The $\alpha_{1B}$-adrenergic receptor decreases the inotropic response in the mouse Langendorff heart model

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Abstract

Objective: $\alpha_1$-Adrenergic receptors (ARs) are known mediators of a positive inotropy in the heart, which may play even more important roles in heart disease. Due to a lack of sufficiently selective ligands, the contribution of each of the three $\alpha_1$-AR subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$) to cardiac function is not clearly defined. In this study, we used a systemically expressing mouse model that overexpresses the $\alpha_{1B}$-AR to define the role of this subtype in cardiac function. Methods: We used the mouse Langendorff heart model to assess changes in contractility under basal and phenylephrine-induced conditions. Results: We find that a 50% increase of the $\alpha_{1B}$-AR in the heart does not change basal cardiac parameters compared to age-matched normals (heart rate, $\Delta dP/dT$ and coronary flow). However, the inotropic response to phenylephrine is blunted. The same results were obtained in isolated adult myocytes. The difference in inotropy could be blocked by the selective $\alpha_{1A}$-AR antagonist, 5-methylurapidil, which correlated with decreases in $\alpha_{1A}$-AR density, suggesting that the $\alpha_{1B}$-AR had caused a compensatory downregulation of the $\alpha_{1A}$-AR. Conclusions: These results suggest that the $\alpha_{1B}$-AR does not have a major role in the positive inotropic response in the mouse myocardium but may negatively modulate the response of the $\alpha_{1A}$-AR.

Keywords: Adrenergic receptor; Heart; Myocyte; Inotropy

1. Introduction

$\alpha_1$-Adrenergic receptors (ARs) mediate the effects of the sympathetic nervous system by binding the catecholamines, epinephrine and norepinephrine. $\alpha_1$-AR subtypes ($\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$) are part of the larger and related family of adrenergic receptors, which include the $\beta$-ARs ($\beta_1$, $\beta_2$ and $\beta_3$) and the $\alpha_2$-ARs ($\alpha_{2A}$, $\alpha_{2B}$ and $\alpha_{2C}$). Adrenergic receptors are also members of the much larger family of G-protein-coupled receptors (GPCRs) of which over 80% of hormones use to transduce their signals.

$\alpha_1$-AR play many roles in the myocardium ranging from positive inotropic and chronotropic effects, cardiac preconditioning, arrhythmogenesis and cardiac hypertrophy [1,2]. $\alpha_1$-AR modulation of cardiac function may become more important in diseased heart where $\beta$-AR responsiveness is often impaired with concomitant upregulation of the $\alpha_1$-AR response (for review, see Ref. [2]). All three $\alpha_1$-AR subtypes are expressed in the heart of a variety of species. Predominant subtypes are the $\alpha_{1A}$- and $\alpha_{1B}$-AR with minor expression (if any) of the $\alpha_{1D}$-AR subtype [1,3]. $\alpha_1$-AR-mediated positive inotropic effects have been well-documented in a number of animal models both in vivo and in vitro, although this response is considered minor in comparison to $\beta$-AR stimulation (25% vs. 75% with norepinephrine stimulation in rat) [4]. However, the role of $\alpha_1$-ARs in mediating cardiac contractility has been controversial due to the substantial variations (i.e. both negative and positive inotropy) between different species and the preparation used.

Elucidating the roles of the individual subtypes pharmacologically has proved difficult with the lack of subtype...
selective ligands and only with the advent of genetic manipulation have we begun to make significant progress. Recent advances in transgenic and knock-out technologies have allowed investigators to dissect out some of the contributions of the various subtypes to physiological responses in the vasculature and cardiac tissues [1]. While some labs have focussed on heart-targeted transgenic models of $\alpha_{1B}$- and $\alpha_{1A}$-AR overexpression, we have developed a transgenic mouse model whereby use of the isogenic mouse $\alpha_{1B}$-AR promoter has allowed us to overexpress a constitutively active mutant (CAM) form of the hamster $\alpha_{1B}$-AR only to tissues that normally express the receptor [5]. Advantages of using this model is that overexpression is targeted to natural cells/tissues that express the subtype, overexpression is not dramatic, and multiple systems are affected and can be studied in the same animal. Of the previous models developed, there is only one report that explores the role of the $\alpha_{1B}$-AR subtype in cardiac function per se with most reports focusing on either tissue contractility, hypertrophy or blood pressure regulation. Heart-targeted overexpression of the wild-type $\alpha_{1B}$-AR leads to decreased ventricular function [6], while overexpression of the $\alpha_{1A}$-AR in the targeted heart was found to increase inotropy dramatically but without evidence of any hypertrophy [7]. We wanted to explore the $\alpha_{1B}$-AR subtype in the heart and determine its role in cardiac function in our systemic mouse model.

