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Augmentation of Cardiac Contractility Mediated by the Human β_3-Adrenergic Receptor Overexpressed in the Hearts of Transgenic Mice

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Background—Stimulation of β_1- and β_2-adrenergic receptors (ARs) in the heart results in positive inotropy. In contrast, it has been reported that the β_3AR is also expressed in the human heart and that its stimulation leads to negative inotropic effects.

Methods and Results—To better understand the role of β_3ARs in cardiac function, we generated transgenic mice with cardiac-specific overexpression of 330 fmol/mg protein of the human β_3AR (TGβ_3 mice). Hemodynamic characterization was performed by cardiac catheterization in closed-chest anesthetized mice, by pressure-volume-loop analysis, and by echocardiography in conscious mice. After propranolol blockade of endogenous β_1- and β_2ARs, isoproterenol resulted in an increase in contractility in the TGβ_3 mice (30%), with no effect in wild-type mice. Similarly, stimulation with the selective human β_3AR agonist L-755,507 significantly increased contractility in the TGβ_3 mice (160%), with no effect in wild-type mice, as determined by hemodynamic measurements and by end-systolic pressure-volume relations. The underlying mechanism of the positive inotropy incurred with L-755,507 in the TGβ_3 mice was investigated in terms of β_3AR–G-protein coupling and adenylyl cyclase activation. Stimulation of cardiac membranes from TGβ_3 mice with L-755,507 resulted in a pertussis toxin–insensitive 1.33-fold increase in [^{35}S]GTPγS loading and a 1.6-fold increase in adenylyl cyclase activity.

Conclusions—Cardiac overexpression of human β_3ARs results in positive inotropy only on stimulation with a β_3AR agonist. Overexpressed β_3ARs couple to G_s and activate adenylyl cyclase on agonist stimulation. **(*Circulation*. 2001; 104:2485-2491.)**

Key Words: signal transduction ■ pharmacology ■ gene therapy ■ inotropic agents

β-Adrenergic receptors (βARs) are members of a family of G protein–coupled receptors that are stimulated by naturally occurring catecholamines. In the heart, both the β_1- and β_2AR subtypes are known to modulate cardiac function by producing positive inotropic and chronotropic effects. A third βAR subtype, the β_3AR,[1,2] has been found primarily in adipose tissue of rodents, in human omental tissue, and in the brown adipose tissue of newborns.[2] In human heart[3,4] and mouse heart,[5] β_3AR transcripts have been detected by sensitive methods, such as RNase protection assays and reverse transcription–polymerase chain reaction assays. Like β_1- and β_2ARs, the β_3AR couples to G_s to activate adenylyl cyclase, which in adipose tissue leads to lipolysis or thermogenesis.[2] It has also been shown that the β_3AR can couple to G_i, resulting in the attenuation of adenylyl cyclase stimulation and in the activation of the mitogen-activated protein kinase (MAPK) pathway.[6,7] Gauthier et al[8] proposed that the β_3AR is present and functional in the human heart. They showed that stimulation of human ventricular endomyocardial biopsies with BRL 37344, a β_3AR agonist, leads to a pertussis toxin (PTX)–sensitive negative inotropic effect, suggesting that in this system the β_3AR is coupled to G_i.[8]

To further explore the physiological consequences of activation of the β_3AR in cardiac contractility, we generated transgenic mice with cardiac-specific overexpression of the human β_3AR (TGβ_3 mice). β_3AR signal transduction was assessed both in vitro in cardiac membranes and in vivo by catheterization in intact mice.

Methods

Transgene Construction

The αMHC-HAβ_3AR transgene was constructed from a 5.5-kb *Sal*I-*Sal*I fragment containing the murine αMHC promoter[9] and the *Eco*RI-*Xba*I fragment containing the human β_3AR coding sequence

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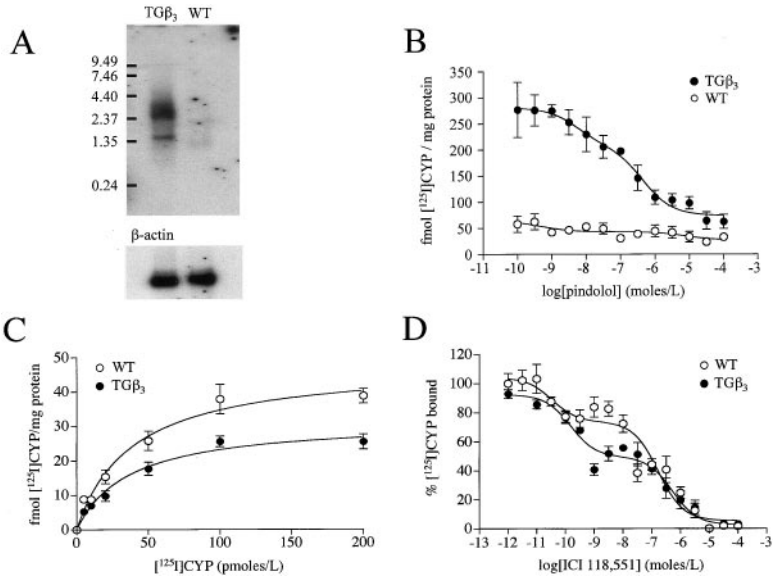

