Regulated Overexpression of the A1-Adenosine Receptor in Mice Results in Adverse but Reversible Changes in Cardiac Morphology and Function

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Background—Both the A1- and A3-adenosine receptors (ARs) have been implicated in mediating the cardioprotective effects of adenosine. Paradoxically, overexpression of both A1-AR and A3-AR is associated with changes in the cardiac phenotype.

Methods and Results—Constitutive A1-AR overexpression caused the development of cardiac dilatation and death within 6 to 12 weeks. These mice developed diminished ventricular function and decreased heart rate. In contrast, when A1-AR expression was delayed until 3 weeks of age, mice remained phenotypically normal at 6 weeks, and >90% of the mice survived at 30 weeks. However, late induction of A1-AR still caused mild cardiomyopathy at older ages (20 weeks) and accelerated cardiac hypertrophy and the development of dilatation after pressure overload. These changes were accompanied by gene expression changes associated with cardiomyopathy and fibrosis and by decreased Akt phosphorylation. Discontinuation of A1-AR induction mitigated cardiac dysfunction and significantly improved survival rate.

Conclusions—These data suggest that robust constitutive myocardial A1-AR overexpression induces a dilated cardiomyopathy, whereas delaying A1-AR expression until adulthood ameliorated but did not eliminate the development of cardiac pathophysiology. Thus, the inducible A1-AR transgenic mouse model provides novel insights into the role of adenosine signaling in heart failure and illustrates the potentially deleterious consequences of selective versus nonselective activation of adenosine-signaling pathways in the heart.

Key Words: heart failure | adenosine | cardiomyopathy | hypertrophy | myocardial contraction | remodeling | receptors

Adenosine is an endogenous purine nucleoside that plays an important role in protecting the heart during stress. In animal models of ischemia, adenosine reduces infarct size,1,2 affords protection from reperfusion injury after prolonged coronary occlusion,3 and facilitates ischemic preconditioning.4 Furthermore, adenosine infusion has been shown to reduce infarct size in patients with a myocardial infarction.5 Because of its pharmacological effects on neurohormone and cytokine activation,6–10 it was hypothesized that adenosine might also affect ventricular remodeling in models of heart failure. Indeed, adenosine reduced cardiac hypertrophy and improved left ventricular (LV) function in mice with transverse aortic constriction.11 In addition, patients with increased muscle adenosine levels due to mutation in at least 1 allele of the adenosine monophosphate deaminase 1 (AMPD1) gene had a longer survival than patients with the wild-type genotype.12–14 Furthermore, in patients with heart failure, increased levels of adenosine were associated with severity of disease.15 These initial studies led investigators to identify the specific adenosine receptor (AR) subtypes that mediated the salutary benefits of adenosine.

Clinical Perspective

The cardiovascular effects of adenosine are modulated by 4 known G-protein–coupled ARs (A1, A2a, A2b, and A3), all of which are expressed in the heart.16 Activation of the A1- and
A1-ARs inhibits adenylyl cyclase and modulates other signaling pathways regulated by Gβγ. By contrast, activation of A2γ-ARs results in coupling to Gβγ proteins and activation of adenylyl cyclase.16–18 Pharmacological studies with receptor-subtype–selective agonists suggested that Aγ- and A2-ARs provide cardioprotection during ischemia and reperfusion,19,20 whereas Aγ-ARs afford protection after ischemia.21–23 Because pharmacological agonists lack selectivity, and because of significant species differences in the pharmacology of ARs,16 recent studies have assessed the role of AR subtypes using selective gene deletion and cardiac-restricted transgenic overexpression.24–27 Although both Aγ- and A2-ARs mediate increased myocardial resistance to ischemia, mice overexpressing modest levels of the Aγ-AR demonstrated a reduction in the response to high doses of catecholamines and an increase in hypertrophy,28 whereas mice with higher levels of A2-AR overexpression did not tolerate myocardial ischemia and had significant atrial arrhythmias.29 Of greater concern was the finding that mice with moderate to high levels of A2-AR overexpression developed a dilated cardiomyopathy.30 These findings raised concerns about the safety of chronic adenosine-AR activation in patients with cardiovascular disease.

