Contraction

Original tracings in Figure 1 demonstrate the effects of the adenosine receptor agonist R-PIA and the parent compound adenosine on force of contraction in isolated electrically driven preparations from human atrial and ventricular myocardium. In atrial myocardium, adenosine and R-PIA produced negative inotropic effects. However, in ventricular tissue, no effect of either compound was observed. In the presence of isoprenaline, adenosine and R-PIA produced negative inotropic effects in atrial and ventricular myocardium. The negative inotropic effect of the purine derivatives in the presence of isoprenaline were more pronounced in atrial myocardium than in ventricular tissue. Adenosine and R-PIA alone produced negative inotropic effects in atrial tissue; in the ventricle, an increase of force of contraction and cellular cAMP levels with isoprenaline was a prerequisite for adenosine and R-PIA to elicit negative inotropic effects. Therefore, the effect of adenosine analogues are termed “direct” in atrial myocardium and “indirect” in ventricular myocardium.

To study whether “direct” and “indirect” effects of R-PIA were mediated by adenosine receptors, concentration-response curves were measured for the effects of the agonist R-PIA alone and in the presence of 1 µmol/l or 30 µmol/l of the adenosine receptor antagonist 8-Phe-Theo. In atrial myocardium (Figure 2a), R-PIA reduced force of contraction with a maximal effect at 10 µmol/l in a
concentration-dependent manner. The effect amounted to 28±7% of the value before application of R-PIA. The EC₅₀ value was 0.09 µmol/l (95% confidence limits, 0.05–0.16; n=9). In the presence of 8-Phe-Theo, there was a parallel shift of the concentration-response curve to the right. The pD₂ values of these experiments are listed in Table 1.

In ventricular myocardium, R-PIA did not reduce force of contraction (not shown) but exerted concentration-dependent negative inotropic effects in the presence of isoprenaline (Figure 2b). This “indirect” negative inotropic effect was maximal at 100 µmol/l and amounted to 56±3% of the value before addition of R-PIA. The EC₅₀ value was 0.46 µmol/l (95% confidence limits, 0.14–1.51; n=7). As in atrial myocardium, there was a concentration-dependent right shift of the concentration-response curve by the adenosine receptor antagonist 8-Phe-Theo. The pD₂ (−logEC₅₀) values are listed in Table 1.

When pA₂ values for the shifts of the concentration-response curves of R-PIA by 1 µmol/l or 30 µmol/l 8-Phe-Theo were calculated according to Arunlakshana and Schild,²⁹ there was a good agreement of the values in atrial and ventricular myocardium (Table 1). Moreover, the slope of the Schild plot roughly estimated from two antagonist-competition curves was 1.45 in atria and 1.36 in ventricular myocardium. This indicates that only one class of receptors is involved in both tissues.

Radioligand Binding Experiments:
Binding of [³H]DPCPX

A typical saturation experiment is shown in Figure 3. [³H]DPCPX bound in a concentration-dependent manner to ventricular cardiac mem-

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FIGURE 1. Original tracings illustrating force of contraction of isolated electrically driven atrial trabeculae (human atrial myocardium) and papillary muscle strips (ventricular myocardium) after application of 1,000 µmol/l adenosine (Ad) or 100 µmol/l R-(−)-N⁶-phenylisopropyladenosine (R-PIA) alone (panel A) or in the presence of 0.03 µmol/l isoprenaline (Iso) plus R-PIA (panel B). Note that Ad and R-PIA alone reduced force of contraction in atrial but not ventricular myocardium. In the presence of Iso, Ad and R-PIA exerted negative inotropic effects in both tissues.
branes of a 49-year-old male patient with ischemic cardiomyopathy who underwent cardiac transplantation. Specific binding was monophasic and clearly saturable. Linearization with the Scatchard plot revealed one class of binding sites. There was no improvement in the goodness of fit when data were analyzed with a two-state model according to De Leen et al.27 Table 2 summarizes the ages, diagnoses, and binding parameters of the hearts from different patients. The $B_{\text{max}}$ values were higher in atrial myocardium (range, 20–34 fmol/mg protein) than in ventricular myocardium (range, 10.2–24.6 fmol/mg protein). To exclude differences in the contamination by nonmyocardial cell membranes, $B_{\text{max}}$ values were related to $[^3\text{H}]$ouabain bound. Essentially the same results were obtained (cf. Table 2). The antagonist affinity of $[^3\text{H}]$DPCPX as judged from the $K_d$ values was similar (atrial myocardium, 1.2–3.2 μmol/l). Nonspecific binding was about 35% at $K_d$ and was similar when the antagonist theophylline (1,000 μmol/l) or the agonist R-PIA (100 μmol/l) was used. Magnesium chloride (0.1–100 μmol/l) or the guanine nucleotides GTP (1–100 μmol/l) or Gpp(NH)p (0.1–100 μmol/l) did not influence antagonist binding of $[^3\text{H}]$DPCPX.

