Clinical and Experimental Emergency Medicine

Clin Exp Emerg Med 2016;3(3):175-180
http://dx.doi.org/10.15441/ceem.16.141

Original Article

eISSN: 2383-4625

Cardiac physiologic regulation of sub-type specific adrenergic receptors in transgenic mice overexpressing β_1- and β_2-adrenergic receptors

Ka Eul Kim[1]*, Hyun-Jin Tae[2,3]*, Petrashevskaya Natalia[4], Jae-Chul Lee[5], Ji Hyeon Ahn[5], Joon Ha Park[5], In Hye Kim[5], Taek Geun Ohk[2], Chan Woo Park[2], Jun Hwi Cho[2], Moo-Ho Won[5]

[1]Department of Biomedical Science, Research Institute of Bioscience and Biotechnology, Hallym University, Chuncheon, Korea
[2]Department of Emergency Medicine, Kangwon National University School of Medicine, Chuncheon, Korea
[3]Bio-Safety Research Institute, College of Veterinary Medicine, Chonbuk National University, Iksan, Korea
[4]CardioPulmonary Genomics Program, Departments of Medicine and Physiology, University of Maryland School of Medicine, Baltimore, MD, USA
[5]Department of Neurobiology, Kangwon National University School of Medicine, Chuncheon, Korea

Objective Combination of β_1-adrenergic receptor (AR) blockade and β_2-AR activation might be a potential novel therapy for treating heart failure. However, use of β-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 β-AR agonist (isoproterenol) in hearts isolated from 6-month-old transgenic (TG) mice overexpressing β_1- and β_2-ARs (β_1- and β_2-AR TG mice, respectively).

Results Cardiac physiologic consequences of β_1- and β_2-AR overexpression resulted in similar maximal response to isoproterenol and faster temporary decline of positive inotropic response in β_2-AR TG mice. β_1-AR TG mice showed a pronounced negative limb of FFR, whereas β_2-AR TG mice showed high stimulation frequencies with low contractile depression during FFR. In contrast, Frank-Starling relationship was equally enhanced in both β_1- and β_2-AR TG mice.

Conclusion Hemodynamic evaluation performed in the present showed a difference in β_1- and β_2-AR signaling, which may be due to the difference in the desensitization of β_1- and β_2-ARs.

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

Capsule Summary

What is already known

Combination of β_1-adrenergic receptor (AR) blockade and β_2-AR activation is a potential novel therapy for treating heart failure. However, use of β-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 between β_1- and β_2-AR signaling, which may be because of a difference in the desensitization of β_1- and β_2-ARs.

Received: 30 March 2016
Revised: 29 April 2016
Accepted: 10 May 2016

Correspondence to:
Jun Hwi Cho
Department of Emergency Medicine, Kangwon National University Hospital, 156 Baengnyeong-ro, Chuncheon 24289, Korea
E-mail: cjhemd@kangwon.ac.kr

Moo-Ho Won
Department of Neurobiology, Kangwon National University School of Medicine, 1 Gangwondaehak-gil, Chuncheon 24341, Korea
E-mail: mhwon@kangwon.ac.kr

*These two authors contributed equally to this work.

How to cite this article:
Kim KE, Tae HJ, Natalia P, Lee JC, Ahn JH, Park JH, Kim IH, Ohk TG, Park CW, Cho JH, Won MH. Cardiac physiologic regulation of sub-type specific adrenergic receptors in transgenic mice overexpressing β1- and β2-adrenergic receptors. Clin Exp Emerg Med 2016;3(3): 175-180.

INTRODUCTION

β_1- and β_2-adrenergic receptors (ARs), expressed on cardiomyocytes, participate in catecholamine-mediated enhancement of cardiac inotropic or chronotropic responses.[1-3] Broad therapeutic spectrum of β_2-AR agonists is the rationale for combining selective β_1-AR blockade and moderate β_2-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 β_2-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 β_2-AR compared with that of β_1-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 β_1- and β_2-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 β-AR agonist (isoproterenol) by using the hearts isolated from TG mice with comparable levels of β_1- and β_2-AR overexpression.