Because activation of the Aγ-AR in pregnant mice inhibits cardiac cell proliferation and leads to cardiac hypoplasia,31 we hypothesized that the changes in the cardiac phenotype that result from moderate Aγ-AR overexpression might be due, at least in part, to activation of the Aγ-AR transgene in the early heart tube.32 To test this hypothesis, we created mice with inducible overexpression of the Aγ-AR using a tetracycline-based system in which expression could be regulated throughout cardiac development.33 Surprisingly, both constitutive and controlled overexpression of the Aγ-AR resulted in the development of a reversible dilated cardiomyopathy.

Methods

Transgenic Mouse Generation

The human Aγ-AR cDNA was cloned into a cardiac-specific and inducible controlled vector (TREMHC composed of a modified mouse α-myosin heavy chain (α-MHC) minimal promoter fused with nucleotide binding sites for tetracycline transactivating factor (tTA).33 Aγ-AR transgenic (TG) mice, engineered on an FVB background (PolyGene, Zurich, Switzerland), were crossed with myocardium membranes from 6-week-old mice were incubated with Aγ-AR antibody. C, Myocardial expression of Aγ-AR by radioligand binding. Myocardium membranes from 6-week-old mice were incubated with Aγ-AR radioligand, [3H]DPCPX. Nonspecific binding was measured in the presence of 100 μmol/L nonselective AR agonist, N6-2-phenylisopropyl-adenosine (PIA). Figures are representative of at least 3 independent experiments. WT indicates wild type.

Mouse Langendorff Heart Perfusion

As we described previously,36–38 anesthetized mice were euthanized by cervical dislocation. The abdominal cavity was opened immediately and the heart cooled with ice-cold perfusion fluid. The aorta was cannulated above the aortic valve, and retrograde perfusion was begun with a modified Krebs-Henseleit bicarbonate buffer, pH 7.4, equilibrated with 95% O2/5% CO2 at 37°C. Buffer composition (in mmol/L) was NaCl 113.8, NaHCO3, 22, KCl 4.7, KH2PO4, 1.2, MgSO4, 1.1, CaCl2, 2.0, and glucose 11.0. The hearts were perfused with a Langendorff apparatus and paced at 400 bpm with a Grass stimulator (9 V, 0.5 ms). All hearts were immersed in a water-jacketed organ chamber to maintain a temperature of 37°C. A constant-pressure protocol was used to compare the acute response between wild-type and Aγ-TG mouse hearts. For hemodynamic measurements, a balloon was inserted into the LV, and the balloon volume was adjusted to 8 to 11 mm Hg of LV end-diastolic pressure (LVEDP) for stabilization. Initially, each isolated heart was perfused with a constant pressure of 55 cm H2O for 45 minutes. After stabilization, no further alterations in heart rate, ECG recordings were obtained with PowerLab (ML866) and Bio Amp (ML136; ADInstruments, Colorado Springs, Colo). Briefly, animals were anesthetized with inhalation of isoflurane, placed in a supine position, and restrained. Mouse returned to consciousness within 2 minutes. An ECG was recorded for 2 minutes and analyzed with Chart 5 software (ADInstruments). To normalize the recordings, mice were trained 3 times a day for 7 days before measurement.
ADInstruments). Coronary perfusion pressure was measured at heart level via a fluid-filled pressure transducer. At the end of the protocol, left and right ventricles were flash-frozen with liquid nitrogen.

