Early and Delayed Consequences of $\beta_2$-Adrenergic Receptor Overexpression in Mouse Hearts

Critical Role for Expression Level

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Background—Transgenic cardiac $\beta_2$-adrenergic receptor (AR) overexpression has resulted in enhanced signaling and cardiac function in mice, whereas relatively low levels of transgenically expressed $G_{\text{max}}$ or $\beta_2$AR have resulted in phenotypes of ventricular failure. Potential relationships between the levels of $\beta_2$AR overexpression and biochemical, molecular, and physiological consequences have not been reported.

Methods and Results—We generated transgenic mice expressing $\beta_2$AR at 3690, 7120, 9670, and 23 300 fmol/mg in the heart, representing 60, 100, 150, and 350 times background $\beta_2$AR expression. All lines showed enhanced basal adenyl cyclase activation but a decrease in forskolin- and NaF-stimulated adenyl cyclase activities. Mice of the highest-expressing line developed a rapidly progressive fibrotic dilated cardiomyopathy and died of heart failure at 25 ± 1 weeks of age. The 60-fold line exhibited enhanced basal cardiac function without increased mortality when followed for 1 year, whereas 100-fold overexpressors developed a fibrotic cardiomyopathy and heart failure, with death occurring at 41 ± 1 weeks of age. Adenyl cyclase activation did not correlate with early or delayed decompensation. Propranolol administration reduced baseline $\Delta dP/dt_{\text{max}}$ to nontransgenic levels in all $\beta_2$AR transgenics except the 350-fold overexpressors, indicating that spontaneous activation of $\beta_2$AR was present at this level of expression.

Conclusions—These data demonstrate that the heart tolerates enhanced contractile function via 60-fold $\beta_2$AR overexpression without detriment for a period of $\geq$1 year and that higher levels of expression result in either aggressive or delayed cardiomyopathy. The consequences for enhanced $\beta_2$AR function in the heart appear to be highly dependent on which signaling elements are increased and to what extent. (Circulation. 2000;101:1707-1714.)

Key Words: receptors, adrenergic, $\beta_2$ ■ cardiomyopathy ■ heart failure

Catecholaminergic activation of cardiac $\beta$-adrenergic receptors ($\beta$ARs) regulates the acute hemodynamic response to stress or injury by increasing myocardial contractility and heart rate. A deleterious effect of chronic $\beta$AR stimulation is suggested by clinical heart failure studies in which sympathomimetic agents are associated with a poor outcome,1–3 by a benefit of sympathomimetic agents in heart failure,4–6 and by reports of catecholamine-induced cardiomyopathies.7–10 Increasing cardiac adrenergic signaling by overexpressing $\beta$ARs or inhibiting the $\beta$AR kinase, however, appears to have therapeutic potential in heart failure, as demonstrated by favorable effects in genetically modified mice. Transgenic overexpression of $\beta_2$ARs in mouse cardiomyocytes increased basal and isoproterenol-stimulated adenyl cyclase activity and enhanced resting cardiac systolic function.11,12 $\beta_2$AR overexpression normalized the characteristic resting systolic dysfunction in the $G_{\text{max}}$ transgenic mouse model of hypertrophy13 but failed to improve a murine genetic dilated cardiomyopathy.14 Interestingly, augmenting $\beta$AR signaling through overexpressing a peptide inhibitor of the $\beta$AR kinase enhanced $\beta$AR-stimulated cardiac function15 and significantly improved function in murine dilated cardiomyopathy14 but not contractile depression in the $G_{\text{max}}$ hypertrophy model.13 From these results, it appears that at least in mice, chronic enhancement of $\beta$AR signaling can augment contractile function in the normal heart and provide functional benefit. However, the favorable effects reported in $\beta_2$AR-overexpressing mice contrast with dilated cardiomyopathy and myocardial fibrosis after overexpression of $\beta_2$AR or $G_{\text{max}}$, the signaling protein that couples $\beta$AR to adenyl cyclase.16,17

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What is lacking in our understanding of pathological effects of enhanced $\beta$AR signaling is a long-term assessment of signaling, structure, and in vivo function of multiple mouse

Received August 13, 1999; revision received October 18, 1999; accepted November 5, 1999.