METHODS

TG mice

TG mice overexpressing cardiac-specific β_1- and β_2-ARs (β_1- and β_2-AR TG mice) were developed, as described previously.[1,2] Briefly, wild-type human β_1- and β_2-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 performing targeted PCR with one primer against the α-myosin heavy chain promoter and one primer against the TG cDNA. β_1- and β_2-AR TG mice were examined at 6 months of age when they developed phenotypes independent of the confounding effects of cardiac growth and of the changes in the functional coupling of β-ARs. Procedures for animal studies were approved by the Institutional Animal Care and Use Committee of the University of Maryland Baltimore.

Isolated work-performing hearts

Experimental conditions used for heart preparations have been described previously.[2] 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 $CaCl_2$, 0.5 mM Na-EDTA, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11 mM glucose saturated with 95% O_2 and 5% CO_2). A polyethelene-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[13] 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.[13] 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 ± 6.7
EDP (mmHg)	6.4 ± 2.1	1.3 ± 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.

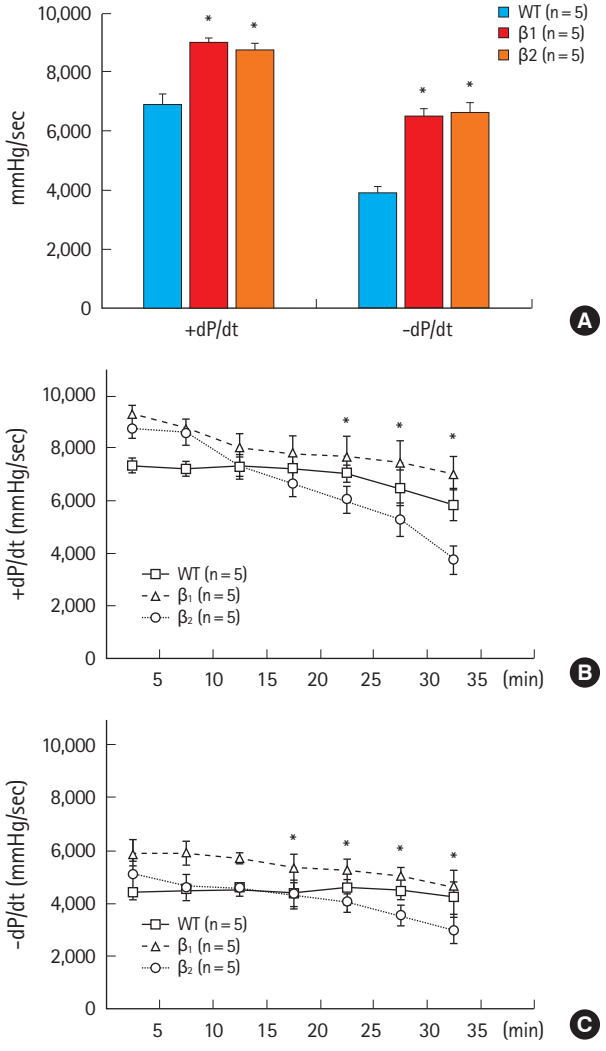

Fig. 1. (A) Maximum inotropic and lusitropic responses to isoproterenol in wild-type (WT) mice and β_1- and β_2-adrenergic receptor (AR) transgenic (TG) mice. All the measurements were obtained under maximal responses after infusion of 10^{-7} M isoproterenol. *$P<0.05$, β_1- and β_2-AR TG mice versus 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_1-AR TG mice were higher than those in β_2-AR TG mice. *$P<0.05$, β_1- versus β_2-AR TG mice. Results are presented as mean ± standard error.

slightly improved in β_2-AR TG mice compared with those in β_1-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 β_1- and β_2-AR TG mice (Table 1). Stimulation of β_1- and β_2-AR TG mice with the β-AR agonist isoproterenol (10^{-7} M) enhanced cardiac contractility and relaxation. Both β_1- and β_2-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 β_2-AR TG mice compared with that in the hearts of wild-type mice and β_1-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. β_1- and β_2-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 β_1- and β_2-AR TG mice were observed in the secondary-phase negative FFR. β_2-AR TG mice showed enhanced contractility (+dP/dt) and relaxation (-dP/dt) indices at frequencies that induced the

