Cardiac physiologic regulation of subtype specific adrenergic receptors in transgenic mice overexpressing $\beta_1$- and $\beta_2$-adrenergic receptors

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Objective Combination of $\beta_1$-adrenergic receptor (AR) blockade and $\beta_2$-AR activation might be a potential novel therapy for treating heart failure. However, use of $\beta$-AR agonists and/or antagonists in the clinical setting is controversial because of the lack of information on cardiac inotropic or chronotropic regulation by AR signaling.

Methods In this study, we performed hemodynamic evaluation by examining force frequency response (FFR), Frank-Starling relationship, and response to a non-selective $\beta$-AR agonist (isoproterenol) in hearts isolated from 6-month-old transgenic (TG) mice overexpressing $\beta_1$- and $\beta_2$-ARs ($\beta_1$- and $\beta_2$-AR TG mice, respectively).

Results Cardiac physiologic consequences of $\beta_1$- and $\beta_2$-AR overexpression resulted in similar maximal response to isoproterenol and faster temporary decline of positive inotropic response in $\beta_2$-AR TG mice. $\beta_1$-AR TG mice showed a pronounced negative limb of FFR, whereas $\beta_2$-AR TG mice showed high stimulation frequencies with low contractile depression during FFR. In contrast, Frank-Starling relationship was equally enhanced in both $\beta_1$- and $\beta_2$-AR TG mice.

Conclusion Hemodynamic evaluation performed in the present showed a difference in $\beta_1$- and $\beta_2$-AR signaling, which may be due to the difference in the desensitization of $\beta_1$- and $\beta_2$-ARs.

Keywords Adrenergic receptors; Transgenic mice; Isoproterenol; Inotropic; Chronotropic

What is already known
Combination of $\beta_1$-adrenergic receptor (AR) blockade and $\beta_2$-AR activation is a potential novel therapy for treating heart failure. However, use of $\beta$-AR agonists and/or antagonists in the clinical setting is controversial because of the lack of information on cardiac inotropic or chronotropic regulation by AR signaling.

What is new in the current study
Results of hemodynamic evaluation performed in the present study showed a difference in $\beta_1$- and $\beta_2$-AR signaling, which may be due to the difference in the desensitization of $\beta_1$- and $\beta_2$-ARs.

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INTRODUCTION

β₁- and β₂-adrenergic receptors (ARs), expressed on cardiomyocytes, participate in catecholamine-mediated enhancement of cardiac inotropic or chronotropic responses.1-3 Broad therapeutic spectrum of β₂-AR agonists is the rationale for combining selective β₂-AR blockade and moderate β₁-AR activation as potential novel therapy for preventing or treating the loss of ventricular function, and for improving adrenergic signaling and responsiveness during heart failure.4-7 However, the use of β₁-AR agonists for treating heart failure symptoms is controversial because of concerns associated with their efficacy, regulation of receptor signaling, and potential adverse effects.8-11 Limited number of studies have assessed therapeutic targeting of β₁-AR compared with that of β₂-AR by using force frequency response (FFR), myofibril length-dependent mechanisms (Frank-Starling relationship), and receptor systems regulating cardiac inotropes in normal and failing hearts.

Therefore, in the present study, we examined the specific contribution of β₁- and β₂-ARs to intrinsic cardiac regulatory mechanisms. We developed transgenic (TG) mice by using a previously described method;12 performed hemodynamic evaluation, including FFR and Frank-Starling relationship assessment; and examined response to a β₂-AR agonist (isoproterenol) by using the hearts isolated from TG mice with comparable levels of β₁- and β₂-AR overexpression.

METHODS

TG mice

TG mice overexpressing cardiac-specific β₁- and β₂-ARs (β₁- and β₂-AR TG mice) were developed, as described previously.12 Briefly, wild-type human β₁- and β₂-AR cDNA was ligated to the SalI site (exon 3) of a full-length 5.5-kb α-myosin heavy chain promoter. The linearized constructs were injected into the male pronuclei of fertilized FVB/N mouse oocytes, and the oocytes were implanted into the oviducts of pseudopregnant female mice. Genomic DNA isolated from mouse tail-cuts was screened for the transgenes by using FFR and Frank-Starling relationship assessment; and examined response to a β₂-AR agonist (isoproterenol) by using the hearts isolated from TG mice with comparable levels of β₁- and β₂-AR overexpression.

