β3-Adrenergic stimulation produces a decrease of cardiac contractility ex vivo in mice overexpressing the human β3-adrenergic receptor

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Abstract

Objectives: The regulation of cardiac function by catecholamines involves three populations of β-adrenoceptor (β-AR). β1- and β2-AR stimulations produce an increase in contractility and β3-AR stimulation mediates a negative inotropic effect in human ventricular muscle. Because of the lack of suitable animal models, we have generated transgenic mice with cardiac-specific expression of the human β3-AR (TGβ3 mice).

Methods: TGβ3 mice were produced by microinjection of the human β3-AR under the control of the α myosin heavy chain promoter. Phenotypic analyses comprised β3-AR mRNA and protein determinations, histological studies, electrocardiogram, contractility and cyclic nucleotide measurements.

Results: TGβ3 mice presented no histological evidence of myocyte hypertrophy or fibrogenesis. In basal conditions, TGβ3 mice were characterized by an increase in heart rate and an acceleration of twitch parameters without modification of its amplitude. β3-AR agonists (CL 316243, SR 58611A) decreased contractility at low concentrations (1–100 nM). At high concentrations, the negative inotropic effect was abolished. Pretreatment with nadolol, a β1/β2-AR blocker, blunted the rebound in peak tension elicited by β3-AR agonists suggesting a non-specific action of these compounds on β1- and β2-AR. The involvement of β3-AR in the negative inotropic effect was confirmed by the pretreatment with bupranolol, a non-selective β-AR antagonist, which fully abolished the effects of SR 58611A. The negative inotropic effect was associated with an increase in intracellular cGMP level.

Conclusions: We conclude that cardiac overexpression of β3-AR in mice reproduces ex vivo the negative inotropic effects obtained with β3-AR stimulation in human ventricular tissues.

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in vivo lack of desensitization following activation with agonists [3]. These features suggest that the expression of \( \beta_1 \)-AR in heart may have pathophysiological significance. Recently, opposite regulation has been described for \( \beta_1 \)-AR and \( \beta_3 \)-AR in the human failing heart [4]. In addition to the classically observed \( \beta_1 \)-AR downregulation [5], an upregulation of \( \beta_3 \)-AR was reported [4]. Despite increased \( \beta_3 \)-AR expression, the negative inotropic effect was mildly reduced in failing heart tissue compared with responses observed in nonfailing samples because of concurrent alterations in post-receptor coupling mechanisms, especially decreased eNOS expression. Nevertheless, the reduction in \( \beta_3 \)-AR response is less than that obtained with \( \beta_1 \)-AR stimulation. Therefore, the functional loss of catecholamine positive control of cardiac contractility during heart failure may result from a shift in the balance between \( \beta_1 \)-AR-mediated positive and \( \beta_3 \)-AR-mediated negative inotropic pathways.

As none of the animal models studied so far showed a negative inotropic effect induced by \( \beta_3 \)-AR agonists comparable to that found in human ventricles [6], we produced transgenic mice with cardiac-specific expression of human \( \beta_3 \)-AR (TG\( \beta_3 \) mice). The physiological consequences of this expression were assessed on heart morphology, electrocardiogram, ex vivo contractility and cyclic nucleotide production.

2. Methods

2.1. Generation of transgenic mice

Studies on transgenic mice were carried out in agreement with French laws and INSERM guidelines on animal care. The \( \alpha \) myosin heavy chain (\( \alpha \)MHC)-\( \beta_3 \)-AR transgene was constructed from a 5.5 kb BamHI–SalI fragment containing the murine \( \alpha \)MHC promoter [7] and a SalI–SalI cDNA fragment containing the human \( \beta_3 \)-AR coding sequence [8]. The \( \alpha \)MHC-\( \beta_3 \)-AR transgene was linearized with NotI, purified by electroelution, concentrated on an elutrap-d column (Schleicher and Schuell) and used for nuclear injection in fertilized eggs of B6D2/F1 hybrid females. The microinjected oocytes were then reinjected in B6CBA/F1 hybrid pseudopregnant foster mothers. Ten micrograms of genomic DNA digested with BamHI were analyzed by Southern blotting with \( ^{32} \)P-labelled \( \beta_3 \)-AR cDNA fragment. For analysis, membranes were subjected to digital imaging (Molecular Dynamics).