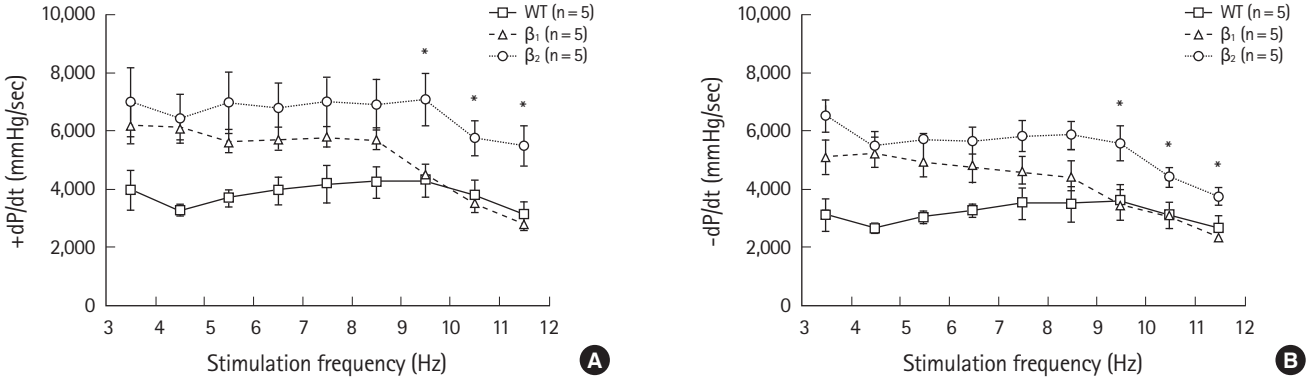

Fig. 2. Force frequency response (FFR) of the work-performing hearts of mice at pacing rates of 4 to 12 Hz (secondary-phase positive and negative FFR). Compared with wild-type (WT) mice, +dP/dt (A) and -dP/dt (B) in β_1- and β_2-adrenergic receptor (AR) transgenic (TG) mice augmented over a range of frequencies of positive FFR (4 to 9 Hz). The β_2-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 β_1- and β_2-AR TG mice. *P<0.05, β_1- versus β_2-AR TG mice.