Isolated work–performing hearts

Experimental conditions used for heart preparations have been described previously.3 Mice were anesthetized by intraperitoneally injecting 100 mg/kg sodium Nembutal and 1.5 units heparin to prevent microthrombus formation (n = 15/group). The heart and aorta were attached to a 20-gauge cannula, and temporary retrograde perfusion was performed using oxygenated Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 0.5 mM Na-EDTA, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, and 11 mM glucose saturated with 95% O₂ and 5% CO₂). A polyethylene-50 catheter was inserted into the apex of the left ventricle to measure intraventricular pressure. The pulmonary vein was connected to another cannula, and antegrade perfusion was performed using a basal workload of 300 mmHg mL/min (6 mL venous return and 50 mmHg mean aortic pressure). The hearts were equilibrated for 20 minutes. Atrial pressure was monitored using the sidearm of the left atrial cannula, and left ventricular pressure signals were digitized at 1 kHz and were analyzed offline by using BioBench software (National Instruments, Austin, TX, USA). The first positive and negative derivatives of the left intraventricular pressure curve (maximal rate pressure development [+dP/dt] and maximal rate pressure decline [-dP/dt]), duration of contraction and relaxation (time to peak pressure [TPP]), and time to half relaxation were calculated. TPP and time to half relaxation were normalized using peak systolic pressure and half relaxation time, respectively, because they depended upon the extent of pressure development. Peak pressure for normalizing TPP was calculated by subtracting end-diastolic pressure from systolic pressure. Half relaxation pressure for normalizing time to half relaxation was calculated using the following formula: (systolic pressure–diastolic pressure)/2.

FFR was measured by pacing the hearts with electrodes connected to aortic and venous return cannulae with a Grass SD9 stimulator (Grass Instruments, West Warwick, RI, USA). A primary-phase negative FFR was induced over a low-frequency range of 1 to 3 Hz and was not used for performing assessments in the present study. The hearts were stimulated from 4 to 12 Hz, with increments of 60 beats/min, to induce FFR over a frequency range similar to the physiological heart rate. These stimulation frequencies induced secondary-phase positive and negative FFRs that were used for analyzing frequency-dependent changes in cardiac +dP/dt and -dP/dt.

Frank-Starling curves were generated by altering ventricular afterloads through a graded aortic flow constriction. Pressure loading was performed by increasing afterloads (aortic resistance) until contractility was no longer elevated and by keeping venous return constant (6 mL/min). Cardiac work at different aortic resistances was calculated and was expressed as mmHg mL/min.
Drug infusion
Cardiac responses to the infusion of a nonselective β-AR agonist isoproterenol (Sigma-Aldrich Co., Saint Louis, MO, USA) were determined after assessing baseline cardiac responses to 10^{-7} M isoproterenol. Stimulation of β_1- and β_2-ARs with isoproterenol produced various inotropic and chronotropic responses whose magnitudes were approximately saturated after the infusion of 10^{-7} M isoproterenol. After determining the maximum response, time courses of pressure-derived parameters (+dP/dt and -dP/dt) were analyzed over 40 minutes, with 5-minute intervals.

Statistical analysis
All data are presented as mean±standard error. Statistical significance of dP/dt was estimated using one- and two-way analysis of variance and repeated measures with IBM SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA). Differences among groups at specific time points, stimulation frequencies (Hz), and pressure (mmHg) were assessed by performing one-way analysis of variance and post hoc Bonferroni test. P < 0.05 was considered statistically significant.