2.2. Total RNA preparation

Total RNA from heart and white adipose tissues of wild-type (WT) and TG\( \beta_3 \) mice were prepared using a single-step guanidinium thiocyanate/phenol/chloroform extraction [9]. To avoid contamination with genomic DNA, 20 \( \mu \)g of each sample were treated with RNase-free DNase I enzyme (Roche-Boehringer), purified by a phenol–chloroform step and submitted to isopropanol precipitation.

2.3. Reverse transcriptase–polymerase chain reaction

To analyze the expression of the mRNA coding for the human \( \beta_3 \)-AR in tissues of TG\( \beta_3 \) mice, we designed a couple of primers that allows specific amplification of the human \( \beta_3 \)-AR cDNA (sense: 5’ GCCGACGCG- CGAGGCAGCGGC 3’, antisense: 5’ ACCC CCTC-GGGCGAGCGCAC 3’). To ensure that the product visualized by electrophoresis through 2% agarose ethidium bromide-stained gel was of a human origin, the gel was blotted onto nylon N+ membrane and hybridized at 65 °C with a human \( \beta_3 \)-AR cDNA radioactive probe [8]. Furthermore, half of the PCR product was digested with AccI, a restriction endonuclease which cuts into two parts the human but not the mouse amplicon (data not shown).

2.4. Northern blotting experiments

Twenty-five micrograms of total heart RNAs from both WT and TG\( \beta_3 \) mice were separated by electrophoresis on a 1% agarose gel, transferred onto a nylon N+ filter by standard techniques [10] and hybridized with the \( ^{32} \)P-labelled human \( \beta_3 \)-AR cDNA probe.

2.5. Western blotting experiments

The expression of the \( \beta_3 \)-AR was examined with a monoclonal antibody raised against the human isoform of this receptor [11]. Heart protein fractions extracted from WT or TG\( \beta_3 \) mice (100 \( \mu \)g) were denatured with 5% \( \beta \)-mercaptoethanol in Laemmli’s sample buffer and electrophoresed on 10% polyacrylamide/SDS gels and transferred onto a Hybond C super membrane (Amersham, France) using an electroblotting apparatus (Bio-Rad, USA). The protein amount was checked by staining with Coomassie. Nonspecific binding was blocked by incubating membranes in non-fat dry milk 5% in TBS. Then they were incubated in milk with 1% TBS alone or with the primary antiserum, washed in TBST (TBS containing 0.1% Tween 20) and incubated with the secondary antibody anti-IgG peroxidase conjugate (Sigma, USA). The membranes were washed with TBS and antibody complexes were revealed by an enhanced chemiluminescence detection procedure (Amersham, France).

2.6. Histological studies

WT or TG\( \beta_3 \) mouse hearts at 3 and 9 months of age, were fixed in 95% ethanol for 48 h and paraffin embedded. The sections (4 \( \mu \)m) were washed in toluene and absolute ethanol to eliminate the paraffin and rehydrated in graded ethanol and finally washed in PBS. A hemalun–phloxine–
saffron trichromic labelling was carried out. Nuclear and cytoplasmic staining was obtained with hemalun and phloxine. Interstitial connective tissues were stained with saffron.

2.7. Electrocardiogram

Surface electrocardiograms (ECGs) were recorded as reported previously [12]. Q-T interval was corrected for heart rate using the formula: Q-Tc=Q-T/(R-R/100)\(^{1/2}\) established for mice with Q-T and R-R measured in milliseconds.