Figure 1. Characterization of overexpression of β_3AR in TGβ_3 and WT mice. A, Northern blot for human β_3AR expression in TGβ_3 mice. mRNA (10 μg) from TGβ_3 and WT hearts was probed with entire coding sequence of human β_3AR clone. Equal loading of sample was confirmed by reprobing filter with human β-actin probe. B, Competition binding assays were performed on membranes prepared from TGβ_3 (n=3) and WT (n=3) hearts incubated with 500 pmol/L [^{125}I]ICYP and various concentrations of pindolol. Nonspecific binding was determined with 0.1 mmol/L isoproterenol and was ≈30% and 70% of total binding in TGβ_3 and WT membranes, respectively. Estimated B_{max} was calculated with GraphPAD software equation for competitive binding to 2 receptor types with different K_d for radioligand (K_ds are constants). K_d for binding [^{125}I]ICYP to β_1/β_2ARs is 0.030 nmol/L,[24] and that for binding β_3ARs is 1 nmol/L.[2] C, TGβ_3 (n=5) and WT (n=5) cardiac membranes were incubated with 5 to 200 pmol/L [^{125}I]ICYP. D, TGβ_3 (n=4) and WT (n=4) cardiac membranes were incubated with 50 pmol/L [^{125}I]ICYP and increasing concentrations of ICI 118,551. Nonspecific binding was determined with 1 μmol/L propranolol. Estimated B_{max} was calculated with GraphPAD software.

(1 to 402 amino acids) with an NH_2-terminal hemagglutinin (HA) tag.[10] The αMHC-HAβ_3AR transgene was digested with *Spe*I-*Sac*I, purified with CsCl, and used for nuclear injection of oocytes by the Duke Comprehensive Cancer Center Transgenic Mouse Facility. One line of C57B6SJL/J mice that expressed the HAβ_3AR transgene was established. Studies were performed on mice 2 to 8 months of age.

Northern Blotting

mRNAs from heart tissue of both TGβ_3 and wild-type (WT) mice were separated by electrophoresis and transferred onto a nylon filter (Schleicher & Schuell) by standard techniques.[11] The filter was hybridized to a random primer radiolabeled probe corresponding to the entire coding region of the human β_3AR clone.

Ligand Binding, GTPγS Loading, and Adenylyl Cyclase Assays

Crude membranes were prepared from excised hearts, and ligand binding assays were performed as previously described.[9] [^{35}S]GTPγS loading and adenylyl cyclase assays were performed as previously described.[12,13]

Transthoracic Echocardiography

2D guided M-mode echocardiography was performed in conscious mice with an HDI 5000 echocardiograph (ATL) as previously described.[14]

Myocyte Isolation

Adult myocytes were isolated from WT and TGβ_3 mice as previously described.[15] After isolation, myocytes were fixed in 3% paraformaldehyde, and length and width were measured with a video edge-detection system (Crescent Electronics).

Hemodynamic Evaluation in Intact Anesthetized Mice

Cardiac catheterization was performed as described previously.[14] Mice were anesthetized with a mixture of ketamine (100 mg/kg IP) and xylazine (2.5 mg/kg IP), and after bilateral vagotomy, a 1.4F high-fidelity micromanometer catheter (Millar Instruments) was inserted into the right carotid artery and advanced retrogradely across the aortic valve.

Experimental Protocols

Protocol 1

Hemodynamic measurements were recorded at baseline and 45 to 60 seconds after the injection of isoproterenol (1000 pg IV). After hemodynamics returned to baseline, propranolol (0.05 μg/g body weight [BW] IV) was administered to block β_1- and β_2ARs. After return to baseline, hemodynamic measurements were again recorded before and after administration of isoproterenol.

Protocol 2

Hemodynamic measurements were recorded at baseline and 90 to 120 seconds after the injection of an incremental dose of L755,507 (0.25 to 4.0 μg IV).