**Real-Time Quantitative Polymerase Chain Reaction**

Real-time quantitative polymerase chain reaction analysis determined both genomic copies of inserted transgene and transgene expression with the human and mouse conserved A1-AR primer set (see Data Supplement). Briefly, 40 ng of genomic DNA from mouse tail was used to quantify the number of transgenes inserted into the genome. Reverse-transcribed cDNA from myocardium RNA was used to determine the expression of A1-AR, atrial natriuretic peptide (ANP), sarcoplasmic reticulum Ca$^{2+}$ ATPase (SERCA), phospholamban, cFos, early growth response factor-1 (EGR-1), and collagen 1a, 3a and 6a genes with specific primer sets (see Data Supplement).

**Membrane Preparation and A1-AR Receptor Binding Assay**

Radioligand binding of A1-AR in crude cardiac membranes was performed as described previously and detailed in the Data Supplement.

**Immunoblotting and Histopathology of Myocardium**

Immunoblotting of ventricular protein extracts was digitally detected with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, Neb) as described previously and detailed in the Data Supplement. Picrosirius red staining for assessment of fibrosis was performed by the Research Animal Diagnostic Laboratory, University of Missouri. To determine fibrosis, 5 independent high-power fields of stained images from each animal were analyzed with Image-Pro Plus software (Media Cybernetics, Silver Spring, Md).

**Surgical Procedure for Aortic Banding**

Six-week-old male wild-type and TG FVB mice were used for aortic banding and are described in the online Data Supplement. Briefly, an aortic band was created by placing a ligature (7-0 nylon suture) securely between the origin of the right innominate and left common carotid arteries with a 27-gauge needle as a guide. The sham procedure was identical except that the aorta was not ligated. Echocardiography and heart harvest were performed 4 weeks after surgery.

**Statistical Analysis**

Analysis was performed with SPSS for Windows (version 11.5, SPSS Inc, Chicago, Ill). Kaplan-Meier survival curves were compared between groups with log-rank tests. The results are presented as mean±SEM. One-way ANOVA was performed with a Student-Newman-Keuls test. For multiple variables, we used a 2-way ANOVA. Categorical differences were analyzed with the Mann-Whitney test. Differences were considered to be statistically significant at $P<0.05$.

The authors had full access to and take full responsibility for the integrity. All authors have read and agree to the manuscript as written.

**Results**

**Inducible and Cardiac Specific Expression of A1-AR**

We generated human A1-TG mice controlled by an inducible cardiac-specific promoter with binding sites for tTA. Gene expression was initiated by crossing 6 founder A1-TG lines with mice that expressed tTA in the heart (MHC-tTA; Figure 1A). By immunoblotting, 4 of the 5 founder lines showed robust A1-AR protein expression (Figure 1B). Expression of A1-AR was confirmed by radioligand binding in cardiac membranes with the A1-AR ligand DPCPX (Figure 1C).

Quantification of immunoblots and radioligand binding indicated that these A1-AR TG lines expressed the A1-AR at 500- to 1000-fold above the endogenous A1-AR level. Specificity was confirmed by competition with A1-AR antigenic peptide and with excess nonradioactive A1-AR binding ligand (Figure 1C and data not shown).

**DOX-Regulated A1-AR Expression**

The stable tetracycline analog DOX inhibits tTA transactivation. We determined the minimum dose of DOX (300 mg/kg of mouse diet) that attenuated A1-AR expression, which in turn prevented cardiomyopathy. As shown in Figure 2A, when DOX was continuously administered to pregnant mothers and subsequently to the offspring, cardiac A1-AR expression was significantly attenuated at 6 weeks of age compared with constitutively expressed A1-AR TG mice (A1-TGCon). To generate inducible A1-AR transgenic mice (A1-TGInd), DOX was removed when mice reached 3 weeks of age. Time-course studies showed that A1-AR was fully reexpressed at 6 weeks of age. At 6 weeks of age, A1-AR protein expression in A1-TGCon and A1-TGInd mice was identical (Figures 2B and 2C). DOX inhibition and A1-AR induction were confirmed by A1-AR binding assays (Figure 2D).