RESULTS
Myocardial hypertrophy and physiological function
β_1- and β_2-AR TG mice showed higher heart/body weight ratios than wild-type mice (3.91 ± 0.17 and 3.78 ± 0.14, respectively, vs. 3.61 ± 0.07 mg/g; P < 0.05); however, this difference was not statistically significant. Myocardial cell diameter showed the same trend as the heart/body weight ratios, with β_1- and β_2-AR TG mice showing greater myocardial diameter than wild-type mice; however, this difference was also not statistically significant (data not shown). Table 1 shows that cardiac-specific overexpression of both β_1- and β_2-AR enhanced cardiac function in TG mice. Cardiac contractility and relaxation and heart rate in β_1- and β_2-AR TG mice were significantly higher than those in wild-type mice. No significant differences were observed between cardiac parameters of β_1- and β_2-AR TG mice; however, these parameters were

| Table 1. Baseline hemodynamic parameters of the isolated work-performing hearts of wild-type mice and β_1- and β_2-AR-overexpressing TG mice |
|-----------------------------------------------|
|                                           |
| Wild-type mice (n = 5) | β_1-AR TG mice (n = 5) | β_2-AR TG mice (n = 5) |
| SP (mmHg) | 132.0 ± 4.5 | 158.9 ± 9.0^* | 167.0 ± 19^* |
| DP (mmHg) | -7.2 ± 3.2 | -32.0 ± 2.4 | -38.2 ± 17.6 |
| EDP (mmHg) | 6.4 ± 2.1 | 6.0 ± 1.0 | 3.7 ± 2.7 |
| +dP/dt (mmHg/sec) | 3.863 ± 85 | 5.718 ± 594^* | 5.901 ± 749^* |
| -dP/dt (mmHg/sec) | 2.852 ± 272 | 5.085 ± 603^* | 5.149 ± 342^* |
| HR | 259 ± 8 | 347 ± 12 | 335 ± 12 |
| TPP (ms/mmHg) | 0.41 ± 0.04 | 0.26 ± 0.02 | 0.30 ± 0.03 |
| TR1/2 (ms/mmHg) | 0.65 ± 0.03 | 0.44 ± 0.04 | 0.46 ± 0.06^* |

Values are presented as mean ± standard error.

AR, adrenergic receptor; TG, transgenic; SP, left ventricular systolic pressure; DP, left ventricular diastolic pressure; EDP, left ventricular end diastolic pressure; +dP/dt, maximal rate pressure development; -dP/dt, maximal rate pressure decline; TPP, time to peak pressure (normalized to peak pressure); TR1/2, half relaxation pressure (normalized to half relaxation pressure).

*P < 0.05, β_1- versus β_2-AR TG mice versus wild-type mice.

Fig. 1. (A) Maximum inotropic and lusitropic responses to isoproterenol in wild-type (WT) mice and β_1- and β_2-adrenergic receptor (AR) transgenic (TG) mice. All the measurements were obtained under maximal responses after infusion of 10^{-7} M isoproterenol. *P<0.05, β_1- and β_2-AR TG mice versus WT mice. (B, C) The time course of left ventricular +dP/dt and -dP/dt responses in β_1- and β_2-AR TG mice to 10^{-7} M isoproterenol infusion. Inotropic responses in β_2-AR TG mice were higher than those in β_1-AR TG mice. *P<0.05, β_1- versus β_2-AR TG mice. Results are presented as mean ± standard error.
slightly improved in β₂-AR TG mice compared with those in β₁-AR TG mice.

AR subtype–specific time-dependent effect of the β-AR agonist on the inotropic responses of the isolated work-performing hearts

Baseline cardiac contractility and relaxation and heart rate were similar between β₁- and β₂-AR TG mice (Table 1). Stimulation of β₁- and β₂-AR TG mice with the β-AR agonist isoproterenol (10⁻⁷ M) enhanced cardiac contractility and relaxation. Both β₁- and β₂-AR TG mice showed similar increases in +dP/dt and -dP/dt after maximum isoproterenol stimulation compared with wild-type mice (Fig. 1A). Next, we analyzed inotropic responses over 30 minutes and observed that time-dependent return to basal contraction rate increased in the hearts of β₂-AR TG mice compared with that in the hearts of wild-type mice and β₁-AR TG mice (Fig. 1B, C).