2.8. Contractility measurements

WT and TG\(β_3\) mice were killed by cervical dislocation. Hearts were immediately excised and placed in Tyrode’s solution (35±0.5 \(°\)C) composed as follows (in mM): 116 NaCl, 5 KCl, 1.5 CaCl\(_2\), 1.1 MgCl\(_2\), 0.35 NaH\(_2\)PO\(_4\), 5 glucose and 27 NaHCO\(_3\). The right ventricle was cut into small pieces. Then the samples were placed in an experimental chamber and superfused with the Tyrode’s solution at a flow rate of 5 ml/min with oxygenation (95% O\(_2\), 5% CO\(_2\)). They were subjected to field stimulation at a heart rate of 1.66 Hz. Stimulus pulse width was 1–2 ms, and amplitude was twice the diastolic threshold. Tension was recorded using a mechano-electric force transducer (Akers, AE 801, SensoNor, Norway), as previously described [1,6]. In order to normalize basal contraction, the samples were stretched gradually to length at which active twitch tension was maximal. Then, the tension was slightly reduced to obtain about 90% of maximal tension. After a 60-min equilibration period, the basal parameters were recorded and cumulative concentration–response curves of \(β_3\)-AR agonists were constructed by superfusion with successive increasing concentrations of the drugs. For all concentrations, tension was measured at steady state.

2.9. Cyclic nucleotide assay

Small ventricular pieces were cut and rapidly placed in tubes containing Tyrode’s solution with 100 \(μ\)M 3-isobutyl-1-methylxanthine (IBMX) for basal condition and either 1 \(μ\)M isoproterenol or 100 nM CL 316243 for stimulated conditions. The fragments were incubated at 37 \(°\)C under gentle agitation during 8 min for isoproterenol or 10 min for CL 316243. To stop the reaction, the fragments were first transferred to tubes containing cold Krebs buffer with 100 \(μ\)M IBMX and 6% (v/v) trichloro-acetic acid and then frozen in liquid nitrogen. Ventricle fragments were crushed in liquid nitrogen. The powder was immediately transferred into the Krebs buffer with IBMX and trichloro-acetic acid and centrifuged. The pellet was stored at −20 \(°\)C for the measurement of protein concentration [13]. Cyclic nucleotide levels were quantified in the supernatant by ELISA (Spi-Bio, France) as previously described [14]. Then, 20 \(μ\)l and 50 \(μ\)l of extracts were used for the quantification of cAMP and cGMP levels, respectively. The mean value was calculated from duplicate measurements of each sample and normalized to total cell protein content.

2.10. Drugs

\((-\)isoproterenol, \((-\)propranolol, trichloro-acetic acid and IBMX were from Sigma–Aldrich (France). Diethyl-ether was from Merck-Eurolab (France). SR 58611A (ethyl [(7)S]−(2R)2-(3-chlorophenyl)-2-hydroxyethyl]amino]-5,6,7,8-tetrahydroanaphthyl-2-yl[oxyacetate hydrochloride) was a generous gift from Sanofi Recherche, France. CL 316243 (5-(2-[2-(3-chlorophenyl)-2-hydroxyethyl]amino)propyl)-1,3-benzodioxole-2,2-dicarboxylate) and bupranolol were generous gifts from American Cyanamid (USA) and from Schwarz Pharma (Germany), respectively.

2.11. Statistical analysis

Results are expressed as means±S.E.M. Unpaired Student’s t-test was carried out to compare the weights, the ECG parameters and the basal contractility parameters of the WT and TG\(β_3\) mice. Paired Student’s t-test was applied to compare cyclic nucleotide production. The statistical significance of the drug effect on contractility was assessed using one-way analysis of variance (ANOVA) followed by a Dunnett’s test. For all tests, a value of \(P<0.05\) was considered significant.

3. Results

3.1. Generation of transgenic mice

To express the human \(β_3\)-AR in hearts of transgenic mice, the \(β_3\)-AR cDNA under the control of the \(α\)MHC promoter was microinjected into mouse oocytes. Southern blot analysis showed five founders with 5–20 transgene copies. Using a RT-PCR analysis on total RNA with human \(β_3\)-AR-specific oligonucleotide primers, the human \(β_3\)-AR mRNA was exclusively detected in heart (data not shown). Only one transcript of 1.4 kb length was detected in TG\(β_3\) heart by Northern blot analysis (Fig. 1A). No expression was observed in the heart of the WT mouse. Equal loading of samples was confirmed by reprobing the filter with a rat \(β\)-actin probe (data not shown).