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lines expressing a range of a given signaling protein. To address this, transgenic mice were generated with β2 AR overexpression ranging from 60 to 350 times background. The results reported here confirm a long-term positive inotropic effect of β2 AR overexpression at 60-fold endogenous βAR levels but also demonstrate that a long-term consequence of β2 AR overexpression at higher levels is progressive myocardial fibrosis, ultimately leading to heart failure.

Methods

Transgenic Mice
The wild-type human β2 AR cDNA was ligated into the Sal-1 site (exon 3) of the full-length 5.5-kb α-myosin heavy chain promoter essentially as previously described.12 The linearized constructs were injected into male pronuclei of fertilized FVB/N mouse oocytes and implanted into pseudopregnant female oviducts. Thirteen founders were identified by genomic Southern analysis.

Analysis of Cardiac Function
In vivo ventricular function was measured with invasive and noninvasive techniques essentially as previously described12,18,19 in lightly anesthetized (ketamine/thiopentobarbital), spontaneously breathing, closed-chest mice. Noninvasive cardiac function was assessed by 2D guided M-mode echocardiography of tribromoethanol-anesthetized mice. In some cases, studies were performed before and after intraperitoneal administration of 100 ng/g isoproterenol.

Assessment of Cardiac Hypertrophy
Hypertrophy was assessed gravimetrically by an analytical balance and by RNA dot-blot Northern analysis of hypertrophy-associated genes as previously described.18,19

βAR Signaling Studies
Radioligand binding with 125I-cyanopindolol to ventricular membranes was performed as previously described.12,13 For adenylyl cyclase activities, ventricular membranes (~10 μg) were coincubated with (mmol/L) phosphoenolpyruvate 2.8, GTP 0.06, ATP 0.12, cAMP 0.1, and ascorbic acid 0.1; and 4 U/mL myokinase, 10 μM pyruvate kinase, and 3×10^6 dpm [α-32P]ATP for 10 minutes at 37°C with various concentrations of isoproterenol, 10 mmol/L NaF, or 100 μmol/L forskolin. Reactions were stopped by dilution with 1.0 mL of a 4°C solution containing excess ATP and cAMP and 25 000 dpm/mL [3 H]cAMP (used for column recovery). [32P]cAMP was separated by chromatography over alumina columns. GRK2 and Gαi2/3 were measured in whole-heart homogenates by immunoblotting using standard techniques and antibodies from Santa Cruz Biotechnology as we have previously described in detail.20,21

Measurement of Calcium Currents
Whole-cell patch clamp studies were performed on ventricular cardiomyocytes as described previously.22,23 In the studies of basic Ca2+ channel kinetics, cells were dialyzed with 5 mmol/L EGTA. For isoproterenol experiments, EGTA was replaced with 10 mmol/L BAPTA to prevent Ca2+-dependent inactivation.24

Statistical Analysis
Unless stated otherwise, data are presented as mean±SEM. Transgenic mice from a single line and nontransgenic littermates were compared by 2-tailed Student’s t test. Multiple comparisons between different lines or at different ages within 1 line were performed with 1-way ANOVA followed by a Bonferroni procedure. In vivo dose-response data were analyzed by a mixed-factor ANOVA, with repeated measures on the second factor. A value of P<0.05 was considered significant.

Results
To better understand the effects of cardiac β2 AR overexpression, we created a series of β2 AR-overexpressing mouse lines in the FVB/N background with a range in transgene copy number. Of 13 founder mice, 1 failed to breed; 3 failed to transmit the transgene to progeny, suggesting that they were
TABLE 1. Characteristics of β2AR-Overexpressing Mouse Lines

