β3-adrenoceptor deficiency blocks nitric oxide–dependent inhibition of myocardial contractility

Paul Varghese, Robert W. Harrison, Robert A. Lofthouse, Dimitrios Georgakopoulos, Dan E. Berkowitz, and Joshua M. Hare

1Department of Medicine, Cardiology Division, and
2Department of Anesthesiology and Critical Care Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland, USA

Address correspondence to: Joshua M. Hare, Johns Hopkins Hospital, Cardiology Division, 600 N. Wolfe Street, Carnegie 568, Baltimore, Maryland 21287-6568, USA. Phone: (410) 614-4161; Fax: (410) 955-3478; E-mail: j hare@mail.jhmi.edu.

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Paul Varghese and Robert W. Harrison contributed equally to this work.

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The cardiac β-adrenergic pathway potently stimulates myocardial performance, thereby providing a mechanism for myocardial contractile reserve. β-Adrenergic activation also increases cardiac nitric oxide (NO) production, which attenuates positive inotropy, suggesting a possible negative feedback mechanism. Recently, in vitro studies suggest that stimulation of the β3-adrenoceptor results in a negative inotropic effect through NO signaling. In this study, using mice with homozygous β3-adrenoceptor deletion mutations, we tested the hypothesis that the β3-adrenoceptor is responsible for β-adrenergic activation of NO. Although resting indices of myocardial contraction were similar, β-adrenergic–stimulated inotropy was increased in β3–/– mice, and similar hyper-responsiveness was seen in mice lacking endothelial NO synthase (NOS3). NOS inhibition augmented isoproterenol-stimulated inotropy in wild-type (WT), but not in β3–/– mice. Moreover, isoproterenol increased myocardial cGMP in WT, but not β3–/–, mice. NOS3 protein abundance was not changed in β3–/– mice, and cardiac β3-adrenoceptor mRNA was detected in both NOS3–/– and WT mice. These findings indicate that the β3-adrenoergic subtype participates in NO-mediated negative feedback over β-adrenergic stimulation.


Introduction

Nitric oxide (NO) inhibition of β-adrenergic–stimulated contractility has been demonstrated in vitro (1) and in vivo (2–5). In addition to activating adenylyl cyclase production of cAMP, a cardio stimulatory pathway, β-adrenergic agonists lead to the production of NO. NO stimulates soluble guanylyl cyclase to produce cGMP, a cyclic nucleotide that opposes the cardiotimulatory effects of cAMP. Cardiodepression due to the NO-cGMP pathway likely has pathophysiologic significance because its activity is increased with heart failure (6, 7), sepsis (8), and aging (9, 10).

Whereas β-adrenergic stimulation has been shown to increase NO production (11), the relative contributions of specific β-adrenoceptor subtypes (1, 2, or 3) in this signal-transduction pathway have not been established. The β3-adrenoceptor, recently appreciated in the mammalian heart (12–14), is widely expressed in adipose, intestinal, and lung tissues (15), where its activation promotes lipolysis and energy expenditure (16–18). β3-adrenoceptor activation in the human heart produces direct negative inotropic responses (12, 13, 19). Observations that these negative inotropic effects are attenuated by NO synthase (NOS) inhibition suggest that the β3-adrenoceptor has a role in NO-mediated inhibition of adrenergic stimulation.

The lack of specific β3-adrenoceptor antagonists has hampered characterization of the precise in vivo role of β3-NO signaling. To address this issue we measured β-adrenergic contractility in mice with a homozygous deletion mutation of the β3-adrenoceptor gene (β3–/–). Using these animals, we tested the hypothesis that β3–/– mice lack the NO negative feedback over β-adrenergic contractility.

Methods

Murine species. β3–/– mice (34 ± 1 g body weight, 7–9 months old), based on an inbred FVB background, were obtained courtesy of Brad Lowell, Beth Israel-Deaconess Medical Center (Boston, Massachusetts, USA) (20–22). FVB wild-type (WT) mice (32 ± 1 g body weight, 7–9 months old) from Taconic Farms (Germantown, New York, USA) were used as control animals. NOS3–/– mice (31 ± 2 g body weight, 7–9 months old) were kindly provided by Paul Huang (Massachusetts General Hospital, Boston, Massachusetts, USA) (23).