3.2. Western blotting experiments

Western blot probed with an antibody directed against human \(β_3\)-AR showed two bands of roughly 50 and 60 kDa that correspond to the two putative glycosylated forms (Fig. 1B). The major signal of 60 kDa was compatible with the immunoreactivity described as a glycosylated
Fig. 1. (A) Northern blot of human β1-AR mRNA expression in hearts from WT and TGβ3 mice. Total RNA (25 μg) was hybridized with a 32P-labeled human β1-AR cDNA. (B) Western blot analysis of human β1-AR expression in hearts from WT and TGβ3 mice. B1: The protein amount was checked by staining with Coomassie. B2: The membrane was incubated without (left panel) or with anti human β1-AR antibody (right panel) and was revealed by chemiluminescence detection.

form of β1-AR which was also observed in human heart [4,11]. No reactivity of the two bands was observed in the absence of anti β1-AR antibody.

3.3. Cardiac morphology and histology

There was no difference in body weights in 4- to 5-month-old TGβ3 compared to WT animals of the same litter. However, the hearts of TGβ3 mice weighed less than those of control mice and the heart/body weight ratio was smaller in TGβ3 than in control animals (Table 1). The histopathological examination of hearts from 3- or 9-month-old mice indicated no differences between WT or TGβ3 mice. In addition, there was no histological evidence of myocyte hypertrophy or fibrogenesis in TGβ3 mice (Fig. 2).

3.4. ECG measurements

Six-lead surface ECGs were recorded and compared between WT and TGβ3 mice. Lead I was further selected as the most appropriate lead allowing discrimination of both T and P waves. Average ECG values are summarized in Table 2. These data demonstrate that P wave and P-R interval durations were comparable between the two groups of mice. P-P, R-R and Q-T intervals were significantly reduced in TGβ3 compared to WT mice. However, the effect on Q-T interval was abolished after correction. The heart rate was significantly increased by 18% in TGβ3 compared to WT mice.

<table>
<thead>
<tr>
<th>Comparison between 4- and 5-month-old WT and TGβ3 mice</th>
<th>WT (n=13)</th>
<th>TGβ3 (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>28.45 ± 1.55</td>
<td>26.88 ± 1.39</td>
</tr>
<tr>
<td>Heart weight (mg)</td>
<td>130 ± 5</td>
<td>108 ± 6*</td>
</tr>
<tr>
<td>Ratio (heart/body)</td>
<td>4.62 ± 0.16</td>
<td>4.01 ± 0.09**</td>
</tr>
</tbody>
</table>

WT, wild-type mice; TGβ3, transgenic mice with cardiac-specific expression of human β1-adrenoceptor.

*P<0.05 and **P<0.01 TGβ3 versus WT.
3.5. Basal contractility parameters

The mean peak tension was not modified in ventricular samples from TGβ3 compared to those obtained in control mice while the other twitch parameters were significantly reduced (Table 3).

3.6. Effects of β3-AR agonists on cardiac contractility

CL 316243 and SR 58611A, two preferential β3-AR agonists, did not significantly modify the peak tension in WT mice for concentrations ranging from 1 nM to 10 μM (Fig. 3A,B). In TGβ3 mice, both compounds produced a biphasic effect. At lower concentrations, they decreased peak tension with a maximum effect at 0.1 μM. At higher concentrations, this negative inotropic effect was abolished (Fig. 3A,B). In order to determine the β-AR subtypes involved in this effect, additional experiments were carried out with SR 58611A in the presence of β-AR antagonists. After pretreatment of samples with 10 μM nadolol, a β1- and β2-AR antagonist, the negative inotropic effect of SR 58611A was observed at all concentrations tested and the maximal effect was obtained at 10 μM (Fig. 3C). In this condition, the biphasic effect was lost. In the presence of 1 μM bupranolol, a β1-, β2- and β3-AR antagonist, the effects of SR 58611A were fully abolished (Fig. 3C). The involvement of β3-AR in the negative inotropic effect obtained in TGβ3 mice was strengthened by experiments carried out with isoproterenol, a non selective β-AR agonist. In TGβ3 mice, isoproterenol increased the peak tension, but the maximal effect was obtained at a higher concentration (1 μM) compared to WT mice (0.1 μM).