Pressure-Volume Measurements

In separate experiments, in vivo pressure-volume (P-V) relations were determined as previously described.[14] Mice were anesthetized as described above and maintained by the administration of 0.5% to 1.0% isoflurane. The space and time resolutions of the sonomicrometry system are 0.015 mm and 0.001 seconds, respectively.

Data and Statistical Analyses

The digitized data were analyzed with a computer algorithm as previously described.[14] Data are expressed as mean±SEM. Unpaired Student's *t* tests and repeated-measures ANOVA were performed for statistical comparisons of the WT and TGβ_3 mice after agonist stimulation. Post hoc analysis was performed with a Scheffé test. For all tests, a value of $P<0.05$ was considered significant.

Results

Generation of β_3AR Overexpressing Mice

To investigate the biochemical and physiological consequences of overexpression of β_3ARs in heart, we generated transgenic mice with cardiac-restricted overexpression of the human β_3AR (TGβ_3). Transgene expression was documented by Northern analysis of mRNA from the heart (Figure 1A). As shown, expression was detected only in TGβ_3 mice and was absent in WT mice. Several transcripts were detected, corresponding to 1.4 kb and 3.0 to 4.0 kb in size. The range in sizes is probably the result of the utilization of a variety of transcriptional termination signals downstream of the integration site.

Characterization of the β_3AR Expression in TGβ_3 Mice

The level of β_3AR expression in cardiac membranes of the TGβ_3 mice was quantified by use of competition ligand-

TABLE 1. Physiological and Basal Hemodynamic Parameters in WT and TGβ_3 Mice

	WT Mice (n=14)	TGβ_3 Mice (n=20)
Heart rate, bpm	436±78	441±58
LVSP, mm Hg	115±18	96±20*
LVEDP, mm Hg	7±4	4±3
LV dP/dt$_{max}$, mm Hg/s	9138±2341	6250±1111†
LV dP/dt$_{min}$, mm Hg/s	−7328±1771	−5729±1153*
BW, g	27.38±4.50	25.46±3.25†
Tibial length, mm	17.97±0.36	18.01±0.31
RV weight, mg	23.17±3.50	18.55±3.03†
LV weight, mg	91.74±14.57	69.56±9.16*
LV/BW, mg/g	3.37±0.27	2.75±0.33*
LV/tibial length, mg/mm	5.10±0.76	3.86±0.48*

LVSP indicates LV left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; LV dP/dt$_{max}$, maximal derivative of LV pressure; LV dP/dt$_{min}$, minimal derivative of LV pressure; and RV, right ventricular weight.

*$P<0.01$, †$P<0.001$ vs WT.

binding assays (Figure 1B) with the radioligand [^{125}I]iodocyanopindolol ([^{125}I]ICYP) and increasing concentrations of unlabeled pindolol. Binding data from membranes prepared from WT and TGβ_3 heart extracts were fit by a biphasic curve with a very small high-affinity component corresponding to β_1- and β_2ARs and a low-affinity phase corresponding to displacement of pindolol from the β_3ARs. From these data, the number of β_3ARs expressed in hearts of TGβ_3 mice (B$_{max}$) was calculated with GraphPAD software and was determined to be 330±36 fmol/mg membrane protein.

To characterize the endogenous expression levels of β_1- and β_2ARs in the transgenic heart, saturation binding experiments were performed with 5 to 200 pmol/L [^{125}I]ICYP. At these relatively low concentrations and because of the low affinity of the β_3ARs for the radioligand, only β_1- and β_2ARs will be bound, with minimal contribution from the β_3ARs. Results in Figure 1C show that the B$_{max}$ for β_1- and β_2ARs is reduced from 53.6±4.1 fmol/mg in the WT mice to 34.5±2.0 fmol/mg in TGβ_3 mice ($P<0.002$ between WT and TGβ_3 mice). Competition binding experiments with the β_2AR-selective antagonist ICI 118,551 were performed to assess the proportion of β_1- and β_2ARs expressed in the heart (Figure 1D). In cardiac membranes from WT mice, the data were fit by a biphasic curve with 33.8±3.4% high-affinity binding sites (β_2AR) and 66.2±3.4% low-affinity sites (β_1AR). In the TGβ_3 mice, however, the proportion was 51.8±0.7% β_2ARs and 48.5±0.8% β_1ARs ($P<0.02$ between WT and TGβ_3 mice), which suggests that the expression of the endogenous β_1ARs was downregulated by ≈50%, from 35.5 fmol/mg in the WT mice to 16.7 fmol/mg in the TGβ_3 mice. These data also demonstrate that there was no compensatory change in the β_2AR expression in TGβ_3 hearts.