Since we have found that our $\alpha_{1B}$-AR transgenic mice display neurodegeneration [5] and a corresponding autonomic dysfunction [8], we decided to look at the effects of $\alpha_{1B}$-AR overexpression in the myocardium ex vivo to try to determine what contribution the $\alpha_{1B}$-AR makes to the inotropic effects of the $\alpha_{1A}$-AR pool of receptors. We find that there is no change in basal cardiac parameters in the transgenic animals compared to controls; however, there is a significant decrease in the response to phenylephrine, suggesting a negative inotropy. We propose that this difference may be attributed to a decrease in $\alpha_{1A}$-AR levels in the transgenic animals due to compensatory effects. Therefore, although the $\alpha_{1B}$-AR may not play a major role in inotropy in the mouse myocardium, its effects may be related to a negative regulation of the $\alpha_{1A}$-AR subtype, the major determinant of cardiac inotropy for $\alpha_{1A}$-AR responsiveness.

2. Materials and methods

2.1. Mice

The generation and genotyping of transgenic mice possessing systemic $\alpha_{1B}$-AR over-activity has been described elsewhere [5]. Briefly, tissue-specific distribution of systemic $\alpha_{1B}$-AR over-activity was achieved by utilizing the murine $\alpha_{1B}$-AR gene promoter [9] to drive overexpression of a transgene containing a cDNA coding for a constitutively active mutant of the $\alpha_{1B}$-AR, called T for triple mutant [10]. The Cleveland Clinic Foundation Transgenic Core Facility injected approximately 200 copies of each transgene into the pronuclei of one-cell B6/CBA mouse embryos, which were surgically implanted into pseudopregnant female mice. Founder mice were identified and subsequent generations were genotyped by Southern analysis of genomic DNA extracted from tail biopsies. Mice are used at approximately 9–10 months of age with equal numbers of male and female mice and their use conforms to NIH guidelines.

2.2. Measurement of cardiac function in intact heart preparations

After intravenous injection of heparin sodium, i.p. (500 U/kg) and intraperitoneal anesthetization with pentobarbital sodium (150 mg/kg), the heart, with all major vessels and lungs attached, was excised. The aorta was then cannulated with a flared PE10 catheter and was positioned above the coronary ostia. A water-filled latex balloon was inserted into the lumen of the left ventricle via the left atrium. The distal end of the balloon-attached catheter was connected to a pressure transducer for measurement of intraventricular pressure and $\pm dP/dt$. The balloon was inflated to a constantly-held diastolic pressure of 3–10 mm Hg. The retrograde perfusion via the aorta was carried out by a perfusion pump maintaining a column of Krebs—Henseleit solution (KHS) composed of (in mM) 118 NaCl, 4.7 KCl, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 0.5 EDTA, 25 NaHCO$_3$, 5 pyruvic acid and 11 glucose; pH 7.4 (following gassing with 95% O$_2$–5% CO$_2$ at 37 °C) to provide a constant coronary perfusion pressure of 75 mm Hg. We confirmed the coronary perfusion pressure by using a pressure transducer connected via a side port to the aorta perfusion cannula. Coronary flow was measured via an inline Transonic flow probe (Transonics 1N) connected to a Transonics flow meter (T106). Drugs were added by infusion pump through an injection port directly above the aortic cannula. Data were continually recorded and displayed on a Powerlab data acquisition system. Cardiac parameters were measured off-line at the end of the experiment and the final steady-state values were used. The preparation was allowed to stabilize for 30 min prior to the start of the experiment. Propranolol (1 $\mu$M), rauwolscine (0.1 $\mu$M) and 5-methylurapidil (1 nM) were administered 20 min before the administration of phenylephrine. Phenylephrine (10 $\mu$M) was infused for a period of 10 min.

2.3. Membrane preparation and binding experiments

Individual hearts were placed in ice cold buffer A composed of 10 mM Hepes (pH 7.4), 250 mM sucrose, 5 mM EGTA, 12.5 mM MgCl$_2$ and a cocktail of protease inhibitors. After a 30-s disruption with a polytron, material was transferred to a dounce homogenizer, diluted 1:7 in buffer A, and homogenized 10 times each with a loose and
were spun for 15 min at 35,000 × g to remove fat and for 5 min at 1250 × g to remove nuclei and then incubated for 15 min at 4 °C in an equal volume of 0.5 M KCl. The KCl wash breaks down myosin, resulting in a purer membrane preparation. Homogenates were spun for 15 min at 35,000 × g to pellet membranes. Pellets were resuspended in ice cold buffer B composed of 20 mM Hepes (pH 7.4), 100 mM NaCl, 5 mM EGTA, 12.5 mM MgCl2 and a cocktail of protease inhibitors. This spin/wash procedure was repeated twice. After resuspension in buffer B containing 10% glycerol, the final pellet was homogenized again, analyzed for protein concentration by Bradford and frozen at −70 °C. Saturation binding was performed using the α1A-AR antagonist 2-[β-(4-hydroxyl-3-[125I]iodophenyl)ethylaminomethyl]-tetralone ([125I]HEAT) as the radioligand and phenolamine (100 μM) to determine the total α1A-AR density. The density of the α1B-AR population was determined by repeating the saturation experiment with 1 mM Ca2+. The filtrate was placed in a 37 °C water bath for 5 min, and then the supernatant was discarded and the wash was repeated. The final pellet was resuspended in a collagenase-free Hepes buffer containing 1 mM Ca2+. Myocytes were used within 4 h of isolation.