Animal preparation. Animals were housed under diurnal lighting conditions and allowed food and tap water ad libitum. Animal treatment and care was provided in accordance with institutional guidelines, and the protocol was approved by the Animal Care and Use Committee of the Johns Hopkins University.
Mice were anesthetized and ventilated as described previously (24). Briefly, mice were anesthetized with a combination of etomidate (3.3 mg/kg), urethane (1000 mg/kg), and morphine (0.5 mg/kg). A tracheotomy was performed, and mice were ventilated at 120 breaths per minute with a peak airway pressure of less than 11 mmHg. After a subternal lateral thoracotomy, a combined micromanometer-conductance catheter (SPR-719; Millar Instruments Inc., Houston, Texas, USA) was advanced retrograde into the left ventricle (LV) through an apical stab wound made with a 25-gauge needle, along the cardiac longitudinal axis; the distal tip was placed in the aortic root and a proximal electrode was placed just within the endocardial wall of the LV apex.

Infusions were administered through the right jugular vein cannulated with a 30-gauge needle. Offset calibration of the recorded volume signal was obtained by the saline wash-in technique (25, 26). Stroke volume calibration was derived from the cardiac output obtained from direct measurements of the aortic blood flow, obtained using a flow probe (AT01RB; Transonic Systems Inc., Ithaca, New York, USA) placed around the aorta, and the flow per minute was recorded (AT106; Transonic Systems Inc.). Pressure, volume, and flow signals were digitized at 1 kHz, stored to a disk, and analyzed with custom software.

**Hemodynamic data analysis.** Indices of myocardial systolic and diastolic performance were derived from pressure-volume data obtained at steady state and during transient loading of the heart with direct occlusion of the abdominal aorta. Cardiac preload was indexed as the left ventricular end-diastolic volume (EDV) and end-diastolic pressure (EDP). Cardiac afterload was evaluated as effective arterial elastance (Ea; ratio of LV systolic pressure to stroke volume) (27, 28). This parameter is not preload dependent and has been validated to closely approximate total afterload, which incorporates systemic vascular resistance, aortic impedance, and the reflected wave properties of the vasculature. Myocardial contractility was indexed by the peak rate of rise in LV pressure (+dP/dt) divided by instantaneous pressure (dP/dt-IP) (29) and the load-independent end-systolic elastance (Ees) (30, 31). Ees is the slope of the end-systolic pressure-volume relationship (ESPVR). Because the ESPVR is nonlinear in mice, we used the formula derived by Mirsky (32) to fit our pressure-volume data:

\[
\frac{\text{Pes} - \text{Pes}_0}{\alpha + \beta \cdot \text{Pes}} = \log \frac{\text{Ves}}{\text{Vo}}
\]

Where \(\alpha\) and \(\beta\) are fitting parameters, \(\text{Pes}\) and \(\text{Ves}\) are the end-systolic pressure and volume, respectively, and \(\text{Vo}\) is the volume of the heart at zero pressure. This formula is derived from the mechanical properties of muscle and has been validated in the canine heart (32). Ees was taken as the average slope of the fitted ESPVR in the pressure range of 90–160 mmHg.

**Expression of \(\beta_3\)-adrenoceptor.** RT-PCR was performed using 1 µg of total RNA (mouse heart and epididymal fat). Total RNA was reverse-transcribed with M-MLV RT (Superscript II) (Life Technologies Inc., Gaithersburg, Maryland, USA) for 30 minutes at 37°C in a solution containing 500 nM oligodT, 0.2 mM of each
dNTP, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, and 10 mM dithiothreitol. RNA template was digested with RNase H. Denatured cDNA (for 5 minutes at 94°C) was submitted to 30 cycles of amplification in a solution containing 0.5 U of Taq polymerase (Perkin Elmer, Norwalk, Connecticut, USA) and 1 mM sense and antisense oligonucleotides corresponding to a 340-bp fragment from 651–991 (forward primer, 5′-3′, GTGGGTTCAGGATCTACAG; reverse primer, 5′-3′, TGTCACCCACCCTTGGCGT) of the mouse β₃-adrenoceptor sequence (36). cDNA from mouse hearts and white epididymal fat (as a positive control) were used as a template for the PCR reactions. To further confirm the presence of β3 transcription, RNase protection was performed using the cloned fragment, as described previously (37). Hearts without reverse transcriptase were used as a control to test for genomic contamination.