Physiological and Basal Hemodynamic Parameters in WT and TGβ_3 Mice

To determine the functional consequences of β_3AR overexpression in the heart, cardiac catheterization was performed and hemodynamic measurements were recorded. As shown in Table 1, TGβ_3 mice showed a significantly lower left ventricular (LV) systolic pressure and reduced LV dP/dt$_{max}$ and LV dP/dt$_{min}$ compared with WT mice. There was no difference in heart rate or LV end-diastolic pressure. Interestingly, in the TGβ_3 mice, LV weight was lower, resulting in a lower LV/BW ratio than in WT mice. Morphometric analysis of the hearts, however, revealed no differences in myocyte size between the WT and TGβ_3 hearts (2365±97 μm^2, n=100, versus 2555±88 μm^2, n=100, respectively, $P<0.147$), suggesting that overexpression of β_3AR results in a decreased number of cells or a reduced amount of nonmyocyte tissue.

TABLE 2. Echocardiographic Parameters in Conscious WT and TGβ_3 Mice

	WT (n=5)	TGβ_3 (n=5)
HR, bpm	687±27	629±18
LVEDD, mm	3.11±0.11	3.17±0.17
LVESD, mm	1.29±0.14	1.27±0.07
FS, %	59±3	60±1
SEPTh, mm	0.59±0.03	0.61±0.05
PWTh, mm	0.61±0.04	0.63±0.03

HR indicates heart rate; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; SEPTh, interventricular septal wall thickness; and PWTh, posterior wall thickness. Mice were 4 months of age when studied. There was no statistical difference in echocardiographic parameters between WT and TGβ_3 mice.

Echocardiography in Conscious WT and TGβ_3 Mice

Because anesthesia can affect the contractile state of the ventricle, we sought to measure echocardiographic parameters in conscious mice. Chamber dimensions, wall thickness, % fractional shortening, and heart rate did not show any difference between WT and TGβ_3 mice (Table 2).

Effect of Isoproterenol on Hemodynamics in WT and TGβ_3 Mice

To determine whether the $\beta_1/\beta_2/\beta_3$AR agonist isoproterenol could augment contractile function in the TGβ_3 mice, hemodynamic measurements were made before and after isoproterenol administration. LV contractility, as assessed by LV dP/dt$_{max}$ at baseline conditions, was lower in TGβ_3 mice than in WT mice (6664±388 mm Hg/s, n=10, versus 9470±921 mm Hg/s, n=9, $P<0.02$, Figure 2A, Table 1), whereas the effect of isoproterenol on LV dP/dt$_{max}$ was comparable between TGβ_3 and WT mice (Figure 2A). To further characterize the increase in ΔLV dP/dt$_{max}$ with isoproterenol, the TGβ_3 mice were pretreated with the nonselective β_1/β_2AR antagonist propranolol. As shown, the positive inotropic effect of isoproterenol was completely abolished by pretreatment with propranolol in WT mice; a small but significant increase in contractility was still observed, however, in the TGβ_3 mice (Figure 2B), indicating that a small fraction of the positive inotropic action of isoproterenol in TGβ_3 mice may be attributed to the stimulation of overexpressed β_3ARs.

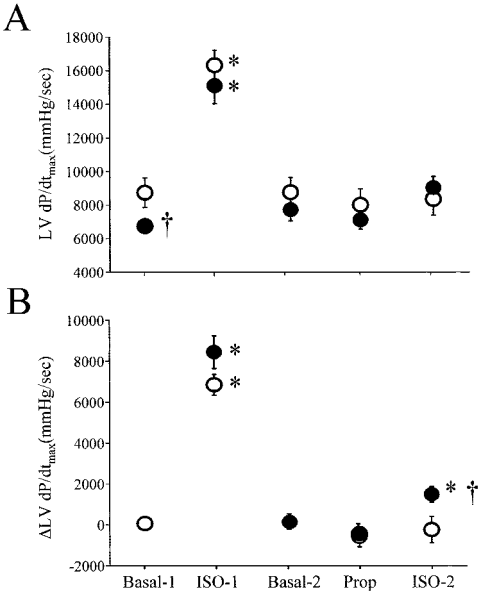

Figure 2. In vivo assessment of LV function in TGβ_3 and WT mice in response to isoproterenol. Effect of isoproterenol on LV dP/dt_{max} (A) and change from basal LV dP/dt_{max} (B) in TGβ_3 (●, n=10) and WT (○, n=9) mice. Basal indicates baseline conditions; ISO, isoproterenol (1000 pg IV); and Prop, propranolol (0.05 μg/g BW IV). *$P<0.001$, TGβ_3 or WT at ISO-1 vs their respective Basal-1 and TGβ_3 at ISO-2 vs Prop. †$P<0.02$, TGβ_3 vs WT under same conditions. By ANOVA, pattern of change between groups was statistically different for (A) LV dP/dt_{max}, $P<0.0005$, and (B) ΔLV dP/dt_{max}, $P<0.02$.