### Kinetic studies.

Figure 4 demonstrates time dependence of association and dissociation of binding of $[^3\text{H}]$DPCPX to cardiac ventricular membranes at 25° C. Association and dissociation were rapid and revealed a $t_{1/2}$ (half-life) of 7.6±1.0 minutes for

### Table 1. Values of $pD_2$ and $pA_2$ Showing the Effect of R-(-)-N$^\text{a}$-Phenylnisopropyladenosine Alone or in the Presence of 8-Phenyltheophylline on Isometric Force of Contraction in Isolated Electrically Driven Preparations From Human Atrial Myocardium or Human Ventricular Myocardium

<table>
<thead>
<tr>
<th></th>
<th>Atrial myocardium</th>
<th>Ventricular myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$pD_2$ value</td>
<td>$pA_2$ value</td>
</tr>
<tr>
<td>R-PIA</td>
<td>7.04±0.11*</td>
<td>...</td>
</tr>
<tr>
<td>R-PIA+8-Phe-Theo</td>
<td>6.67±0.16†</td>
<td>6.13</td>
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<tr>
<td>(1 μmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-PIA+8-Phe-Theo</td>
<td>6.09±0.11†</td>
<td>5.42</td>
</tr>
<tr>
<td>(30 μmol/l)</td>
<td></td>
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</table>

Values are mean±SEM. $pD_2$, $-\log EC_{50}$; $pA_2$, competitive antagonist affinity constant; R-PIA, R-(-)-N$^\text{a}$-phenylisopropyladenosine; 8-Phe-Theo, 8-phenyltheophylline. Myocardial adenosine receptors mediate "direct" effects on atrial myocardium and "indirect" effects on ventricular myocardium.

* $p<0.05$ vs. R-PIA plus 8-Phe-Theo (1 μmol/l).
† $p<0.05$ vs. R-PIA.
**A1 RECEPTORS IN HUMAN VENTRICULAR MYOCARDIUM**

- Ischemic Cardiomyopathy
- $B_{\text{max}} = 16.1 \text{ fmol/mg protein}$
- $K_D = 2.0 \text{ nmol/l}$

**Figure 3.** Graph showing binding of [$^3$H]-8-cyclopentyl-1,3-dipropylxanthine ([$^3$H]-DPCPX) to cardiac membranes of a 49-year-old patient with ischemic cardiomyopathy. Inset: Linear transformation. [$^3$H]-DPCPX (fmol) bound per milligram protein was plotted as a function of the ratio of bound-to-free (B/F×10$^{-6}$ [$^3$H]-DPCPX). The intercept with the abscissa is the maximal number of binding sites ($B_{\text{max}}$); the slope is the apparent affinity ($K_D$). Data points are the mean of triplicate observations. Note that binding is monophasic and represents one class of binding sites.