FFR and response to loading of isolated work-performing hearts

Data obtained using different frequencies (4 to 11 Hz) for inducing the secondary phase are shown in Fig. 2. β₁- and β₂-AR TG mice showed a flattened secondary phase at frequencies 4 to 9 Hz, with a critical decline observed at the limb of 9 Hz. Negative FFR of the isolated work-performing hearts was induced at a high-frequency range (9 to 12 Hz). Differences between the hearts of β₁- and β₂-AR TG mice were observed in the secondary-phase negative FFR. β₂-AR TG mice showed enhanced contractility (+dP/dt) and relaxation (-dP/dt) indices at frequencies that induced the

Fig. 2. Force frequency response (FFR) of the work-performing hearts of mice at pacing rates of 4 to 12 Hz (secondary-phase positive and negative FFR). Compared with wild-type (WT) mice, +dP/dt (A) and -dP/dt (B) in β₁- and β₂-adrenergic receptor (AR) transgenic (TG) mice augmented over a range of frequencies of positive FFR (4 to 9 Hz). The β₂-AR TG mice demonstrate an augmented contractility and relaxation over the positive and negative phases of the FFR at stimulation frequencies from 4 to 14 Hz in the WT, and β₁- and β₂-AR TG mice. *P<0.05, β₁- versus β₂-AR TG mice.

Fig. 3. Responses of the isolated work-performing hearts to preload over a range of cardiac work from 100 to 600 mmHg/mL min. Baseline recordings were obtained under similar conditions: mean aortic pressure (afterload, 50 mmHg) and venous return (preload, 6 mL/min; left ventricular minute work 300 mmHg/mL min. +dP/dt (A) and -dP/dt (B) of experimental groups were plotted against gradually increasing afterloads at a constant venous return. In both β₁- and β₂-adrenergic receptor transgenic mice, +dP/dt and -dP/dt increased at different cardiac workloads, indicating augmented contractility at low and high cardiac workloads.
contractile depression of the heart. Although no differences were observed in +dP/dt and -dP/dt among mice in the three groups at the initial frequency of 4 Hz, β2-AR TG mice showed significantly higher +dP/dt than β1-AR TG and wild-type mice (4,665 ± 384, 2,805 ± 245 mmHg/sec, P < 0.05).

**Frank-Starling**

The capacity of the ventricle to adjust the force of contraction as a function of cardiac load is called Frank-Starling mechanism. Cardiac minute work varied from 50 mmHg mL/min to the maximal level of mean aortic pressure that was generated at a given venous return of 6 mL/min to determine the extent to which β1- and β2-AR TG mice could be subjected to increasing workload. The slope of the initial part of the Frank-Starling left ventricular functional curve (range, 0 to 250 mmHg mL/min) was calculated by performing linear regression analysis. This slope reflected the changes in myofibril length-dependent activation, and its γ-intercept indicated the contractile status under low cardiac load.13 Wild-type mice and both β1- and β2-AR TG mice showed a strong positive correlation among +dP/dt, -dP/dt, and cardiac work (Fig. 3). At all workloads, the hearts of wild-type mice showed lower absolute values of +dP/dt and -dP/dt than those of β1- and β2-AR TG mice. The slope of workload response was also higher for both β1- and β2-AR TG mice than for wild-type mice, reflecting steeper functional response to high workloads (intercepts [+dP/dt]: β1-AR TG mice, 4,168 ± 450; β2-AR TG mice, 4,123 ± 266; wild-type mice, 1,778 ± 158; slopes: β1-AR TG mice, 27.6 ± 7.1; β2-AR TG mice, 22.9 ± 6.2; wild-type mice, 12.5 ± 2.5; intercepts [-dP/dt]: β1-AR TG mice, 4,027 ± 604; β2-AR TG mice, 4,580 ± 635; wild-type mice, 2,367 ± 126; slopes [-dP/dt]: β1-AR TG mice, 36.8 ± 9.2; β2-AR TG mice, 28.8 ± 6.9; wild-type mice, 16.5 ± 2.3).