<table>
<thead>
<tr>
<th></th>
<th>NTG</th>
<th>β2-60</th>
<th>β2-100</th>
<th>β2-150</th>
<th>β2-350</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAR expression, fmol/mg</td>
<td>66±19</td>
<td>3690±540*</td>
<td>7120±320*</td>
<td>9670±950*</td>
<td>23300±2400*</td>
</tr>
<tr>
<td>Adenylyl cyclase activity, pmol · min⁻¹ · mg⁻¹</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Basal</td>
<td>43±4.7</td>
<td>73±5.2*</td>
<td>42±6.6</td>
<td>49±3.4</td>
<td>45±4.5</td>
</tr>
<tr>
<td>Isoproterenol, max</td>
<td>87±9.5</td>
<td>91±10.0</td>
<td>70±11</td>
<td>72±3.7</td>
<td>73±8.5</td>
</tr>
<tr>
<td>NaF 10 mmol/L</td>
<td>148±17.9</td>
<td>110±8.4</td>
<td>65±6.9*</td>
<td>94±7.6*</td>
<td>97±10*</td>
</tr>
<tr>
<td>Forskolin 100 μM</td>
<td>477±53</td>
<td>324±13</td>
<td>204±10*</td>
<td>301±13</td>
<td>262±30</td>
</tr>
<tr>
<td>Isoproterenol, % NaF</td>
<td>60±2.7</td>
<td>82±2.8*</td>
<td>107±10*</td>
<td>78±5.8*</td>
<td>74±2.2*</td>
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<tr>
<td>Morphometry</td>
<td></td>
<td></td>
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<tr>
<td>Body wt, g</td>
<td>24±1</td>
<td>26±1</td>
<td>29±1</td>
<td>23±1</td>
<td>27±1</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>160±5</td>
<td>155±10</td>
<td>190±8</td>
<td>155±17</td>
<td>226±34*</td>
</tr>
<tr>
<td>Lung wt, mg</td>
<td>155±12</td>
<td>186±6</td>
<td>172±2</td>
<td>146±2</td>
<td>212±3</td>
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<td>H/B wt</td>
<td>6.9±0.2</td>
<td>6.0±0.3</td>
<td>6.4±0.7</td>
<td>6.8±0.4</td>
<td>8.1±0.2*</td>
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<tr>
<td>L/B wt</td>
<td>6.8±0.2</td>
<td>7.2±0.3</td>
<td>6.0±0.2</td>
<td>6.5±0.4</td>
<td>7.1±0.3</td>
</tr>
<tr>
<td>Echocardiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% FS</td>
<td>44±1</td>
<td>58±2*</td>
<td>56±4*</td>
<td>54±1*</td>
<td>32±3*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>443±33</td>
<td>460±30</td>
<td>440±31</td>
<td>432±28</td>
<td>470±26</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>1.6±0.2</td>
<td>1.2±0.2</td>
<td>1.2±0.2</td>
<td>1.4±0.1</td>
<td>2.5±0.2*</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>3.2±0.2</td>
<td>2.9±0.2</td>
<td>2.7±0.2</td>
<td>3.0±0.2</td>
<td>3.7±0.2*</td>
</tr>
<tr>
<td>Gene expression (fold NTG)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>αMHC</td>
<td>1</td>
<td>1.1</td>
<td>0.9</td>
<td>0.9</td>
<td>0.6</td>
</tr>
<tr>
<td>βMHC</td>
<td>1</td>
<td>0.8</td>
<td>1.1</td>
<td>0.7</td>
<td>2.3*</td>
</tr>
<tr>
<td>ANF</td>
<td>1</td>
<td>0.7</td>
<td>0.8</td>
<td>0.9</td>
<td>6.7*</td>
</tr>
<tr>
<td>α-skeletal actin</td>
<td>1</td>
<td>0.6</td>
<td>1.2</td>
<td>0.8</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Comparative βAR morphometric, echocardiographic, and molecular characteristics of 4 cardiac β2AR-overexpressing mouse lines and nontransgenic controls (NTG). Results shown are mean values of 4 to 14 determinations each. NTG values represent aggregate of nontransgenic siblings from each βAR line. βAR expression indicates 125I-CYP maximal binding capacity; H/B wt, heart weight indexed to body weight; L/B wt, lung weight indexed to body weight; % FS, left ventricular fractional shortening; HR, heart rate; ESD, left ventricular end-systolic dimension; EDD, left ventricular end-diastolic dimension.

*P<0.05 vs respective nontransgenic sibling controls for each line.

chimeric; independent lines were established for 5 founders; 4 other founders died before breeding; and 1 established line was lost before the present studies. The 4 founders that died had massive cardiac enlargement with dilated atria (Figure 1), pulmonary congestion, and pleural effusions.