Levels of cGMP. FVB (n = 8) and β₃–/– (n = 13) mice were anesthetized and ventilated as described above and infused intravenously with either normal saline or isoproterenol (5 ng/kg/min) and resuspended in sodium acetate buffer for analysis with an enzyme immunoassay (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA).

Statistics. All results are reported as mean ± SEM. A one-way ANOVA was used to compare base-line hemodynamics in WT, β₃–/–, and NOS3–/– mice. For statistical analysis of isoproterenol concentration–effect relationships, a one-way ANOVA with repeated measures was used to analyze responses per strain. A two-way ANOVA with terms for treatment and strain was used to analyze responses per strain. A two-way ANOVA for repeated measures was used to compare the effects of isoproterenol with and without l-NMMA or l-NAME in WT and β₃–/– mice. The effects of isoproterenol on cGMP concentration were compared using an unpaired t test with a Bonferroni correction. All statistical analyses were performed using SAS software (Cary, North Carolina, USA). Differences were considered significant at P values less than 0.05.

Results

Effect of β₃-adrenoceptor and NOS3 deletion on base-line hemodynamic parameters. We used a combined micromanometer-conductance catheter to assess the determinants of cardiovascular performance in WT, β₃–/–, and NOS3–/– mice (Table 1). β₃–/– mice had similar base-line indices of myocardial contractility and afterload compared with WT mice. Both the β₃–/– and NOS3–/– mice had smaller chamber sizes (lower EDV) than the WT mice, which was accompanied by impaired relaxation (higher tau) in the β₃–/– mice. Whereas all three strains of mice had similar body weights, the NOS3–/– mice had elevated heart weights resulting in increased heart-weight/body-weight ratio. As described previously (23), NOS3–/– mice had hypertension reflected not only by elevated systemic blood pressure but also by increased Ea, a measure of arterial tone. Also, the NOS3–/– mice had increased heart rate compared with the WT and β₃–/– mice.

Effect of intravenous isoproterenol on myocardial contractility. To assess the β-adrenergic inotropic response we infused isoproterenol (1, 10, 100 ng/kg/min) to anesthetized mice. As shown in Figure 1, isoproterenol produced an augmented contractile response in β₃–/– mice (58 ± 7.9% increase in dP/dt-IP at peak isoproterenol response; P < 0.001) compared with WT mice (37 ± 5.9% peak dP/dt-IP increase; P < 0.001 vs. base line, P < 0.01 vs. β₃–/– mice).

The effects on contractility, as assessed by dP/dt-IP, were not related to changes in preload or afterload after isoproterenol infusion, because EDP, EDV, Ea, and systolic blood pressure were not changed after isoproterenol infusion in either WT or β₃–/– mice. Heart rate increased similarly in both the β₃–/– mice (21.7 ± 3.2%; P < 0.01 vs. base line) and WT mice (29.0 ± 4.3%; P < 0.01 vs. base line, P = not significant [NS] vs. β₃–/–). Effect of NOS-inhibition on β-adrenergic stimulation. To determine the role of NO in β-adrenergic contractility, we examined the impact of NOS inhibition with l-NMMA on β-adrenergic responses. Mice were administered isoproterenol (5 ng/kg/min) followed by the NOS inhibitor l-NMMA (10 mg/kg/h). At this concentration of isoproterenol, dP/dt-IP increased in both WT (10.1 ± 2.6%; P < 0.05 vs. base line; Figure 2a) and β₃–/– mice.