Table 2
Average ECG data in WT and TGβ3 mice

<table>
<thead>
<tr>
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<th>WT (n=12)</th>
<th>TGβ3 (n=16)</th>
</tr>
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<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>455±26</td>
<td>535±15**</td>
</tr>
<tr>
<td>P (ms)</td>
<td>26±1</td>
<td>25±1</td>
</tr>
<tr>
<td>P-R (ms)</td>
<td>35±2</td>
<td>35±1</td>
</tr>
<tr>
<td>P-P (ms)</td>
<td>135±7</td>
<td>113±3**</td>
</tr>
<tr>
<td>R-R (ms)</td>
<td>136±8</td>
<td>113±3**</td>
</tr>
<tr>
<td>Q-T (ms)</td>
<td>100±7</td>
<td>85±3*</td>
</tr>
<tr>
<td>Q-Tc</td>
<td>85±4</td>
<td>80±2</td>
</tr>
</tbody>
</table>

All measurements were carried out on lead I under general anesthesia with etomidate. P is the P wave duration. Q-Tc is the Q-T duration corrected with formula adapted to mouse. WT, wild-type mice; TGβ3, transgenic mice with cardiac-specific expression of human β3-adrenoceptor; ECG, electrocardiogram.

*P<0.05 and **P<0.01 TGβ3 versus WT.

Table 3
Comparison of twitch parameters obtained from 15- to 40-week-old WT and TGβ3 mice

<table>
<thead>
<tr>
<th></th>
<th>WT (n=28)</th>
<th>TGβ3 (n=43)</th>
</tr>
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<tbody>
<tr>
<td>Peak tension (μN/mg of tissue)</td>
<td>154.66±18.89</td>
<td>159.51±15.61</td>
</tr>
<tr>
<td>Total duration (ms)</td>
<td>185.96±4.54</td>
<td>167.81±4.75**</td>
</tr>
<tr>
<td>Time-to-peak (ms)</td>
<td>57.21±0.92</td>
<td>49.74±1.25**</td>
</tr>
<tr>
<td>Half-contraction time (ms)</td>
<td>23.11±0.39</td>
<td>20.88±0.58**</td>
</tr>
<tr>
<td>Half-relaxation time (ms)</td>
<td>39.93±0.90</td>
<td>33.35±1.03**</td>
</tr>
</tbody>
</table>

WT, wild-type mice; TGβ3, mice, transgenic mice with cardiac-specific expression of human β3-adrenoceptor.

**P<0.01 TGβ3 versus WT.
addition, the maximal increase was smaller in TGβ3 mice (+69.0±17.8%; n=4) than in WT mice (+97.7±16.7%; n=5), albeit this effect was not significant.

3.7. Cyclic nucleotide measurements

Basal cAMP levels were measured in ventricular biopsies. They were not statistically different between WT and TGβ3 animals (44.67±7.66 and 54.47±9.91 pmol/min per mg of protein, respectively). After stimulation of the ventricular samples with 1 μM isoproterenol, a non-selective β-AR agonist, cAMP levels increased 1.79±0.14-fold (P<0.05) and 2.28±0.45-fold (P<0.05) over basal values for WT and TGβ3 mice, respectively (Fig. 4A). In the presence of 0.1 μM CL 316243, cAMP level was significantly increased in TGβ3 (2.20±0.31-fold; P<0.05) but not in WT mice (1.48±0.19-fold). The production of cGMP was evaluated in the same ventricular samples as
those used for cAMP. Basal values were approximately fivefold lower for cGMP than cAMP both in WT and TGβ3 mice: 8.43±2.42 and 10.34±3.14 pmol/min per mg of protein, respectively. In WT animals, neither isoproterenol nor CL 316243 induced an increase in cGMP level. In TGβ3 mice, isoproterenol and CL 316243 produced a rise of 2.13±0.33 and 2.35±0.33-fold (P<0.05), respectively (Fig. 4B).