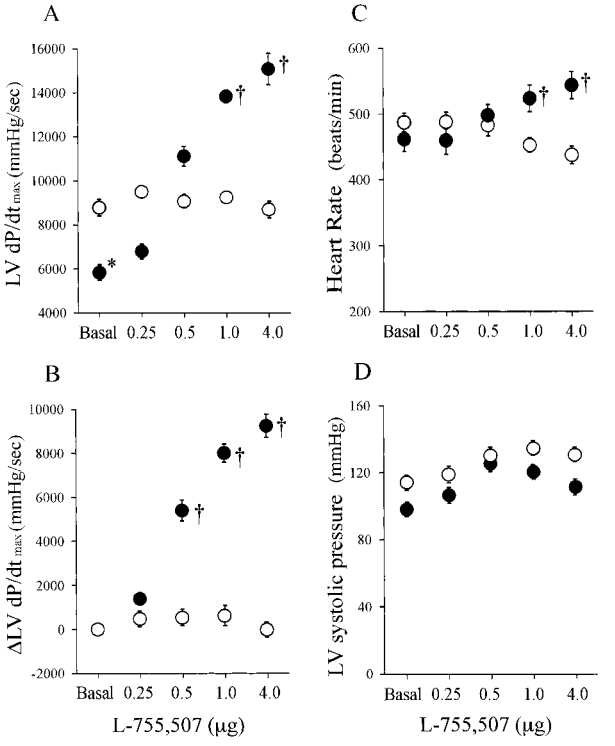

Figure 3. In vivo assessment of LV function in TGβ_3 and WT mice in response to β_3AR agonist L-755,507. Effect of selective human β_3AR agonist L-755,507 on LV dP/dt_{max} (A), change from basal LV dP/dt_{max} (B), heart rate (C), and LV systolic pressure (D) in TGβ_3 (●, n=10) and WT (○, n=5) mice. Post hoc testing was done with Scheffé's F test (*$P<0.05$ and †$P<0.0005$, TGβ_3 vs WT). A significant between-group main effect in response to L-755,507 was found for (A) LV dP/dt_{max}, $P<0.005$; (B) ΔLV dP/dt_{max}, $P<0.00001$; and (D) LV systolic pressure, $P=0.05$. Pattern of change between groups was statistically different for (A) LV dP/dt_{max}, $P<0.00001$; (B) ΔLV dP/dt_{max}, $P<0.00001$; and (C) heart rate, $P<0.00001$.

Effect of the Selective β_3AR Agonist L755,507 on Hemodynamics in WT and TGβ_3 Mice

To test directly whether stimulation of β_3ARs could augment contractility, hemodynamic parameters in WT and TGβ_3 mice were measured in response to the selective human β_3AR agonist L-755,507. This compound is >1000-fold more selective for the activation of the β_3AR than for the β_1AR and has no measurable β_2AR agonist activity.[16] As shown in Figure 3A and 3B, L-755,507 (0.25 to 4.0 μg IV) led to a marked increase in cardiac contractility in TGβ_3 mice (n=10) that was completely absent in WT mice (n=5). Similarly, a large dose-dependent increase in heart rate in response to L-755,507 was also observed in TGβ_3 mice and was again absent in WT mice (Figure 3C). There was no significant difference in the response of LV pressure to L-755,507 between WT and TGβ_3 mice (Figure 3D). These results indicate that L-755,507 acts selectively on the human β_3AR and exerts positive inotropic and chronotropic actions in TGβ_3 mice.

P-V Loops in WT and TGβ_3 Mice

To rigorously investigate whether basal function was different in TGβ_3 mice and WT mice, as suggested by Table 1 and Figures 2A and 3A, we obtained end-systolic P-V relations for both groups (Figure 4B and 4C). Under basal conditions, the end-systolic P-V relation was curvilinear and the slope, E_{max}', of TGβ_3 mice at baseline was comparable to that of WT mice (Table 3). The administration of L-755,507 resulted in a steeper and more curvilinear end-systolic P-V relation only in the TGβ_3 mice (Figure 4B and 4C, Table 3). Furthermore, no significant difference in the volume intercept of the end-systolic P-V relation, LV end-systolic volume, or LV end-diastolic volume was observed between WT and TGβ_3 mice. These data show that overexpression of the β_3AR does not affect the basal contractile state of the ventricle but can result in a significant enhancement of contractility with administration of L-755,507. Interestingly, whereas baseline LV dP/dt_{max} suggested depressed contractility in TGβ_3 mice, a more rigorous analysis using P-V data showed basal contractility similar to that of WT mice. This is in agreement with known limitations of using LV dP/dt_{max} as an index of contractile function.[14]