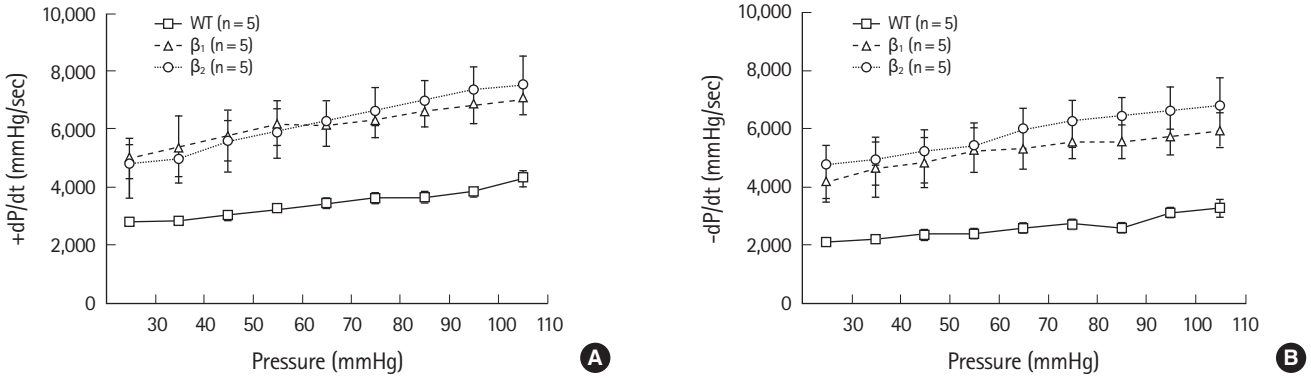

Fig. 3. Responses of the isolated work-performing hearts to preload over a range of cardiac work from 100 to 600 mmHg/mL min. Baseline recordings were obtained under similar conditions: mean aortic pressure (afterload, 50 mmHg) and venous return (preload, 6 mL/min; left ventricular minute work 300 mmHg/mL min. +dP/dt (A) and -dP/dt (B) of experimental groups were plotted against gradually increasing afterloads at a constant venous return. In both β_1- and β_2-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, β_2-AR TG mice showed significantly higher +dP/dt than β_1-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 β_1- and β_2-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 y-intercept indicated the contractile status under low cardiac load.[13] Wild-type mice and both β_1- and β_2-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 β_1- and β_2-AR TG mice. The slope of workload response was also higher for both β_1- and β_2-AR TG mice than for wild-type mice, reflecting steeper functional response to high workloads (intercepts [+dP/dt]: β_1-AR TG mice, 4,168±450; β_2-AR TG mice, 4,123±266; wild-type mice, 1,778±158; slopes: β_1-AR TG mice, 27.6±7.1; β_2-AR TG mice, 22.9±6.2; wild-type mice, 12.5±2.5; intercepts [-dP/dt]: β_1-AR TG mice, 4,027±604; β_2-AR TG mice, 4,580±635; wild-type mice, 2,367±126; slopes (-dP/dt): β_1-AR TG mice, 36.8±9.2; β_2-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 β_2-AR TG mice. Moreover, we compared the functional effects of β_1- and β_2-AR overexpression in the hearts of TG mice and established its physiological effects based on the differences in AR signaling.

Petrashevskaya et al.[12] reported that inotropic stimulation mediated by β_1- and β_2-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 β_2-AR TG mice did not salvage the loss of agonist responsiveness in later life, which was similar to that in 2-month-old β_1-AR TG mice. In the present study, 6-month-old β_2-AR TG mice showed rapid functional desensitization of ARs compared with 6-month-old β_1-AR TG mice. These findings suggested that compared with β_1-AR overexpression, the effect of β_2-AR overexpression was bifurcated at the level of Gi proteins, with more prominent Gi2 upregulation in β_2-AR TG mice, indicating that Gi2 contributed to the prolonged survival of and delayed cardiac pathology in β_2-AR TG mice.[14] However, downstream signaling effectors connecting the β_2-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 β_2-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 β_1- and β_2-AR overexpression differed at the negative descending limb of the secondary FFR. At higher frequencies (9 to 12 Hz), β_2-AR TG mice showed less inotropic depression than β_1-AR TG mice, resulting in a secondary-phase negative FFR. Endoh[13] 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 β_1- and β_2-AR TG mice in the present study.

In contrast, the Frank-Starling curves, which primarily reflect myofibril length-dependent changes in Ca^{2+} sensitivity of myofibrillar force, were steeper for both β_1- and β_2-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 Ca^{2+} sensitivity.[15-17] Myofibril length-dependent changes in Ca^{2+} 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 β_1-and β_2-ARs enhances cardiac function during acute increases in afterload, which is partly mediated by the Frank-Starling mechanism.[17] Thus, both β_1-and β_2-ARs may contribute to more efficient Ca^{2+}-myofibril interaction, actin- and myosin-binding protein C phosphorylation, and steeper ventricular function curves.

Together, these results indicated that both β_1- and β_2-AR TG mice showed enhanced maximal response to the β-AR agonist. However, inotropic support was significantly downregulated in the hearts of β_2-AR TG mice after long-term exposure to the β-AR agonist, which may have contributed to the accelerated functional desensitization of β_2-AR. Cardiac contractility (+dP/dt) and

relaxation (-dP/dt) were higher in β_2-AR TG mice at stimulation frequencies. Frank-Starling responses were steeper in both β_1- and β_2-AR TG mice. Thus, hemodynamic evaluation performed in the present study indicated a difference in β_1- and β_2-AR signaling and indicated that this difference was caused by the differential desensitization of β_2- and β_1-ARs. Moreover, our results provided evidence that selective β_1-AR blockade and β_2-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.

ACKNOWLEDGMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2014R1A1A2057263). Petrashevskaya Natalia was supported by an NIH grant HL077101 awarded to S. Liggett.

REFERENCES

1. Liggett SB, Tepe NM, Lorenz JN, et al. Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: critical role for expression level. Circulation 2000;101:1707-14.
2. Mialet Perez J, Rathz DA, Petrashevskaya NN, et al. Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 2003;9:1300-5.
3. Xiao RP, Zhu W, Zheng M, et al. Subtype-specific beta-adrenoceptor signaling pathways in the heart and their potential clinical implications. Trends Pharmacol Sci 2004;25:358-65.
4. Ahmet I, Krawczyk M, Heller P, Moon C, Lakatta EG, Talan MI. Beneficial effects of chronic pharmacological manipulation of beta-adrenoreceptor subtype signaling in rodent dilated ischemic cardiomyopathy. Circulation 2004;110:1083-90.
5. Dorn GW 2nd, Tepe NM, Lorenz JN, Koch WJ, Liggett SB. Low- and high-level transgenic expression of beta2-adrenergic receptors differentially affect cardiac hypertrophy and function in Galphaq-overexpressing mice. Proc Natl Acad Sci U S A 1999;96:6400-5.
6. Du XJ, Gao XM, Jennings GL, Dart AM, Woodcock EA. Preserved ventricular contractility in infarcted mouse heart overexpressing beta(2)-adrenergic receptors. Am J Physiol Heart Circ Physiol 2000;279:H2456-63.
7. Shah AS, Lilly RE, Kypson AP, et al. Intracoronary adenovirus-mediated delivery and overexpression of the beta(2)-adrenergic receptor in the heart: prospects for molecular ventricular assistance. Circulation 2000;101:408-14.
8. Brodde OE, Bruck H, Leineweber K. Cardiac adrenoceptors: physiological and pathophysiological relevance. J Pharmacol Sci 2006;100:323-37.
9. Du XJ, Autelitano DJ, Dilley RJ, Wang B, Dart AM, Woodcock EA. beta(2)-adrenergic receptor overexpression exacerbates development of heart failure after aortic stenosis. Circulation 2000;101:71-7.
10. Vinge LE, Raake PW, Koch WJ. Gene therapy in heart failure. Circ Res 2008;102:1458-70.
11. Yoo B, Lemaire A, Mangmool S, et al. Beta1-adrenergic receptors stimulate cardiac contractility and CaMKII activation in vivo and enhance cardiac dysfunction following myocardial infarction. Am J Physiol Heart Circ Physiol 2009;297:H1377-86.
12. Petrashevskaya N, Gaume BR, Mihlbachler KA, Dorn GW 2nd, Liggett SB. Bitransgenesis with beta(2)-adrenergic receptors or adenylyl cyclase fails to improve beta(1)-adrenergic receptor cardiomyopathy. Clin Transl Sci 2008;1:221-7.
13. Endoh M. Force-frequency relationship in intact mammalian ventricular myocardium: physiological and pathophysiological relevance. Eur J Pharmacol 2004;500:73-86.
14. Foerster K, Groner F, Matthes J, Koch WJ, Birnbaumer L, Herzig S. Cardioprotection specific for the G protein Gi2 in chronic adrenergic signaling through beta 2-adrenoceptors. Proc Natl Acad Sci U S A 2003;100:14475-80.
15. Hanft LM, McDonald KS. Sarcomere length dependence of power output is increased after PKA treatment in rat cardiac myocytes. Am J Physiol Heart Circ Physiol 2009;296:H1524-31.
16. Komukai K, Kurihara S. Length dependence of Ca(2+)-tension relationship in aequorin-injected ferret papillary muscles. Am J Physiol 1997;273(3 Pt 2):H1068-74.
17. van der Velden J, de Jong JW, Owen VJ, Burton PB, Stienen GJ. Effect of protein kinase A on calcium sensitivity of force and its sarcomere length dependence in human cardiomyocytes. Cardiovasc Res 2000;46:487-95.