Pharmacological Properties

The 4 successfully propagated α-myosin heavy chain (αMHC)–β2AR transgenic lines exhibited a range of β2AR expression (125I-cyanopindolol binding; Table 1) ~60-, 100-, 150-, and 350-fold greater than nontransgenic and were so designated (β2-60, etc.). Results from adenylyl cyclase studies are shown in Figure 2 and Table 1. When normalized to NaF stimulation (Figure 2B), basal activities were increased ~3-fold in the 2 lowest-expressing lines and ~2-fold in the 2 higher-expressing lines. The extent of maximal isoproterenol stimulation was also increased with each line compared with nontransgenic. However, the maximal increase was not commensurate with the increase in basal, so the fold stimulation over basal was in fact decreased. Absolute levels of activity (pmol · min⁻¹ · mg⁻¹) are shown in Figure 2A and Table 1. As can be seen, only the β2-60 line had higher basal activities when the data are expressed in this way. Both NaF- and forskolin-stimulated adenylyl cyclase activities were depressed to similar extents in all transgenic lines (Figure 2, C and D), suggesting that a compensatory event distal to the receptor occurs with chronic overexpression of β2AR at these levels. We therefore assessed by immunoblotting possible changes in expression of βAR kinase (GRK2) or Gαi, both of which are known to alter βAR signaling when increased in the heart. Neither βAR kinase nor Gαi levels were increased in any of the β2AR overexpressors (data not shown). Immunoreactivity of adenylyl cyclase type V/VI was not sufficient for accurate determination, so it is not possible to exclude a change in adenylyl cyclase expression with β2AR overexpression.

Early Effects

Initial phenotypic characterizations and interline comparisons of mice 10 to 15 weeks old demonstrated pathology in β2-350 mice. Gross morphological analysis revealed a 25% increase in heart weight of the β2-350 line compared with the other β2AR lines or with nontransgenic littermates (Table 1). Whereas echocardiographic left ventricular fractional shortening was enhanced in the 3 lower-expressing β2AR lines

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(Table 1), $\beta_2$-350 exhibited impaired left ventricular function with ventricular enlargement (Table 1). Increased cardiac $\beta$MHC and atrial natriuretic factor gene expression, characteristic features of cardiac hypertrophy, were also detected only in $\beta_2$-350 mice (see Table 1 and below). Furthermore, whereas the 3 lower-expressing $\beta_2$ AR lines showed no histological evidence of fibrosis or cardiomyocyte hypertrophy (not shown), $\beta_2$-350 hearts showed replacement fibrosis (see below). Thus, young adult mice expressing $\beta_2$ AR at 350 times normal levels developed cardiomegaly, increased expression of hypertrophy-associated genes, and depressed systolic function, whereas multiple transgenic lines with lower $\beta_2$ AR expression levels had normal cardiac size and gene expression with enhanced systolic function.

Late Effects

Because cardiomegaly, fetal gene expression, and fibrosis in young adult mice were observed only in the highest-expressing $\beta_2$ AR transgenic line, we considered that a longitudinal analysis of $\beta_2$ AR overexpressors might detect additional phenotypic features related to duration of transgene expression. Cohorts of 20 to 30 mice from each of 3 lines ($\beta_2$-60, -100, and -350) were therefore prospectively followed for 1 year. No increase in all-cause mortality was found in $\beta_2$-60 mice compared with nontransgenic littermates during this period (Figure 3). Early mortality was, however, observed in $\beta_2$-350 mice, which died at 25 \pm 1 weeks (Figure 3), suggesting an association between high levels of $\beta_2$ AR expression and early death. The cause of death appeared to be left heart failure, as shown by pulmonary congestion (increased lung weights) and massive cardiac enlargement (Table 2). $\beta_2$-100 mice also developed cardiac enlargement, heart failure, and premature death, but death occurred at 41 \pm 1 weeks (Figure 3, Table 2). Thus, these longitudinal mortality data demonstrate delayed deleterious effects of $\beta_2$ AR overexpression that are proportional in severity and rapidity of progression to the level of cardiac $\beta_2$ AR.

**TABLE 2. Characteristics of $\beta_2$-AR-Overexpressing Mice That Develop Heart Failure**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NTG</th>
<th>$\beta_2$-100</th>
<th>$\beta_2$-350</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at death, wk†</td>
<td>25 \pm 1</td>
<td>41 \pm 1</td>
<td>25 \pm 1</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>25 \pm 2</td>
<td>25 \pm 1</td>
<td>25 \pm 1</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>185 \pm 9</td>
<td>338 \pm 14*</td>
<td>425 \pm 39*</td>
</tr>
<tr>
<td>Lung wt, mg</td>
<td>172 \pm 5</td>
<td>492 \pm 30*</td>
<td>360 \pm 75*</td>
</tr>
<tr>
<td>h/B wt</td>
<td>7 \pm 1</td>
<td>14 \pm 2*</td>
<td>17 \pm 2*</td>
</tr>
<tr>
<td>L/B wt</td>
<td>7 \pm 1</td>
<td>20 \pm 3*</td>
<td>15 \pm 4*</td>
</tr>
</tbody>
</table>

Morphometric features of $\beta_2$ AR mice with terminal congestive cardiomyopathy. Abbreviations as in Table 1.