Table 1: Base-line conditions

<table>
<thead>
<tr>
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<th>WT</th>
<th>β₃–/–</th>
<th>NOS3–/–</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>541 ± 23</td>
<td>559 ± 15</td>
<td>629 ± 19</td>
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<td>Body weight (g)</td>
<td>32.4 ± 0.7</td>
<td>33.7 ± 0.6</td>
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<td>Heart weight (mg)</td>
<td>145 ± 9</td>
<td>158 ± 15</td>
<td>192 ± 9</td>
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<td>Heart-weight/body-weight ratio (mg/g)</td>
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<td>4.7 ± 0.4</td>
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<tr>
<td>Afterload</td>
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<td>Systolic blood pressure (mmHg)</td>
<td>101 ± 4</td>
<td>108 ± 5</td>
<td>132 ± 58</td>
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<td>Ea (mmHg/µL)</td>
<td>6.4 ± 1.2</td>
<td>8.7 ± 0.6</td>
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<td>Preload</td>
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<td></td>
<td></td>
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<tr>
<td>EDP (mmHg)</td>
<td>9.9 ± 1.1</td>
<td>9.4 ± 0.9</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>EDV (µL)</td>
<td>25.9 ± 4.0</td>
<td>14.1 ± 1.0</td>
<td>11.5 ± 1.6</td>
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<td>Contractility</td>
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<tr>
<td>+dP/dt (mmHg/s)</td>
<td>11,575 ± 921</td>
<td>11,407 ± 714</td>
<td>11,639 ± 675</td>
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<tr>
<td>+dP/dt-IP (s⁻¹)</td>
<td>194.8 ± 10.8</td>
<td>184.8 ± 8.1</td>
<td>162.9 ± 8.5</td>
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<td>Ees (mmHg/µL)</td>
<td>12.0 ± 3.0</td>
<td>16.8 ± 3.8</td>
<td>15.4 ± 0.5</td>
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<tr>
<td>Diastole</td>
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<tr>
<td>Tau (ms)</td>
<td>4.9 ± 0.2</td>
<td>6.0 ± 0.4</td>
<td>5.2 ± 0.3</td>
</tr>
<tr>
<td>–dP/dt (mmHg/s)</td>
<td>–8,915 ± 836</td>
<td>–8,816 ± 881</td>
<td>–11,309 ± 795</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SEM. *P < 0.05 vs. other two strains using ANOVA. **P < 0.01 vs. other two strains using ANOVA.
Proterenol was administered intravenously at rates of 1, 10, and 100 ng/kg/min to WT mice (FVB, n = 12) and mice with homozygous β3-adrenoceptor-deletion mutations (β3−/−; n = 7). Peak positive dP/dt divided by the instantaneous left-ventricular pressure (dP/dt-IP) was used as an index of contractility and is displayed as a percentage of change from baseline. As shown, the inotropic concentration-effect relationship was highly significant response to isoproterenol was augmented in change from base line.)

**Figure 1**

β-Adrenergic concentration-effect curves in β3−/− and WT mice. Proterenol was administered intravenously at rates of 1, 10, and 100 ng/kg/min to WT mice (FVB, n = 12) and mice with homozygous β3-adrenoceptor-deletion mutations (β3−/−; n = 7). Peak positive dP/dt divided by the instantaneous left-ventricular pressure (dP/dt-IP) was used as an index of contractility and is displayed as a percentage of change from baseline. As shown, the inotropic concentration-effect relationship was highly significant response to isoproterenol was augmented in change from base line. As shown, the inotropic concentration-effect relationship was highly significant by one-way ANOVA (P < 0.01). Data are reported as mean ± SEM. *P < 0.01, β3−/− vs. FVB, by two-way ANOVA.

The contractile effects observed with a co-infusion with L-NMMA did not augment the already elevated positive inotropic effect in β3−/− mice (dP/dt-IP increased 26.4 ± 5.6% over base line; P < 0.001 vs. base line, P = NS vs. isoproterenol alone; Figure 2a). A different NOS inhibitor, L-NAME, was also used in additional experiments. In similar fashion to L-NMMA, L-NAME augmented the isoproterenol inotropic response in WT, but not β3−/− mice, relative to FVB. Each concentration-effect relationship was highly significant by one-way ANOVA (P < 0.01). Data are reported as mean ± SEM. *P < 0.01, β3−/− vs. FVB, by two-way ANOVA.