As reported previously, the MHC-tTA mouse line expressed tTA at very low levels. We found tTA expression in wild-type mice did not affect mouse heart weight and function up to 12 weeks. Similarly, the amount of DOX used in the study did not affect wild-type mouse heart size or function (Data Supplement Figures III and IV).

**Constitutive and Induced Overexpression of A1-AR**

As shown by the induction scheme (Figure 3A), A1-AR was either expressed constitutively in the absence of DOX (A1-TGCon) or expression was induced (A1-TGInd) by removing DOX at 3 weeks of age. Constitutive A1-AR overexpression in 2 A1-TG lines led to development of a dilated cardiomyopathy and high mortality rate in both male and female mice. Almost all A1-TGCon mice died within 6 to 12 weeks, depending on the founder line (Figure 3B). The higher mortality rate of line B was associated with a higher A1-AR protein expression (Figure 1B). Mice died of apparent congestive heart failure (postmortem cardiac hypertrophy and dilation; pleural effusion). Both male and female mice showed similar mortality rates and phenotypes (data not shown). In contrast, when A1-AR expression was delayed until mice reached 3 weeks of age, >90% of A1-TGInd mice survived 30 weeks or longer despite comparable levels of A1-AR overexpression. We chose to use line C for the remaining physiological and biochemical studies because this
line had the lowest transgene expression level and afforded longer survival.

We assessed cardiac functions and physical cardiac parameters when A1-TGCon or A1-TGInd mice reached 6 weeks of age. A1-TGCon mice developed early and profound cardiac dilatation, diminished ventricular function, and marked bradycardia (Figure 4A; Table 1). However, we did not detect significant arrhythmia using surface ECG measurements (data not shown). In addition, we determined the expression of genes frequently associated with cardiomyopathy. Data showed that expression of ANP and collagen genes was enhanced in A1-TG myocardium (Figure 4A; Table 1). On the other hand, expression of the calcium-handling genes, SERCA and phospholamban, was decreased (Table 1). These mice also demonstrated extensive fibrosis by picrosirius red staining and enhanced expression of collagen genes (Figure 4A). In contrast, when A1-AR induction was delayed until 3 weeks of age, A1-TGInd mice had a normal phenotype at 6 weeks of age, as demonstrated by ventricular weight and cardiac function. There was, however, a small but statistically significant decrease in heart rate (Table 1). The marked reduction in heart rate was detected in both anesthetized resting mice and in conscious restrained mice. When A1-AR expression was delayed until mice reached 3 weeks of age, A1-TGInd mice showed a significantly improved heart rate and cardiovascular parameters compared with A1-TGCon mice. To assess the effects of A1-AR overexpression on Akt phosphorylation, we probed heart extracts from 6-week-old male mice using antibodies that recognize phosphorylated Akt at Thr308. A1-AR overexpression decreased significantly in basal Akt phosphorylation in both A1-TGCon and A1-TGInd mice (Figure 4C). Consistently, phosphorylation of Akt Ser473 was also decreased (Data Supplement Figure V). In contrast, phosphorylation of map kinases (Erk1/2, JNK1/2, and p38) was not altered (Data Supplement Figure V).

**Effect of Induced A1-AR Expression Responding to Pressure Overload**

Because A1-TGInd mice had normal cardiac morphology and function at 6 weeks, we assessed whether A1-AR overexpression was cardioprotective in the presence of pressure overload induced by aortic banding. We measured LV systolic pressure immediately before and after banding, and pressure...
gradients between wild-type and A1-TGInd mice were similar (data not shown). As shown in Figures 5A, 5B, and 5C, 4 weeks after surgery, aortic banding accelerated the hypertrophic response to pressure overload and further decreased fractional shortening as compared with nontransgenic controls. In addition, aortic banding markedly reduced the level of expression of the calcium-handling genes SERCA and phospholamban (Figure 5D), markedly enhanced fibrosis, and effected a significant decrease in heart rate in mice overexpressing the A1-AR transgene (Figure 5E).