**Agonist and antagonist competition experiments.**

**Table 2. Age, Diagnosis, and Binding Data of Patients Undergoing Cardiac Surgery**

<table>
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<tr>
<th>Patient</th>
<th>Age (yr)/sex</th>
<th>Diagnosis</th>
<th>$B_{\text{max}}$ (fmol/mg protein)</th>
<th>$B_{\text{max}}$ (fmol/pmol [H]-ouabain)</th>
<th>$K_D$ (nmol/l)</th>
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</thead>
<tbody>
<tr>
<td><strong>Atrial myocardium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.E.</td>
<td>37/M</td>
<td>DCM, AS</td>
<td>28.6</td>
<td>5.6</td>
<td>3.2</td>
</tr>
<tr>
<td>D.B.</td>
<td>49/F</td>
<td>DCM</td>
<td>26.0</td>
<td>5.2</td>
<td>2.2</td>
</tr>
<tr>
<td>G.W.</td>
<td>51/M</td>
<td>DCM, CHD</td>
<td>20</td>
<td>5.0</td>
<td>2.1</td>
</tr>
<tr>
<td>J.U.</td>
<td>58/M</td>
<td>DCM</td>
<td>34</td>
<td>7.9</td>
<td>3.1</td>
</tr>
<tr>
<td>D.S.</td>
<td>50/M</td>
<td>CHD</td>
<td>26</td>
<td>6.3</td>
<td>1.2</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>49±3.4</td>
<td></td>
<td>26.9±2.3*</td>
<td>6.0±0.5*</td>
<td>2.2 (1.4–3.0)</td>
</tr>
</tbody>
</table>

| **Ventricular myocardium** | | | | | |
| F.E.    | 37/M        | DCM, AS   | 15.3                                | 3.0                                      | 3.2          |
| D.S.    | 49/M        | CHD       | 16.1                                | 3.9                                      | 2.0          |
| S.U.    | 22/M        | DCM       | 21.5                                | 3.1                                      | 1.5          |
| D.P.    | 41/M        | CHD       | 10.2                                | 2.5                                      | 1.5          |
| K.W.    | 49/M        | DCM       | 14.9                                | 2.1                                      | 1.8          |
| Mean±SEM | 39.6±5.0   |           | 15.6±1.6                            | 2.9±0.4                                  | 1.8 (0.1–3.0) |

$B_{\text{max}}$, maximal density; $K_D$, apparent affinity of binding sites; DCM, dilated cardiomyopathy; CHD, coronary heart disease; AS, aortic stenosis. For $K_D$ geometric means are shown with 95% confidence limits in parentheses.

*p<0.05 vs. ventricular myocardium.
monophasic and steeper and reflects competition at one site. The data for the antagonists DPCPX, 8-Phe-Theo, and theophylline are summarized in Table 3. The slope factor (pseudo-Hill coefficient), reflecting the more shallow curves for the agonist, was significantly smaller for agonists than for antagonists in atrial and ventricular myocardium. Analysis of the biphasic agonist competition curve identified similar proportions of the high and low affinity states with the different agonists. Moreover, the proportion of high affinity states was similar in atrial and ventricular myocardium and amounted to 61.3% in atrial and to 63.0% in ventricular myocardium. The rank order of potency for agonists was R-PIA>S-PIA>NECA in atrial and ventricular myocardium. The KH values (affinity constant of the receptors in the high affinity state) did not differ in both parts of the heart. Antagonist competition showed a potency order of DPCPX>8-Phe-Theo>theophylline.

Interactions of A1-Adenosine Receptors With G-Proteins

Interactions of receptors with G-proteins can be studied by the influence of guanine nucleotides on agonist binding. Figure 6 shows competition of R-PIA to [3H]DPCPX binding in atrial myocardial membranes. Without the guanine nucleotide Gpp(NH)p, there was a biphasic curve with a high and low affinity component. In the presence of Gpp(NH)p, the curve shifts to the right and becomes monophasic with a pseudo-Hill coefficient of 1.02±0.2. As judged from ratio of the IC50 values of R-PIA alone and R-PIA in the presence of Gpp(NH)p, the shifts were similar in atrial and ventricular myocardium (compare with Table 3). Gpp(NH)p influenced agonist competition in atrial and ventricular myocardium but not the binding of the antagonist [3H]DPCPX to cardiac membranes.