4. Discussion

We have established a transgenic mice model in which human β1-AR is specifically over-expressed in hearts, as confirmed by Northern and Western blotting. This over-expression of β1-AR does not modify cardiac morphology but is associated with an increase in basal heart rate and an acceleration of basal contractility ex vivo. Stimulation with β1-AR agonists produces a negative inotropic effect. In TGβ3 mice, as also recently described by Kohout et al. [15], heart weight was lower, resulting in a lower heart weight/body weight ratio compared with WT mice. Furthermore, in our model of TGβ3 mice, there was no histological evidence of myocyte hypertrophy or fibrogenesis. These results are in agreement with those obtained in mice over-expressing the β2-AR in heart [16], but they are different to those obtained in transgenic mice over-expressing β1-AR [17]. In this latter model, hearts display major morphological and functional alterations, in particular, a hypertrophy of myocytes associated with fibrosis. These discrepancies between models of mice overexpressing different subtypes of β-AR could be explained either by the best coupling of the β1-AR subtype to positive inotropic response or by subtype-specific signaling pathways. Moreover, the increased workload due to a higher basal cardiac performance in mice overexpressing β1-AR could also play a role.

In basal conditions, heart rate was significantly increased in TGβ3 mice compared to WT mice. Several hypothesis could explain this modification. In in vivo studies, several preferential β1-AR agonists such as BRL 37344 and CL 316243 induced positive chronotropic effects in dogs and rats [18–20]. However, these effects probably resulted from reflex mechanisms rather than from a direct stimulation of cardiac β1-AR since they were abolished after sinoaortic denervation in conscious dogs [18] or after β1- and β2-AR blockade in dogs and rats [20]. Likewise, in humans, the positive β1-AR-related chronotropic effects described by Wheeldon and colleagues were prevented by β1- or β2-AR antagonists and were likely due to baroreflex activation secondary to the vasodilation induced by the β1-AR agonist [21,22]. The increase in heart rate that we observed in TGβ3 mice could result from the overexpression of β1-AR. The β1-AR belong to the superfamily of G protein-coupled receptors. These receptors are known to exist in the cell membrane in two sub-populations: (i) an inactive sub-population that requires agonist occupancy for coupling to G protein; (ii) a constitutively active sub-population that can couple to G protein in the absence of agonist. An increase in the constitutively active sub-population has been reported for receptors over-expressed at a high level. The over-expression of the wild-type β2-AR in myocardial cells of transgenic mice produced an elevation of baseline heart rate and baseline adenylyl cyclase activity [16]. A comparable behavior has been reported with β1-AR so that basal adenylyl cyclase activity increased with β1-AR density in CHO cells [23]. Moreover, in our study, the increase in basal heart rate could result from an activation of a potassium channel, QvLQT1, as has been described for the human β1-AR in a recombinant system [24]. The activation of QvLQT1 which is expressed in the mice conduction system [25], could produce an acceleration in heart rate. In a recent study carried out in the guinea-pig heart, it has been shown that β3-AR stimulation inhibits the slow delayed rectifier potassium current Iks [26]. As Iks is absent in adult mice heart [27], its modification could not explain the effects obtained in heart rate. Then, future studies will be needed to clarify the role of β1-AR in the regulation of heart rate as well as the involvement of potassium channels in this effect.

At baseline, we observed an acceleration of the different parameters of the cardiac contraction in TGβ3 mice. Similar effects have been previously described for the activation of β1-AR by preferential β1-AR agonists in the human heart [1]. However, the amplitude of the contraction was not significantly modified in mice.