**Figure 2**

(a) Contractile effects of isoproterenol and L-NMMA in WT, β3−/−, and NOS3−/− mice. Isoproterenol was administered intravenously at a rate of 5 ng/kg/min for 4 minutes, followed by a co-infusion with L-NMMA at 10 mg/kg/h for 5 minutes. Contractility was indexed by dP/dt-IP and is shown as a percentage of change from base line. The β3−/− mice (n = 10) had greater responses to isoproterenol than did the WT mice (n = 8), but did not show any further augmentation after NOS inhibition with L-NMMA. Similarly, NOS3−/− mice (n = 15) were hyper-responsive to isoproterenol. L-NMMA augmented the isoproterenol response in WT mice to the level observed in β3−/− mice. (b) The effect of an additional NOS inhibitor, L-NAME. L-NAME had an effect similar to L-NMMA, augmenting the response to isoproterenol in WT, but not β3−/− mice. Data are reported as mean ± SEM. *P < 0.05 vs. respective base line, †P < 0.01 vs. respective base line, ‡P < 0.05 vs. WT isoproterenol response by one-way ANOVA.

Discussion

In this study we have shown that mice lacking the β3-adrenoceptor do not exhibit NO-mediated inhibition of cardiac function. Proterenol was administered intravenously at rates of 1, 10, and 100 ng/kg/min to WT mice (FVB, n = 12) and mice with homozygous β3-adrenoceptor-deletion mutations (β3−/−; n = 7). Peak positive dP/dt divided by the instantaneous left-ventricular pressure (dP/dt-IP) was used as an index of contractility and is displayed as a percentage of change from baseline. As shown, the inotropic concentration-effect relationship was highly significant response to isoproterenol was augmented in change from base line. As shown, the inotropic concentration-effect relationship was highly significant by one-way ANOVA (P < 0.01). Data are reported as mean ± SEM. *P < 0.01, β3−/− vs. FVB, by two-way ANOVA.

Concentration of cGMP. To test the impact of altered NO signaling we assessed concentrations of the NO second messenger cGMP. Basal cGMP was elevated in β3−/− (10.7 ± 2 pmol/g, n = 7) vs. WT (2.4 ± 0.4 pmol/g, n = 3; P < 0.05 vs. β3−/−). In WT mice, isoproterenol-treated (5 ng/kg/min) hearts had a higher level of cGMP (6.5 ± 0.8 pmol/g, n = 5; P < 0.05) than hearts treated with normal saline. β3−/− mice did not exhibit increased cGMP with isoproterenol (10.9 ± 1.7 pmol/g with isoproterenol, n = 6; P = NS vs. normal saline).

**NOS3 protein abundance.** Western blot analysis of myocardial tissue revealed no change in NOS3 protein levels, relative to p-38 or actin, between WT and β3−/− mice (Figure 4a). Protein abundance of NOS3 was similar between WT and β3−/− mice (85 ± 5 vs. 87 ± 3 arbitrary units, n = 8 and 9, respectively). Moreover, when corrected for abundance of either p-38 (1.03 ± 0.06 vs. 1.11 ± 0.10 P = NS) or actin (0.84 ± 0.08 vs. 1.06 ± 0.13, P = NS), NOS3 was similarly unchanged. Whereas NOS3 protein was absent in the NOS3−/− mice, expression of p-38 and actin was present in similar quantities to the other strains of mice (Figure 4a).

Expression of β3-adrenoceptor. Expression of β3-adrenoceptor mRNA was detected by RT-PCR in heart and epidermal fat of both WT and NOS3−/− mice, but not β3−/− animals (Figure 4b). RT-PCR in reverse-transcribed negative reactions revealed little or no PCR product, confirming minimal contamination with genomic DNA. RNase protection assay confirmed the presence of β3-adrenoceptor mRNA in WT and NOS3−/− mice (Figure 4c).

Discussion

In this study we have shown that mice lacking the β3-adrenoceptor do not exhibit NO-mediated inhibition of
β-adrenergic–stimulated inotropic responses. The cardiovascular effects of β3-adrenoceptor deletion in mice were apparent only during sympathetic stimulation. Under baseline conditions, the β3−/− and WT mice exhibited similar heart rates, loading conditions, and contractile states. However, when stimulated with isoproterenol, a nonspecific β-adrenergic agonist, the β3−/− mice had a greater positive inotropic response than did the WT mice. In WT mice the inotropic response to isoproterenol was augmented by L-NMMA and L-NAME, NOS inhibitors, to the level seen in β3−/− mice with isoproterenol alone. Augmentation by L-NMMA or L-NAME was absent in the β3−/− mice. Similarly, mice lacking NOS3 were hyper-responsive to isoproterenol infusion, lack augmentation with NOS inhibition, and yet have similar indices at baseline. These observations suggest that the β3-adrenoceptor plays a major role in NO attenuation of β-adrenergic–stimulated positive inotropy.