**Figure 4.** Graph showing time dependence of [3H]-8-cyclopentyl-1,3-dipropylxanthine ([3H]DPCPX) binding and its reversibility in cardiac ventricular membranes at 25° C. The association reaction was started with membranes and terminated by rapid vacuum filtration at the indicated time points. The dissociation reaction was started by addition of 1 mmol/l theophylline and also terminated by vacuum filtration. Inset A shows the pseudo first-order plot for the association, and inset B shows the first-order plot for the dissociation. B, [3H]DPCPX bound; B0, [3H]DPCPX bound at equilibrium. Analysis gave a Koff rate of 0.021 nmol/min and Kaoff rate of 0.035/min.

**Figure 5.** Graphs showing competition for [3H]-8-cyclopentyl-1,3-dipropylxanthine ([3H]DPCPX) binding to membranes from human atrial myocardium (panel A) and human ventricular myocardium (panel B) by R-(-)-N6-phenylisopropyladenosine (R-PIA) and 8-phenyltheophylline. The concentration of [3H]DPCPX was 2 nmol/l. Data are means of triplicate determinations in four different atria or ventricles. The ordinate represents specific binding (% of maximal [3H]DPCPX bound. The abscissa represents concentrations of R-PIA or 8-phenyltheophylline (nmol/l). Note that the competition curve of the agonist R-PIA was biphasic and shallower than the competition curve with the antagonist 8-phenyltheophylline, which was monophasic.
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TABLE 3. Displacement of [3H]-8-Cyclopentyl-1,3-dipropylxanthine Binding With R-(-)-N\(^{\text{a}}\)-Phenylisopropyladenosine Alone and in the Presence of Guanylylimidodiphosphate, S- (±)-N\(^{\text{a}}\)-Phenylisopropyladenosine, and 5'- (N-Ethylcarboxamido)adenosine as Agonists and 8-cyclopentyl-1,3-dipropylxanthine, 8-Phenylethylphosphine, and Theophylline as Antagonists in Membranes From Human Atrial and Ventricular Myocardium

<table>
<thead>
<tr>
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<th>One-site fit</th>
<th>Two-site fit</th>
<th>Slope factor ((nM))</th>
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<td></td>
<td>(K_r (\text{nmol/l}))</td>
<td>(R_h (%))</td>
<td>(K_{H} (\text{nmol/l}))</td>
</tr>
<tr>
<td>Atrial myocardium</td>
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<tr>
<td>R-PIA</td>
<td>56.0±1.7</td>
<td>0.75 (0.3-2)</td>
<td>89.8 (47-168)</td>
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<tr>
<td>R-PIA+Gpp(NH)p</td>
<td>286 (146-557)</td>
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<tr>
<td>S-PIA</td>
<td>63.4±5.1</td>
<td>3.75 (2.06-6.5)</td>
<td>344 (336-460)</td>
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<td>NECA</td>
<td>64.5±4.3</td>
<td>6.81 (2.75-16.8)</td>
<td>493 (372-654)</td>
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<td>DPCPX</td>
<td>0.36 (0.12–0.94)</td>
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<td>. . .</td>
</tr>
<tr>
<td>8-Phe-Theo</td>
<td>100 (36-207)</td>
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<td>. . .</td>
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<tr>
<td>Theo</td>
<td>4,070 (2,020–7,300)</td>
<td>. . .</td>
<td>. . .</td>
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<tr>
<td>Ventricular myocardium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-PIA</td>
<td>61.6±2.2</td>
<td>0.51 (0.25–0.9)</td>
<td>49 (35-49)</td>
</tr>
<tr>
<td>R-PIA+Gpp(NH)p</td>
<td>130 (56-336)</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>S-PIA</td>
<td>59.4±9.7</td>
<td>2.89 (0.63-12.6)</td>
<td>179 (101-317)</td>
</tr>
<tr>
<td>NECA</td>
<td>72±1.9</td>
<td>6.16 (4.08-9.3)</td>
<td>217 (68-690)</td>
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<tr>
<td>DPCPX</td>
<td>0.41 (0.33–0.51)</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>8-Phe-Theo</td>
<td>183 (46-729)</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Theo</td>
<td>9,000 (2,100–63,000)</td>
<td>. . .</td>
<td>. . .</td>
</tr>
</tbody>
</table>