Characterization of β_3AR Signaling in TGβ_3 Hearts

To investigate the mechanism by which the selective stimulation of the β_3AR in the TGβ_3 mice enhances contractility, biochemical analysis of the physical coupling of the receptor to its cognate G protein and measurement of adenylyl cyclase activity were performed. The agonist-mediated activation of the G protein α-subunit was measured by the quantitative binding of the radiolabeled nonhydrolyzable GTP analog [^{35}S]GTPγS. Figure 5A shows that stimulation with

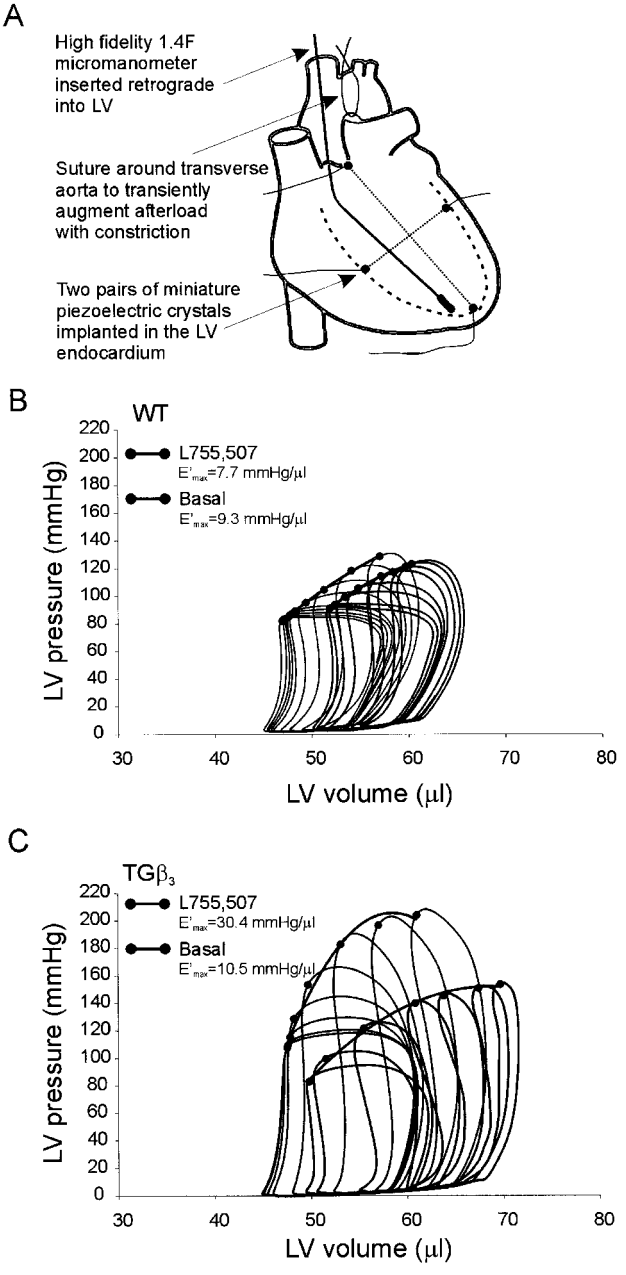

Figure 4. In vivo assessment of contractility with LV P-V relations. Schematic of instrumented heart (A) and representative P-V loops under basal conditions (black) and after administration of L-755,507 (gray) in WT (B) and TGβ_3 (C) mice. P-V loops were recorded during transient constriction of transverse aorta to augment afterload. Curvilinear end-systolic P-V relations in TGβ_3 mice were shifted upward and to left after L-755,507, indicating enhanced contractility.

L-755,507 results in GTPγS loading only in TGβ_3 membranes (1.33±0.04-fold over basal) and not in WT. To determine whether L-755,507–mediated GTPγS loading in TGβ_3 membranes is due to incorporation into G_s or G_i, TGβ_3 mice were treated with PTX (0.1 μg/g BW) overnight. GTPγS loading stimulated by the G_i-coupled A_1 adenosine receptor agonist N^6-cyclopentyladenosine (CPA) was completely abrogated in the PTX-treated sample, confirming G_i blockade by PTX (Figure 5A). Stimulation of the PTX-treated TGβ_3 membranes with L-755,507 resulted in GTPγS loading that was not significantly different from that observed in the untreated TGβ_3 membranes. These data show that the overexpressed human β_3ARs in the TGβ_3 mice are coupled mostly to non-G_i proteins.