These present findings agree with those of Gauthier et al. (12, 13, 19) that direct stimulation of β3-adrenoceptors produces a negative inotropic response due to NO-cGMP signaling. These previous studies were unable to address the impact of inhibiting the β3-NO pathway because of the lack of availability of β3-specific antagonists. The present study, by examining the β3-NO pathway in animals lacking the β3-adrenoceptor, has established the importance of this receptor in NO-mediated negative feedback over β-adrenergic inotropic responses. In WT mice, NOS inhibition augments β-adrenergic–stimulated positive inotropic effects (2, 3). β3−/− mice, on the other hand, are hyper-responsive to isoproterenol infusion, lack augmentation with NOS inhibition, and yet have similar indices at base line. These observations suggest that β3-adrenoceptor

Figure 3
Left ventricular pressure-volume data in WT and β3−/− mice. A combined micromanometer-conductance catheter was inserted into the LV through the apex. Transient occlusion of the descending aorta was used to generate the end-systolic pressure-volume relationship (loops not shown). Depicted are (a) example steady-state loops and their respective ESPVR (from which Ees is determined) at baseline after receiving isoproterenol (5 ng/kg/min) and after receiving isoproterenol and l-NMMA (10 mg/kg/h). Also shown is (b) pooled data of the augmentation of isoproterenol-stimulated inotropy by l-NMMA in WT (n = 8) and β3−/− (n = 10) mice. Isoproterenol-induced increases in Ees were augmented by NOS inhibition in WT, but not in β3−/− mice. Data are reported as mean ± SEM. *P < 0.05 vs. base line by paired t test; †P < 0.05 vs. WT by unpaired t test.

Figure 4
Abundance of NOS3 protein and β3−/− mRNA in myocardium. (a) Western blot of NOS3 from mouse heart tissue. Equal amounts of protein extracts were resolved on agarose gels, transferred to nitrocellulose, and exposed to an anti-NOS3 Ab or anti-p-38 Ab. The FVB and β3−/− mice had similar NOS3 abundance relative to p-38 MAP kinase, and NOS3 was absent in the NOS3−/− mice. (b) Representative ethidium-stained agarose gel demonstrating expression of β3-adrenoceptor mRNA in different mice strains: mRNA is expressed in both heart (H) and epididymal fat (F) of NOS3−/− and FVB control mice, but not β3−/− mice. Little or no PCR product is amplified in reactions lacking reverse transcriptase (H−), confirming minimal genomic contamination of mRNA. (c) Autoradiograph of RNase protection assay confirming the expression of β3-adrenoceptor in the myocardium of FVB and NOS3−/− mouse hearts. β3AR P, β3-adrenoceptor probe; β3AR PF, β3-adrenoceptor–protected fragment.
stimulation is coupled to NOS activation, that this coupling offers significant negative feedback over sympathetic activation, and that the β3-adrenoceptor exerts its influence primarily during sympathetic activation. 

β3−/− animals had elevated myocardial cGMP levels compared with FVB controls, but only WT animals demonstrated increased cGMP concentrations with exposure to β-adrenergic agonists. The former suggests that the β3-adrenoceptor negatively modulates basal NO-cGMP production and is consistent with several studies showing that receptors that are coupled to NO inhibit its activity by a mechanism involving caveolin, the scaffolding protein found in membrane caveolae (see ref. 38 for review and ref. 4). Alternatively, it must be considered that other pathways capable of stimulating cGMP production (e.g., atrial natriuretic peptides; ANP) may be upregulated in these animals. In this regard, Gyrurko and colleagues have demonstrated recently increased ANP in NOS3−/− mice, which results in cGMP levels similar to WT controls (5). The observation that isoproterenol increases cGMP is consistent with β3-agonist linkage to NO resulting in cGMP elevation (11). The absence of this response in β3−/− is further evidence of a signaling linkage between the β3-adrenoceptor and NOS.