\(K_r\): dissociation constant of receptor-inhibitor complex; \(R_h\): receptors in the high affinity state; \(K_{H}\): dissociation constant for high affinity site; \(n_H\): pseudo Hill coefficient; \(R-PIA, R-(-)-N^{a}-\text{Phenylisopropyladenosine; Gpp(NH)p, guanylylimidodiphosphate; S-PIA, S-(±)-N^{a}-\text{Phenylisopropyladenosine; NECA, 5'-N-Ethylcarboxamido} \text{adenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; 8-Phe-Theo, 8-phenylethylphosphine; Theo, theophylline. For \(K_{H}\) and \(K_I\), geometric means are shown with 95% confidence limits in parentheses. RH values are given as mean±SEM of four to five experiments with triplicate determinations.}

\(K_h\): dissociation constant of receptor-inhibitor complex; \(R_h\): receptors in the high affinity state; \(K_{H}\): dissociation constant for high affinity site; \(n_H\): pseudo Hill coefficient; \(R-PIA, R-(-)-N^{a}-\text{Phenylisopropyladenosine; Gpp(NH)p, guanylylimidodiphosphate; S-PIA, S-(±)-N^{a}-\text{Phenylisopropyladenosine; NECA, 5'-N-Ethylcarboxamido} \text{adenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; 8-Phe-Theo, 8-phenylethylphosphine; Theo, theophylline. For \(K_{H}\) and \(K_I\), geometric means are shown with 95% confidence limits in parentheses. RH values are given as mean±SEM of four to five experiments with triplicate determinations.}

Discussion

Adenosine receptors that mediate either stimulatory or inhibitory effects on adenylate cyclase exist in a variety of cell types. According to van Calker et al., inhibitory adenosine receptors are termed \(A_1\) receptors, and adenosine receptors mediating stimulation of the adenylate cyclase are classified as \(A_2\) receptors. In the heart, the situation is different. In ventricular myocardium, adenosine receptors mediated an "indirect" negative inotropic effect that has been attributed to an inhibition of the adenylate cyclase with a concomitant decrease in the cellular cAMP levels. However, other authors reported a lack of inhibition of adenylate cyclase or a failure to reduce cAMP in intact preparations by adenosine. Moreover, in atrial myocardium, adenosine produces "direct" negative inotropic effects without affecting cAMP formation. This "direct" effect has been shown to be due to an activation of a potassium outward current and is more efficacious than the "indirect" effect in ventricular heart muscle. Since the mechanisms of the "direct" and "indirect" effects are different and, moreover, the situation of the mechanism in the ventricle is controversial, adenosine receptors were classified in human atrial and ventricular myocardium with radioligand binding experiments.

The adenosine receptor antagonist [3H]DPCPX is a novel radiolabeled ligand that selectively binds to \(A_1\) receptors with high affinity. Moreover, it possesses a high specific activity and, therefore, allows detection of adenosine receptors in tissues with a low receptor density. With [3H]DPCPX, adenosine receptors could be detected in human myocardium because the density is 1.8 times greater in atrial tissue. Similar data were obtained in rat atria and ventricles by Linden et al. These authors reported a density of 30 fmol/mg protein and 23 fmol/mg protein in atria and ventricles, respectively, with [125I] aminobenzyladenosine. As judged from the \(K_d\) values, the antagonist binding properties of the human atrial and ventricular adenosine receptors are similar. Adenosine and the adenosine receptor agonist R-PIA produce "direct" and "indirect" negative inotropic responses in isolated electrically driven atrial and ventricular preparations. The concentration-response curves were shifted to the right by the adenosine receptor antagonist 8-Phe-Theo similarly in atrial and ventricular myocardium; this shift indicates that both effects are mediated by adenosine receptors. This supports the conclusion that, not only from the biochemical but also from the functional point of view, the same type of adenosine receptor exists in atrial and ventricular myocardium. The effectiveness of R-
PIA was greater in atrial myocardium than in ventricular heart muscle. The explanation for this difference could be the greater density of A1 adenosine receptors in the atrium than in the ventricle. Similar conclusions were drawn from data in the rat myocardium in which similar differences were observed between both parts of the heart. Moreover, a facilitated coupling of the activated G-protein to potassium channels could also play a role in the greater sensitivity of the atrial A1 adenosine receptors to agonists.