On the basis of these results, we evaluated cardiac morphology and function in older A1-TGInd mice. Consistent with the maladaptive effects of A1-AR overexpression in young mice with aortic banding, at 20 weeks of age, A1-TGInd mice developed ventricular enlargement, fibrosis, decreased fractional shortening, and changes in the expression of calcium-handling genes (Figure 4B; Table 2). Importantly, at this age, a marked reduction in heart rate was detected in anesthetized resting mice compared with heart rates of 6-week-old mice (Table 2). Interestingly, heart rates in conscious restrained mice did not differ between the 2 age groups.

Finally, to obviate the effects of heart rate on the changes in cardiac biology in the transgenic mice, we assessed the expression of early-response genes, cFos and EGR-1, in Langendorff-perfused A1-TGInd hearts. Under identical pacing conditions, hemodynamic parameters (LV developed pressure, diastolic pressure, +dp/dt, and −dp/dt) were similar between wild-type control hearts and A1-TGInd hearts (Data Supplement Table 1). In preliminary studies, real-time quantitative polymerase chain reaction showed that serum rapidly induced cFos and EGR-1 expression in a neonatal myocyte-derived cell line, H9C2, within 30 minutes (data not shown). As seen in Figures 5F and 5G, at steady state, levels of cFos and EGR-1 in mouse hearts were low, and no differences were observed between wild-type and A1-TG mice. However, the administration of a high perfusion pressure and therefore of increased cardiac load resulted in the rapid induction of cFos and EGR-1 expression. Importantly, the induction of cFos and EGR-1 expression was twice as high in A1-TG mice as in wild-type controls.

Effects of DOX Treatment
By 3 weeks after birth, A1-TGCon mice demonstrated enlargement of the LV cavity and fibrosis (Figure 6A). To determine whether the cardiomyopathy induced by A1-AR overexpression was reversible, A1-TGCon mice were fed DOX beginning at 3 weeks to inhibit A1-AR transgene expression (Figure 6B). Assessment of cardiac function at 12 weeks demonstrated
that attenuation of A1-AR expression in the A1-TGCon mice normalized ventricular weight, increased fractional shortening, and modulated LV dimension (Figures 6C and 6D). In addition, DOX reversed collagen staining, corrected the gene expression profile toward normal levels, and enhanced the survival of A1-TGCon mice (Figures 6E and 6F and Data Supplement Figure VII), which indicates that the functional pathology in this model was largely reversible.

**Discussion**

To understand the effects of temporal changes in A1-AR expression on cardiac morphology and function, we created TG mice in which A1-AR expression could be temporally regulated, by crossing mice that harbor the α-MHC promoter, which drives very low levels of tTA, with mice harboring a TG construct that consists of the human A1-AR gene linked to a constitutive A1-AR overexpression could be partially reversed by DOX. To address concerns that the level of A1-AR overexpression in the present experiments might be “superphysiological,” we evaluated A1-TG mice in which expression was activated at 3 weeks, were not able to tolerate pressure overload because banding resulted in markedly enhanced hypertrophy and diminished cardiac function, changes that were not observed in banded wild-type nontransgenic controls. These physiological changes were accompanied by diminished expression of calcium-handling genes and enhanced expression of ANP and collagen genes.

Importantly, the profound adverse consequences of constitutive A1-AR overexpression could be partially reversed by “turning off” the A1-AR transgene with the administration of DOX. To address concerns that the level of A1-AR overexpression in the present experiments might be “superphysiological,” we evaluated A1-TG mice in which 80% of the transgene was suppressed using a suboptimum DOX dose. Data showed that mice with lower A1-AR expression had a normal phenotype up to 20 weeks, but these mice remained unable to tolerate the stress of pressure overload (data not shown). However, we cannot exclude the possibility that there is a narrow therapeutic window for A1-AR overexpression, and therefore, it is possible that lower levels of overexpression may have limited or no adverse effects. Nevertheless, these studies represent the first successful effort to develop “controlled” overexpression of a cardiac G-protein-coupled receptor.