*P<0.05 vs nontransgenics.

†NTG were euthanized at 25 weeks.
Because these studies indicated that mortality in β2-350 mice was a function of the age of the animal, histological, morphometric, and molecular analyses were performed at 7, 11, and 17 weeks of age. These studies showed that the decline in left ventricular function was associated with cardiomyocyte dropout and fibrotic replacement. Fibrosis was not evident at 7 weeks, was apparent at 11 weeks, and was marked by 17 weeks of age (Figure 4A). The pattern of fetal cardiac gene expression remained constant over time (Figure 4B).

**In Vivo Hemodynamics**

To more rigorously assess the effects of β2-AR density and duration of expression on cardiac functional status, we performed in vivo hemodynamic studies on 12- and 20- to 24-week β2-350 mice compared with 12-week β2-60 mice. Whereas baseline heart rates were significantly increased in β2-60, heart rates were not increased in either 12- or 20-week-old β2-350 mice (Table 3). Basal +dP/dt was doubled in β2-60 mice and increased in 12-week-old but not ≈20-week-old β2-350 mice. Basal +dP/dt was doubled in β2-60 mice and increased in 12-week-old but not ≈20-week-old β2-350 mice.
old β2-350 mice (Table 3). Thus, enhanced basal cardiac systolic function was observed in mice expressing lower levels of β2-AR and in younger mice expressing high levels of β2-AR. With development of cardiomegaly in the latter mice, resting systolic function decreased.

Because cardiac phenotypes resulting from β2-AR overexpression may be a consequence of either enhanced agonist-stimulated βAR function or an increase in spontaneous receptor activation, we assessed hemodynamic responsiveness to isoproterenol. As shown in Table 3 and Figure 5A, inotropic and chronotropic responses to isoproterenol were generally blunted in 2-350 mice. HR indicates heart rate; MAP, mean arterial pressure; LVDP, left ventricular end-diastolic pressure; +dP/dt, maximal rate of increase in left ventricular pressure; Iso, value after infusion of 32 ng · g⁻¹ · min⁻¹ isoproterenol.

**TABLE 3. Invasive Hemodynamic Studies of β2-AR Overexpressors**

<table>
<thead>
<tr>
<th></th>
<th>NTG</th>
<th>β2-60</th>
<th>β2-350/12 wk</th>
<th>β2-350/20 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR Basal</td>
<td>392±36</td>
<td>540±13†</td>
<td>463±23</td>
<td>450±37</td>
</tr>
<tr>
<td>Iso</td>
<td>544±14*</td>
<td>547±11</td>
<td>521±11</td>
<td>514±25</td>
</tr>
<tr>
<td>MAP Basal</td>
<td>74±2</td>
<td>77±9</td>
<td>71±5</td>
<td>66±2</td>
</tr>
<tr>
<td>Iso</td>
<td>51±6*</td>
<td>65±17</td>
<td>60±8</td>
<td>63±9</td>
</tr>
<tr>
<td>LVDP Basal</td>
<td>95±3</td>
<td>101±7</td>
<td>95±4</td>
<td>91±4</td>
</tr>
<tr>
<td>Iso</td>
<td>93±5</td>
<td>98±12</td>
<td>89±4</td>
<td>89±5</td>
</tr>
<tr>
<td>+dP/dt Basal</td>
<td>8470±650</td>
<td>15 644±2126†</td>
<td>12 908±590†</td>
<td>9752±815</td>
</tr>
<tr>
<td>Iso</td>
<td>17 188±1145*</td>
<td>17 181±3739</td>
<td>12 531±561†</td>
<td>9751±1223†</td>
</tr>
</tbody>
</table>

Comparative hemodynamic features of 12-week-old nontransgenic (NTG) low-expressing β2-60, high-expressing β2-350, and 20-week-old high-expressing β2-350 mice. HR indicates heart rate; MAP, mean arterial pressure; LVDP, left ventricular end-diastolic pressure; +dP/dt, maximal rate of increase in left ventricular pressure; Iso, value after infusion of 32 ng · g⁻¹ · min⁻¹ isoproterenol.