**DISCUSSION**

In this study, we examined the accelerated temporal decline in inotropic cardiac response after acute infusion of isoproterenol, a nonselective β-AR agonist, in β2-AR TG mice. Moreover, we compared the functional effects of β1- and β2-AR overexpression in the hearts of TG mice and established its physiological effects based on the differences in AR signaling.

Petrashevskaya et al.13 reported that inotropic stimulation mediated by β1- and β2-ARs decreased in 2-month-old TG mice after long-term exposure to β-AR agonists, which was similar to that observed in the present study. They also showed that faster functional desensitization in response to acute agonist stimulation in 2-month-old β2-AR TG mice did not salvage the loss of agonist responsiveness in later life, which was similar to that in 2-month-old β1-AR TG mice. In the present study, 6-month-old β1-AR TG mice showed rapid functional desensitization of ARs compared with 6-month-old β2-AR TG mice. These findings suggested that compared with β1-AR overexpression, the effect of β2-AR overexpression was bifurcated at the level of Gi proteins, with more prominent Gi2 upregulation in β2-AR TG mice, indicating that Gi2 contributed to the prolonged survival of and delayed cardiac pathology in β2-AR TG mice.14 However, downstream signaling effectors connecting the β2-AR/Gi2 axis to cardiac protection have not been established. Potentially, it may mitigate the deleterious effects of catecholamine signaling and contribute to different aspects of protective changes associated with β2-AR/Gi coupling or may decrease cardiac responsiveness to various Gq protein-related pro-growth factors.

FFR as well as the Frank-Starling mechanism are essential for adjusting cardiac contractile function to hemodynamic needs.8,13 In the present study, we observed that the effect of β1- and β2-AR overexpression differed at the negative descending limb of the secondary FFR. At higher frequencies (9 to 12 Hz), β2-AR TG mice showed less inotropic depression than β1-AR TG mice, resulting in a secondary-phase negative FFR. Endoh13 reported that an enhanced positive limb of FFR was observed upon acute activation of ARs. However, this was not detected in the hearts of both β1- and β2-AR TG mice in the present study.

In contrast, the Frank-Starling curves, which primarily reflect myofibril length-dependent changes in Ca2+ sensitivity of myofilibrillar force, were steeper for both β1- and β2-AR TG mice in the present study. Protein kinase A (PKA) mediates the acute effects of the phosphorylation of troponin I and troponin C, with different effects on the sarcomere length dependence of Ca2+ sensitivity.15-17 Myofilibril length-dependent changes in Ca2+ sensitivity were unchanged in both normal and failing cardiomyocytes after acute incubation with PKA, indicating that PKA-mediated phosphorylation was not involved in sarcomere length-dependent force development in the failing heart.17 Long-term activation of both β1- and β2-ARs enhances cardiac function during acute increases in afterload, which is partly mediated by the Frank-Starling mechanism.17 Thus, both β1- and β2-ARs may contribute to more efficient Ca2+-myofibril interaction, actin- and myosin-binding protein C phosphorylation, and steeper ventricular function curves.

Together, these results indicated that both β1- and β2-AR TG mice showed enhanced maximal response to the β-AR agonist. However, inotropic support was significantly downregulated in the hearts of β2-AR TG mice after long-term exposure to the β-AR agonist, which may have contributed to the accelerated functional desensitization of β2-AR. Cardiac contractility (+dP/dt) and
relaxation (-dP/dt) were higher in β₂-AR TG mice at stimulation frequencies. Frank-Starling responses were steeper in both β₁- and β₂-AR TG mice. Thus, hemodynamic evaluation performed in the present study indicated a difference in β₁- and β₂-AR signaling and indicated that this difference was caused by the differential desensitization of β₂- and β₁-ARs. Moreover, our results provided evidence that selective β₁-AR blockade and β₂-AR activation may be a novel therapy for treating heart failure.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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