In WT mice, β1-AR agonists induced no significant effects on contractility. In contrast, in TGβ3 mice, β1-AR agonists (CL 316243 and SR 58611A) produced a biphasic effect on contractility. The second part of this effect, which restored the contractility to the control level, is due to a nonspecific activation of β1- and β2-AR by these compounds because it was lost after pretreatment with nadolol. In these latter conditions, SR 58611A produced a negative inotropic effect at all concentrations tested. The involvement of β1-AR in the negative inotropic effect was confirmed by the pretreatment with bupranolol, which fully abolished the effects of SR 58611A. In addition, we observed a reduction in the maximal effect induced by isoproterenol, which also activates β2-AR. Our present findings agree with previous results, which showed that stimulation of endogenous β1-AR in isolated human [1], dog [6] and guinea pig [28] hearts produced a decrease in contractility. In β2-AR knockout mice, isoproterenol produced an increased contractile response in comparison to WT mice suggesting that activation of β2-AR attenuates β-adrenergic-stimulated positive inotropy [29]. In contrast, in a recent study carried out in vivo on a similar TGβ3 mouse model, another β1-AR agonist, L-755,507, produced a positive inotropic effect [15]. The different experimental approaches, ex vivo versus in vivo, and the
genetic background of the mice (B6D2/F1 hybrid in our study vs. B6S/F1 hybrid in Ref. [15]) could partly explain the discrepancy. A single line of mice was investigated in Ref. [15] whereas we have collected data from several transgenic lines. Moreover, the nature and concentration of new compounds acting on human cardiac myosin heavy chain genes. J Mol Biol 1991;266:9180–9185. 

negative inotropic effects obtained with β3-AR agonists like CL 316243 used at high concentrations in vivo could interact with β3- and β2-AR or another receptor and mask the negative inotropic effect. In our model of TGβ3 mice, the decrease in contractility observed at low concentrations of β3-AR agonists could result from the intracellular calcium decrease. The involvement of this mechanism in the decrease in contractility by β3-AR stimulation has been described both in isolated cardiomyocytes and heart. In the beating guinea-pig heart, very low concentrations of BRL 37344 induced a negative inotropic effect via a reduction in calcium transients which was partly independent of activation of endothelial nitric oxide synthase (eNOS) [28]. In canine ventricular myocytes, Cheng et al. [31] reported that BRL 37344 caused a decrease in the amplitude of contraction and in the peak systolic $[Ca^{2+}]_i$ transient. At higher concentrations of β3-AR agonists, the abolition of the negative inotropic effect that we observed, could be explained by the over-expression of β3-adrenoceptors, which could lead to activation of several G proteins. The activation of Gs leads to a stimulation of adenylly cyclase as suggested by the slight, but not significant, increase in cAMP level in TGβ3 mice compared to WT mice.

In TGβ3 mice, the negative inotropic effect of CL 316243 was associated with an increase in intracellular cGMP level. This effect on cGMP was also obtained with a non-selective β-AR agonist, isoproterenol, which produced no effect in WT mice. These data corroborate our previous results obtained in human ventricle in which β3-AR stimulation produced a decrease in contractility by activation of NO pathway and an increase in intracellular cGMP level [2]. The fact that CL 316243 increased intracellular cAMP could be due either to an indirect effect: the increase in cGMP could inhibit the cGMP-inhibited phosphodiesterase which hydrolyses only cAMP leading to an increase in cAMP level, and/or to its non-specific action on β1- and β2-AR as indicated by the cancelling of the negative inotropic effect at higher concentrations of CL 316243.

5. Conclusions

The present work demonstrates that cardiac overexpression of human β3-AR in mice reproduces ex vivo the negative inotropic effects obtained with β3-AR stimulation in human ventricular tissues. As it is difficult to obtain human cardiac tissues, our model of TGβ3 mice may be useful to investigate the pharmacology and the signalling pathways of this receptor as well as to perform screening of new compounds acting on human β3-AR prior to clinical studies.

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References