*P<0.05 vs basal; †P<0.05 vs NTG.

Ca²⁺ Channel Activity
To confirm that the observed perturbations in cardiac function, myocyte signaling, and adenylyl cyclase activity did not simply reflect increased fibrotic content of β2-350 hearts, patch-clamp studies of inward calcium currents (I_{Ca}) were performed on ventricular cardiomyocytes from 2-60 and 24-week-old β2-350 mice. Cardiomyocyte capacitance, a measure of cell size, was significantly increased compared with nontransgenic siblings at 12 weeks (159.1±4.2 pF, n=129 versus 143.7±4.8 pF, n=67) and 24 weeks (274.7±14 pF, n=41 versus 145.4±5.8 pF, n=59; P<0.05), consistent with the molecular and morphometric indices of

**Figure 5. Hemodynamic consequences of high- and low-level β2AR overexpression. A,** Left ventricular dP/dt_max for β2-60 and β2-350 mice (12 and 20 weeks) as a function of intravenous isoproterenol dose. Each point represents mean of results from 4 animals per group. B, βAR blockade with intravenous propranolol reverses basal enhancement of dP/dt in 12-week-old β2-60 mice but not 12-week-old β2-350 mice.
cardiac hypertrophy noted above. Whereas the voltage dependence of $I_{Ca}$ in $\beta_2$-350 cells was not altered, $I_{Ca}$ density (shown in Figure 6A) was significantly reduced compared with that in nontransgenic cells (5.0 ± 0.3 pA/pF, n = 24 versus 8.9 ± 0.4 pA/pF, n = 38 at 12 weeks; 5.2 ± 0.5 pA/pF, n = 12 versus 10.3 ± 0.6 pA/pF, n = 26 at 24 weeks, $P < 0.05$). Myocytes from ≈24-week $\beta_2$-350 mice showed significantly reduced isoproterenol responsiveness (≈50% of control) without any change in $EC_{50}$ (Figure 6B). These results demonstrate progressive cardiomyocyte $\beta$AR-Ca$^{2+}$ dysfunction in aging $\beta_2$-350 mice.

Discussion

The objective of these studies was to establish a “transgene dose-response” for $\beta$AR expression in the heart. In pursuit of this objective, we created a series of cardiac-specific $\beta_2$AR transgenic mice and found that the range of $\beta_2$AR expression was associated with a spectrum of phenotypes: (1) enhanced contractile function in young mice with $\beta_2$AR levels 60 times normal; (2) rapidly progressive fibrosis, cardiac hypertrophy, and heart failure with $\beta_2$AR levels 350 times normal; and (3) early enhanced function with delayed development of fibrotic cardiomyopathy at intermediate expression levels. Enhanced baseline function accruing from $\beta_2$AR overexpression at levels 60 times normal occurred in the absence of any detectable pathological consequences over a 1-year period. Although the $\beta_2$-60 overexpressing mice are similar to $\beta_2$AR transgenic mice initially reported by Lefkowitz and coworkers$^{11}$ and by us,$^{12}$ it is of course not possible to exclude the possibility that pathological characteristics may eventually develop in $\beta_2$-60 mice as they continue to age. The particularly important findings of the present studies, however, pertain to the $\beta_2$-100 and -350 lines, which exhibited progressive ventricular dysfunction, directly related in severity and rapidity of progression to the level of $\beta_2$AR expression. With all lines, we observed a decrease in NaF- and forskolin-stimulated activities, but the $\beta$AR signaling appears to be even further dampened in the higher-expressing lines. Physiologically, this is manifested as lower systolic function with a lack of responsiveness to agonist, which is common in both. This further loss of ventricular function may be due to a decrease in L-type Ca$^{2+}$ channel density, as observed in the $\beta_2$-350 mice, or some other post-receptor-effector derangement.

Another important distinction between the rapidly progressive $\beta_2$-350 and $\beta_2$-60 lines is the evidence for intrinsic receptor signaling activity. Catheterization-based hemodynamic studies in $\beta_2$-350 mice showed no effect of $\beta_2$AR blockade with propranolol, whereas in $\beta_2$-60 mice, propranolol normalized the basal enhanced contractile function. As a neutral antagonist, propranolol would be expected to block endogenous catecholamine activation of the receptor but not the spontaneous transition of a proportion of $\beta_2$AR to the active (R*) conformation. It is interesting to speculate that the unrestricted signaling activity of R* in the $\beta_2$-350 line contributes to its aggressive cardiomyopathy. Thus, expression of $\beta_2$AR at a level that enhances the in vivo response to agonists but does not cause in vivo ligand-independent signaling may improve cardiac function without deleterious effects. However, exceeding the putative threshold for ligand-independent $\beta_2$AR receptor signaling may cause rapid development of the cardiomyopathic syndrome reported here, as well as additional counterregulatory effects not observed with the other lines.