### TABLE 1. Organ Weights and Echocardiographic and Real-Time PCR Data of 6-Week-Old Mice

<table>
<thead>
<tr>
<th>Organ weights</th>
<th>WT</th>
<th>A1-TGCon</th>
<th>A1-TGInd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>21.8±0.8</td>
<td>17.8±0.6*</td>
<td>22.6±0.8†</td>
</tr>
<tr>
<td>Ventricular/body weight ratio</td>
<td>4.13±0.09</td>
<td>7.45±0.28*</td>
<td>4.17±0.18†</td>
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<tr>
<td>Lung/body weight ratio</td>
<td>6.85±0.22</td>
<td>10.51±0.47*</td>
<td>6.90±0.21†</td>
</tr>
</tbody>
</table>

**Echocardiographic data**

| Heart rate, bpm | 411±11 | 108±8* | 341±13† |
| Heart rate, bpm (conscious) | 580±11 | 257±7* | 345±10† |
| LVESD, mm | 3.50±0.07 | 5.56±0.30* | 3.62±0.07† |
| LVESD, mm | 1.90±0.07 | 4.14±0.37* | 2.20±0.08† |
| Fractional shortening, % | 45.7±2.1 | 26.3±3.6* | 39.4±1.5† |

**Real-time PCR (relative expression)**

<table>
<thead>
<tr>
<th>n</th>
<th>5</th>
<th>5</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERCA</td>
<td>1.00±0.06</td>
<td>0.12±0.01*</td>
<td>0.59±0.05†</td>
</tr>
<tr>
<td>PLB</td>
<td>1.00±0.08</td>
<td>0.23±0.01*</td>
<td>0.86±0.05†</td>
</tr>
<tr>
<td>ANP</td>
<td>1.00±0.3</td>
<td>248±52*</td>
<td>7.9±1.5†</td>
</tr>
</tbody>
</table>

PCR indicates polymerase chain reaction; WT, wild type; LVESD, LV end-systolic dimension; LVESD, LV end-diastolic dimension; and PLB, phospholamban.

Organ weights and echocardiography and gene expression data in mice constitutively expressing A1-AR (A1-TGCon) and after DOX was removed from TG mice at 3 weeks of age to induce A1-AR expression (A1-TGInd). Mice were examined at 6 weeks of age.

*P<0.01 vs WT; †P<0.01 vs A1-TGCon.
The present studies differ from earlier reports in which higher or comparable levels of constitutive overexpression of the A1-AR had less deleterious effects on cardiac morphology or function. For example, 1000-fold constitutive overexpression of rat A1-AR resulted in a decrease in the intrinsic heart rate of Langendorff-perfused hearts of 7- to 9-week-old TG mice (248 versus 318 bpm) and a lower developed tension.26 A1-AR overexpression in these mice also dampened the contractile response to high doses of catecholamines and significantly increased the heart weight/body weight ratio; however, no morphological changes were noted.28 Lower levels of constitutive A1-AR overexpression (300-fold) did not appear to be associated with changes in cardiac morphology or function and provided cardioprotection in globally ischemic, isolated heart models of ischemia-reperfusion injury. However, when studied in vivo, these mice had mildly decreased conscious resting heart rates compared with wild-type controls (620 versus 713 bpm) and died during even brief coronary occlusion.25