Sympathetic activation of the β-adrenergic pathway in the heart produces positive inotropic, chronotropic, and lusitropic responses. These responses are mediated through the activation of the β1- and β2-adrenoceptors coupled to a stimulatory G protein (Gs) (39), which in turn stimulates cAMP production by adenyl cyclase (AC) (39). cAMP, through cAMP-dependent protein kinase-A (PKA), directly augments the l-type Ca++ current (ICa), which enhances excitation-contraction coupling (40). The best-understood signaling pathway opposing β-adrenergic cardiac activation is mediated by muscarinic-cholinergic stimulation of the heart (41, 42). Muscarinic receptors are coupled to an inhibitory G protein (Gi) that both inhibits AC production of cAMP and stimulates NO production via NOS3 (42). NO, which stimulates the production of cGMP, can have both cGMP-dependent (43–47) and independent (48–50) inotropic effects. Thus, the traditional view of cardiac inotropic regulation by the autonomic nervous system is that of two limbs of the autonomic nervous system acting through separate receptor/signaling pathways, exerting opposing forces on myocardial regulation.

Description of β3-adrenoceptors in the heart (13) activated by traditional β-agonists, leading to negative inotropic effects, has raised the possibility that there may be negative feedback over contractility within the sympathetic pathway itself. Initially, β3-adrenoceptors were found to be expressed in adipose tissues, where their activation promotes lipolysis and energy expenditure, and in gastrointestinal tissues (16–18), where they regulate motility. Recently, unique β3-selective agonists have been shown to cause negative inotropic effects in the heart (12, 13, 19, 51) that are accompanied by increases in NO and production of cGMP (12). Inhibition of NO attenuates the negative inotropic effects of β3-selective agonists (12), suggesting that β3-adrenoceptor activation is coupled to NOS stimulation. Activation of NOS by the β3-adrenergic pathway may be through a Gαo protein, similar to the muscarinic system (42), since the negative inotropic effects of β3-adrenergic stimulation are attenuated by pertussis-toxin treatment (12, 13). Unlike the muscarinic-cholinergic pathway, the β3 pathway may serve as negative feedback over sympathetic activation as β3 receptors are activated by the same agonists that activate β1,2-adrenoceptors. These findings suggest a role for the β3-adrenoceptor in inhibition of β1,2-adrenergic–stimulated positive inotropic effects in a pathway that is NO mediated.

Recent studies demonstrating that the β3-adrenoceptor is upregulated in humans (52) and animals (53) with heart failure (52) suggest that the β3-NO pathway has implications in the pathophysiology of heart failure. The failing heart is characterized by downregulation of both the β1- and β2-adrenoceptors (54), through protein kinase A or β-adrenoceptor kinase (55), and subsequent attenuation of catecholamine induced positive inotropy. Unlike the β1,2 subtypes, the β3-adrenoceptor is unlikely to be downregulated by these mechanisms (56). Given the high sympathetic tone associated with heart failure, the increased ratio of β3 to β1,2-adrenoceptors and their respective inotropic effects could have profound consequences important for myocardial function in the failing heart.

This study uses a miniature combined manometer-conductance catheter to acquire pressure-volume loops in mice, thus enabling integrated measurement of cardiovascular performance. This method allows for the simultaneous determination of load-independent measures of contractility (Ees, for example), preload, afterload, and heart rate. The combined conductance-manometer catheter permits accurate determination of cardiovascular performance and has been used to characterize cardiomyopathy in transgenic mice (24, 57). Use of this technique has also shown that β3−/− and NOS3−/− mice have smaller chamber sizes than WT mice. The latter can be attributed to myocardial hypertrophy (increased heart-weight/body-weight ratio), whereas the former appears to be related to diastolic filling abnormalities (increased tau). The pressure-volume data illustrate that these differences do not influence the contractile parameters reported here (i.e., Ees and dP/dt-IP are independent of chamber size). Diastolic abnormalities mediated by the β3-NO pathway are under active investigation by our laboratory.

In conclusion, we have established a physiologic role for the β3-adrenoceptor in the heart. Using transgenic mice lacking the β3-adrenoceptor, we have shown that the β3-adrenoceptor is responsible for NO-mediated negative feedback over β-adrenergic–stimulated positive inotropy.

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