The pathological and physiological characteristics exhibited by the older $\beta_2$-350 and $\beta_2$-100 mice reproduce some features of catecholamine cardiomyopathy as described in human subjects with pheochromocytomas.$^{7–10}$ The pathophysiology of catecholamine-mediated cardiomyopathy has been postulated to result from ischemic necrosis secondary to intense vasospasm or from direct toxic effects of oxidized catecholamines. Our studies demonstrate that none of these putative mechanisms are necessary for development of cardiomyopathy, because $\beta_2$ARs were selectively overexpressed in cardiac myocytes, and there is no reason to believe that circulating or local catecholamine levels are increased in these mice. Rather, these studies and those with the previously reported $\beta_2$AR- and $G_{\alpha_{s}}$-overexpressing models$^{16,17}$ support a direct effect of chronic unrestricted $\beta$AR signaling on cardiomyocytes, a notion consistent with catecholamine cardiomyocyte toxicity demonstrated in some tissue culture studies.$^{25}$

Figure 6. $\beta_2$-350 cardiomyocyte electrophysiological properties. A, Representative whole-cell $I_{Ca}$ recorded in nontransgenic (NTG) and $\beta_2$-350–overexpressing myocytes at 24 weeks of age. B, Concentration-dependent effects of isoproterenol (Iso) on $I_{Ca}$ for NTG and $\beta_2$AR-overexpressing myocytes. $EC_{50}$s were 26.5 ± 6.9, 8.2 ± 4.7, and 43.0 ± 30 nmol/L for NTG, $\beta_2$-350–overexpressing mice at 12 weeks, and the same at 24 weeks, respectively. Data are mean±SEM from 6 to 40 cells.
The variability in adenylyl cyclase responsiveness in the various β2-AR overexpressors suggests that regulatory mechanisms may be evoked by certain levels of long-term overexpression that seem to partially desensitize receptor signaling. Changes in the expression of βAR kinase,26 Gaα, and adenylyl cyclase27 in the heart have been reported to be associated with decreased βAR signaling. An increase in βAR kinase would be expected to exclusively alter receptor-mediated stimulation, which is not the case in these β2-AR overexpressors, in which we find forskolin- and NaF-stimulated activities also depressed, and βAR kinase protein expression was not altered in any of the β2-AR lines. An increase in Gaα could theoretically alter basal, βAR-mediated, and NaF-mediated signaling; however, we found no evidence of such an increase. Finally, we also considered that a decrease in adenylyl cyclase expression could serve to decrease signaling at baseline and in response to agonist, forskolin, and NaF. Indeed, given the above, a decrease in adenylyl cyclase expression seems to be quite a reasonable candidate. However, we are unable to quantitatively assess type V/VI adenylyl cyclase expression in the mouse heart, so we cannot reach a conclusion in this regard.

We have shown that cardiac β2-AR overexpression 60 times background results in enhanced in vitro and in vivo signaling without apparent pathological consequences in mice up to 1 year old. Higher levels of expression result in delayed (β2-100) or rapidly progressive (β2-350) cardiomyopathies. That deleterious effects can occur at some level of β2-AR expression is not altogether surprising. The obverse finding, that moderate levels of overexpression are apparently not detrimental, is contrary to the notion heralded by some that enhanced β2-AR overexpression is observed at 5 to 15 times endogenous β2-AR levels,17 whereas a 60-fold increase in β2-AR appears to be well tolerated. Thus, overly broad generalizations regarding potential deleterious effects of βAR signaling via increased β2-AR, β2-AR, or Gaα or via inhibition of βARK may be overly simplistic, because there appear to be fundamental differences in signaling evoked by these mechanisms.

Acknowledgments

This study was supported by grants GM-54169, HL-58010, HL 22619, and P50-HL-52318 from the National Institutes of Health.

References


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Circulation. 2000;101:1707-1714
doi: 10.1161/01.CIR.101.14.1707

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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