The disparity between the marked adverse effects of even moderate constitutive overexpression of A1-AR in the present experiments and the limited toxicity associated with moderate overexpression in earlier studies may be due to 2 fundamental differences in the studies: (1) Earlier studies created TG mice in the C57/B16 background (G.P. Matherne, personal communication), whereas the present studies were performed in the FVB strain; and (2) earlier studies utilized rat A1-AR cDNA, whereas the present studies used human A1-AR cDNA. It is unlikely that differences between the present results and those reported previously are due to the use of human rather than rat (or mouse) cDNA, because the A1-AR cDNA from mice and humans shared 95% amino acid identity (Data Supplement Figure VI). That the disparities could be explained by the difference in strain is supported by the fact that the genetic background of TG mice can significantly alter morphology, function, and survival. For example, survival was markedly better in mice overexpressing tumor necrosis factor-α/H9251 on an FVB background than on a mixed FVB/C57/Bl6 background.42,43 In addition, strain can markedly influence cardiac responsiveness to adrenergic and cholinergic agents. For example, baseline heart rates are significantly higher in C57/Bl6 mice than in FVB mice.43 Importantly, whereas isoproterenol and atropine increase heart rate in C57/Bl6 mice, isoproterenol and atropine have
TABLE 2. Organ Weights and Echocardiographic and Real-Time PCR Data of 20-Week-Old Mice

<table>
<thead>
<tr>
<th>Organ weights</th>
<th>WT</th>
<th>A1-TGInd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ weights</td>
<td>n = 5</td>
<td>n = 10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>30.0 ± 1.4</td>
<td>28.6 ± 0.7</td>
</tr>
<tr>
<td>Ventricular/body weight ratio</td>
<td>3.94 ± 0.17</td>
<td>5.12 ± 0.19*</td>
</tr>
<tr>
<td>Lung/body weight ratio</td>
<td>6.06 ± 0.17</td>
<td>6.45 ± 0.12</td>
</tr>
<tr>
<td>Echocardiographic data</td>
<td>n = 7</td>
<td>n = 5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>467 ± 31</td>
<td>144 ± 3*</td>
</tr>
<tr>
<td>Heart rate, bpm (conscious)</td>
<td>591 ± 4</td>
<td>333 ± 8*</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.64 ± 0.10</td>
<td>5.03 ± 0.13*</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.81 ± 0.15</td>
<td>3.43 ± 0.10*</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>50.4 ± 3.2</td>
<td>31.8 ± 1.1*</td>
</tr>
<tr>
<td>Real-time PCR (relative expression)</td>
<td>n = 5</td>
<td>n = 5</td>
</tr>
<tr>
<td>SERCA</td>
<td>1.00 ± 0.05</td>
<td>0.53 ± 0.02*</td>
</tr>
<tr>
<td>PLB</td>
<td>1.00 ± 0.03</td>
<td>0.68 ± 0.05*</td>
</tr>
<tr>
<td>ANP</td>
<td>1.00 ± 0.1</td>
<td>138.5 ± 31.9*</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1. Organ weights and echocardiographic and gene expression data in 20-week-old mice after DOX was removed from TG mice at 3 weeks of age to induce A1-AR expression (A1-TGInd).

*P<0.01 vs WT.

The development of cardiac hypertrophy and dilatation in FVB mice overexpressing A1-AR is closely related to the A1-AR–induced decrease in heart rate. Indeed, the profound bradycardia seen in mice with both constitutive and inducible overexpression of A1-AR may be the most likely explanation for the development of cardiomyopathy in these animals. This finding is consistent with other reports in which selective overexpression of A1-AR or A3-AR appears to have salutary effects on cardioprotection without adversely affecting cardiac morphology or function when heart rate does not change; however, cardiac dysfunction is present when heart rate is either moderately or markedly decreased. Assessment of the role of heart rate in affecting changes in cardiac function and morphology in the presence of A1-AR overexpression is challenging, because pacemakers small enough for a mouse are not commercially available. However, we took advantage of the earlier finding that natriuretic peptides (ANP and brain natriuretic peptide) were induced in the presence of increased wall stress and cardiac hypertrophy and that their expression is regulated by the early-response genes such as cFos and EGR-1. We hypothesized that in the presence of increased load, A1-AR TG hearts would demonstrate a rapid and robust increase in early-response gene expression that was independent of heart rate. Consistent with this hypothesis, in the presence of ventricular pacing, Langendorff-perfused hearts from both A1-AR TG and wild-type mice demonstrated an increase in cFos and EGR-1 expression; however, the level of expression induced in the A1-AR TG mice was twice that seen in the wild-type controls (Figures 5F and 5G). Thus, intrinsic molecular changes occur in response to an increase in pressure load in the presence of A1-AR overexpression that are independent of heart rate, and these changes are associated with alterations in the fetal gene program that are a characteristic feature of the heart failure phenotype. However, heart-rate related changes in cardiac...
function and morphology may also contribute to the cardiomyopathy observed in these animals, particularly the profound cardiac dysfunction seen in older animals.