To assess the functional coupling of the overexpressed human β_3AR to G_s in the TGβ_3 mice, L-755,507–stimulated adenylyl cyclase activity was measured in cardiac membranes prepared from WT and TGβ_3 hearts. The L-755,507–stimulated adenylyl cyclase activity in TGβ_3 membranes was 1.6-fold over basal, whereas there was no stimulation in WT membranes (Figure 5B). In addition, there was no measurable increase in basal cyclase activity in the TGβ_3 membranes compared with WT controls (Figure 5B, legend). This observation suggests that despite the marked overexpression of β_3ARs, they are functionally inactive until specifically stimulated with a β_3 agonist.

Discussion

Numerous studies have shown that stimulation of β_1- and β_2ARs in intact animals or cardiac preparations can lead to positive chronotropic and inotropic effects. Conversely, from initial work,[5,8,17] it appears that stimulation of β_3ARs can lead to a negative inotropic effect. To study the potential for the β_3AR to affect cardiac function, a transgenic mouse model was constructed that overexpresses the human β_3AR. The most salient feature of this model is enhanced cardiac contractility after intravenous injection of a selective β_3AR agonist, L-755,507. The increase in LV dP/dt_{max} is attributable to the overexpression of β_3AR, because WT animals showed no enhancement of contractility with administration of the β_3AR agonist. Furthermore, in TGβ_3 mice, administration of isoproterenol could overcome the blockade produced by pretreatment with the selective β_1- and β_2AR antagonist propranolol. Unfortunately, the unavailability of a specific β_3AR antagonist without partial agonist activity hampers the ability to further dissect the inotropic effect of isoproterenol in the TGβ_3 mice. Another characteristic of the TGβ_3 mouse is the downregulation of its endogenous β_1ARs by 50%. This result was not totally unexpected, because in the β_3AR knockout mouse,[18] the mRNA levels for β_1AR are upregulated in white and brown adipose tissues, whereas those for β_2AR are unchanged, suggesting a compensatory regulation of β_1- and β_3AR gene expression.

Gauthier et al[8] initially described functional coupling of the β_3AR to G_i when stimulated with the β_3AR agonist BRL37344, resulting in negative inotropic effects in the human heart. Subsequently, negative inotropic effects of BRL37344 have been demonstrated in the isolated guinea pig heart.[17] A similar conclusion was inferred from studies in β_3AR-knockout mice, in which isoproterenol produced an augmented contractile response in comparison to WT mice.[5] In our study, however, β_3AR-G_s coupling is clearly evident in the TGβ_3 mouse model from the PTX-insensitive GTPγS loading, the activation of adenylyl cyclase in cardiac membranes, and the in vivo positive inotropic effect caused by selective β_3AR stimulation. The reason for the apparent disparity between the results presented in this work and those of Gauthier et al[8] is unknown. They may be attributable,

TABLE 3. P-V Parameters Before and After L755,507 Administration in WT and TGβ_3 Mice

	WT Mice (n=4)		TGβ_3 Mice (n=4)	
	Basal	L755,507	Basal	L755,507
E_{max}, mm Hg/μL	12.6±2.4	11.0±1.8	13.9±4.0	27.0±2.0*
Coefficient a, mm Hg/μL^2	−0.288±0.135	−0.218±0.081	−0.440±0.212	−1.017±0.128*
V_0, μL	27±3	28±3	33±6	32±5
r^2	0.998±0.001	0.998±0.001	0.997±0.001	0.997±0.009
Heart rate, bpm	395±55	394±57	386±60	468±52†
V_{es}, μL	30±5	34±6	43±6	36±5
V_{ed}, μL	41±5	45±6	54±7	49±6

E_{max} indicates slope of end-systolic P-V relation (ESPVR) at volume intercept; a, curvilinearity coefficient; V_0, volume intercept of ESPVR; *r*, correlation coefficient of the nonlinear fitting of ESPVR; V_{es}, LV end-systolic volume; and V_{ed}, LV end-diastolic volume. Mice were 4 months of age at the time of study.