The question arises whether these adenosine receptors can be classified as A1 or A2 receptors or none of these subtypes. A1 and A2 receptors can be distinguished with agonist competition experiments since the rank order of potency for agonists is different at both receptor subtypes (R-PIA > 5'- S-NECA at A1 receptors; NECA>R-PIA>5'- S-PIA at A2 receptors). In membranes from atrial and ventricular myocardium, the agonist competed for [3H]DPCPX binding with a rank order of potency characteristic for the A1 receptor subtype. Analysis of the competition curves revealed a high and low affinity state of the adenosine receptor. The properties of high affinity receptors were similar in atrial and ventricular myocardium. As judged from the dissociation constants for the high affinity (Kd) and low affinity (Kd') sites, the affinity of the different agonists at each affinity state of the receptors was similar in atrial and ventricular membranes. The difference between the Kd and the Kd' values is 60-100-fold in atrial and ventricular tissue compared with a factor of 15-30-fold in bovine myocardium. Therefore, the relative number of high affinity sites in the human heart is higher than in the chick heart in which 20% of the receptors were detected in the high affinity state. This difference might be explained by the use of the agonist radioligand [35S]N6-(p-azidobenzy)-adenosine. Moreover, species differences might also play a role. The competition curves for the antagonists were steeper and revealed one class of binding sites. Again, the dissociation constant of the receptor-inhibitor complex (Kd values) are similar in atria and ventricles and are typical for A1 adenosine receptors; of these DPCPX is the most potent antagonist. Taken together, binding characteristics of adenosine receptors of the human heart provide evidence that the same adenosine receptors of the A1 subtype mediate different effects in atrial and ventricular myocardium.

G-proteins play a crucial role in mediating the effects of certain receptors on ionic channels or adenylate cyclase. Pertussis toxin treatment of atrial cells or laboratory animals abolishes the "direct" effects of adenosine. In rat ventricular myocardial cells, Hazeki and Uji reported that pertussis toxin prevented the inhibition of cAMP formation by adenosine. These findings indicate that "direct" and "indirect" effects mediated by adenosine receptors involve G-proteins that are subject to pertussis toxin-catalyzed ADP ribosylation. In atrial and ventricular myocardium, the nonhydrolyzable guanine nucleotide Gpp(NH)p converted the high and low affinity state of the A1 receptor to one low affinity state. Taken together, these findings indicate that atrial tissue and ventricular tissue process the same amount of adenosine receptors in the high affinity state that is converted by Gpp(NH)p to the low affinity state.

Adenosine is released from the heart during pathological conditions such as ischemia, increased cardiac workload, or heart failure. Since adenosine is released from the heart after application of catecholamines, its "indirect" antiadrenergic effect has been suggested to serve as an endogenous feedback modulator to protect the heart from over-stimulation with catecholamines. Moreover, a defective adenosine-mediated feedback inhibition
resulting in an unopposed β-adrenergic stimulation of the heart has been suggested to play a causal role in the pathogenesis of hypertrophic cardiomyopathy in humans.42 "Direct" effects in human atrial19 and "indirect" effects in human ventricular tissue20 were previously observed. However, this is the first direct demonstration of adenosine receptors in the human heart. Since adenosine receptors can be detected in human ventricular tissue, it is not unreasonable to assume that they could serve as modulators of force of contraction in the human heart. Although differences between diseased and nondiseased myocardium cannot be ruled out in this study, the use of the highly selective antagonist radioligand [3H]DPCPX provides a useful tool in detection of alterations in the number or coupling of adenosine receptors in human myocardial diseases.

In summary, the present study shows that adenosine receptors exist in the human heart and mediate "direct" and "indirect" effects on force of contraction. The adenosine receptors in the atrium and in the ventricle are of the A1 adenosine receptor subtype and are coupled to different effectors beyond receptor occupation, such as potassium channels or adenylyl cyclase, in a similar guanine nucleotide-dependent fashion.

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KEY WORDS • human myocardium • adenosine • \( A_1 \) adenosine receptor • G-protein • heart failure
Characterization of A1 adenosine receptors in atrial and ventricular myocardium from diseased human hearts.
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