Although the A1-AR TG mice displayed profound heart rate reduction, several cardiomyopathy mouse models, such as Gq overexpression, calcineurin overexpression, and tumor necrosis factor-α overexpression, were also associated with significant but less profound heart rate reduction.43,58,59 In contrast, heart rate was not altered in cardiomyopathies caused by calsequestrin, a mutant MHC, and muscle LIM protein knockout.60–63 Interestingly, these cardiomyopathy models with unaffected heart rate all responded favorably to β1-adrenergic pathway inhibition with a β1-adrenergic receptor kinase inhibitor.60–63 In contrast, β1-adrenergic receptor kinase inhibition did not improve cardiomyopathy caused by Gq overexpression.59 Thus, it remains to be determined whether the coupling of heart rate and cardiomyopathy alters the response to β-adrenergic blockade therapeutics. These findings have important implications on the potential therapeutic use of selective AR agonists in patients with cardiovascular disease. For example, when used chronically, doses of selective adenosine agonists that do not decrease heart rate should be chosen for clinical investigation, and individual patients should be observed carefully to ensure that variations in genetic background do not result in unique effects on heart rate in selected patient populations. In addition, the present results suggest that therapeutic agents that have a more balanced effect, i.e., those that activate both A1- and A2-ARs, may be safer in patients with normal or compromised cardiac function. This hypothesis will require further evaluation in experimental animals.

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Disclosures

None.

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51. Hautala N, Tenhunen O, Szokodi I, Ruskoaho H. Direct left ventricular dysfunction in these transgenic mice was associated with a decrease in heart rate suggests that heart rate should be carefully monitored in patients undergoing investigational studies of agents that alter the balance between activation of the various adenosine receptor subtypes.


**CLINICAL PERSPECTIVE**

Adenosine is an endogenous nucleoside that plays a critical role in the process of ischemic preconditioning through activation of a group of G-protein–coupled receptors that includes the A1-, A2a-, and A3-adenosine receptors. Acute administration of either adenosine or of an A1-selective adenosine receptor agonist decreases infarct size after coronary ligation in experimental animals, the same salutary benefit that is seen in transgenic mice in which the A1-adenosine receptor is overexpressed in the heart. However, far less is known about the effects of chronic activation of selective adenosine receptor pathways on the heart’s ability to withstand non-ischemic stress. In the present study, we demonstrate that cardiac-selective overexpression of the A1-adenosine receptor after maturity markedly impairs the heart’s ability to withstand the hemodynamic stress of aortic banding. Furthermore, prolonged overexpression results in a marked decrease in heart rate and in cardiac function. However, the decrease in function appears to be independent of the change in heart rate. These results raise important cautions about the use of selective adenosine receptor agonists (or potentially selective antagonists) in patients with heart muscle dysfunction. Furthermore, the finding that the development of cardiac dysfunction in these transgenic mice was associated with a decrease in heart rate suggests that heart rate should be carefully monitored in patients undergoing investigational studies of agents that alter the balance between activation of the various adenosine receptor subtypes.
Regulated Overexpression of the A₁-Adenosine Receptor in Mice Results in Adverse but Reversible Changes in Cardiac Morphology and Function


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