*$P<0.01$, †$P<0.005$ TGβ_3 at L755,507 vs TGβ_3 at Basal. No significant differences were found for TGβ_3 at basal vs WT at basal.

however, to the use of different β_3AR agonists or to different experimental models, ie, overexpression of human β_3AR in a mouse versus endogenous β_3AR in human endomyocardial biopsies or in guinea pig heart. Nonetheless, the β_3AR-G_s coupling described in the TGβ_3 mouse is consistent with reports of β_3AR dually coupling to G_s and G_i in a variety of cell types.[6,7] Stimulation of β_3ARs in these models led to increased adenylyl cyclase activity, despite potential inhibitory effects from its coupling to G_i.[2]

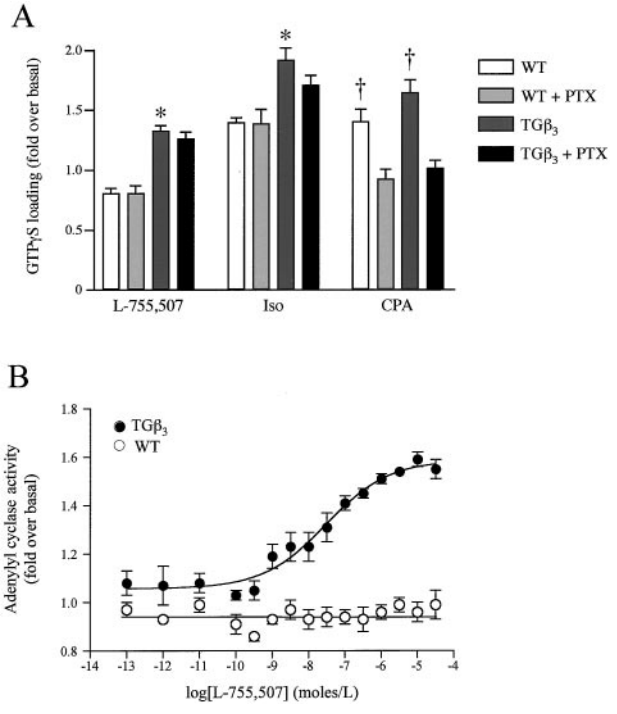

Figure 5. Characterization of β_3AR signaling in cardiac membranes from TGβ_3 and WT mice. A, WT (n=7), PTX-treated WT (n=8), TGβ_3 (n=8), and PTX-treated TGβ_3 (n=6) cardiac membranes were stimulated with 10 μmol/L L-755,507, 10 μmol/L isoproterenol (Iso), or 10 μmol/L CPA and assayed for [^{35}S]GTPγS loading. Activation by L-755,507 and isoproterenol was significantly different between WT and TGβ_3 membranes (*$P<0.004$) and by CPA in TGβ_3 vs TGβ_3+PTX and WT vs WT+PTX membranes (†$P<0.0004$). Basal levels of GTPγS bound were 35.0±8.0 (WT), 21.5±2.5 (WT+PTX), 22.5±4.5 (TGβ_3), and 20.0±3.0 (TGβ_3+PTX) fmol/mg protein. B, TGβ_3 (n=4) and WT (n=4) cardiac membranes were stimulated with various concentrations of L-755,507 and assayed for adenylyl cyclase activity. Basal activities of TGβ_3 and WT membranes were 10.8±1.0 and 10.4±0.6 pmol cAMP · min^{-1} · mg protein^{-1}, respectively.

In addition to the studies describing β_3AR activation in heart, there is evidence of a vasodilatory effect after β_3AR agonist treatment.[19] In conscious dogs, β_3AR stimulation with selective agonists induced marked peripheral vasodilation and positive inotropic and chronotropic effects.[20,21] It is notable that in WT mice, the selective rodent β_3AR agonist CL316243 leads to a hypotensive response, and in β_1-/β_2AR knockout mice, this effect is augmented.[22] These effects do not appear to be directly related to β_3AR stimulation of cardiac myocytes, however, because CL316243 has no chronotropic or inotropic effects in atrial or ventricular preparations from these knockout animals.[22] The variability of the cardiac and vascular responses to β_3AR agonists in different species highlights the fact that the function of the β_3AR in these tissues is still poorly understood.

It has been shown that during chronic heart failure, the cardiac β_1-adrenergic receptors are downregulated, leading to a deficiency in contractility.[23] We show that in this transgenic mouse model with cardiac-restricted overexpression, the human β_3AR is quiescent until stimulated with a selective agonist, at which point there is a marked augmentation in LV contractility. In addition, because the β_3AR is relatively insensitive to catecholamines, it would be minimally activated by endogenous catecholamines. Taken together, this approach could have important therapeutic potential in patients with heart failure, in which delivery of the human β_3AR by gene therapy approaches to the heart could provide a functionally inactive signaling protein that becomes activated only when a highly selective agonist is exogenously administered.

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