2.5. Myocyte contractility measurements

Myocytes were incubated on a laminin-coated glass coverslip in a recording chamber (RC-24, Warner Instrument, Hamden, CT) and mounted onto the stage of an inverted microscope (IX-70, Olympus America, Melville, NY). Myocytes were bathed in Hepes buffer (1 mM Ca2+) at a rate of 1 ml/min (Masterflex L/S; Cole Parmer, Vernon Hills, IL) from an inline heater (37 °C, TS 28; Warner Instrument, Hamden, CT) and field stimulated (0.5 Hz, SD9 stimulator; Grass Instruments, Quincy, MA). Myocytes were imaged with a charge-coupled device camera, and the changes in cell-length were quantified by an edge-motion detection with a video dimension analyzer (Coyote Bay, Manchester, NH). The myocyte twitch amplitude was defined as a percentage of the diastolic length. The baseline measurements were quantitated (Inspector 3.0; Matrox Electronic Systems, Canada) after a 5-min equilibrium period. The effect of increasing concentrations of phenylephrine (1 nM to 10 μM) and isoproterenol (1 nM to 10 μM) on twitch amplitude were analyzed in two cell types [normal (n=24 myocytes from 3 hearts) and transgenic (n=25 from 4 hearts)]. The effect on twitch amplitude was digitally recorded after 2 min of drug perfusion and later quantitated with Matrox Inspector 3.0.

2.6. Statistics

Significant differences are obtained using unpaired Student’s t-test.

3. Results

3.1. Baseline parameters

To determine if the transgenic mice displayed changes in baseline values, we first determined physiological parame-

Table 1
Baseline physiological parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (n = 6)</th>
<th>Transgenic (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
<td>37 ± 1</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>39 ± 2</td>
<td>28.3 ± 2*</td>
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<tr>
<td>Dry ht. wt. (g)</td>
<td>0.19 ± 0.01</td>
<td>0.15 ± 0.02</td>
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<td>Ht. wt./body wt. (ng/g)</td>
<td>5.0 ± 0.3</td>
<td>5.6 ± 0.3</td>
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<tr>
<td>CF/h. wt. (ml/g)</td>
<td>10.44 ± 0.55</td>
<td>11.20 ± 0.47</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M.; ht., heart; wt., weight; CF, coronary flow.

*P<0.05 vs. normal.

Table 2
Baseline cardiac parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (n = 6)</th>
<th>Transgenic (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>345 ± 11</td>
<td>354 ± 12</td>
</tr>
<tr>
<td>DP (mm Hg)</td>
<td>93 ± 7</td>
<td>89 ± 5</td>
</tr>
<tr>
<td>+dP/dt (mm Hg ms⁻¹)</td>
<td>2.86 ± 0.37</td>
<td>2.74 ± 0.14</td>
</tr>
<tr>
<td>−dP/dt (mm Hg ms⁻¹)</td>
<td>1.72 ± 0.16</td>
<td>1.68 ± 0.11</td>
</tr>
<tr>
<td>CF (ml min⁻¹)</td>
<td>2.08 ± 0.19</td>
<td>1.98 ± 0.25</td>
</tr>
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</table>

Values are mean ± S.E.M.; HR, heart rate; DP, developed pressure; +dP/dt, maximal rates of the rise and fall in ventricular pressures; CF, coronary flow.
ters (Table 1). The age matched groups (≈ 9 months) have a significant difference in body weight with the transgenic mice having a reduced body weight (28%) compared to normals. Heart weight, when normalized to body weight, is slightly greater in the transgenic group; however, this is not significant. There were also no differences in the coronary flow between the two when corrected for heart weight differences.

We next determined changes in baseline cardiac parameters (Table 2). There was no significant difference in baseline cardiac parameters between the normal and transgenic hearts. Heart rate (≈ 350 bpm) and developed pressure (≈ 90 mm Hg) parameters are similar to previously reported values obtained using this isolated heart preparation [12].

3.2. Cardiac parameters in response to phenylephrine

Fig. 1A shows a typical response of hearts from control animals to phenylephrine (10 μM), an α₁-AR selective agonist, in the presence of propranolol (1 μM) and rauwolscine (0.1 μM) to block any effects due to β- or α₂-ARs. There is a triphasic response with an initial increase in LVP, dP/dT, HR and CF followed by a rapid

Fig. 1. Recorded changes in cardiac parameters after the addition of phenylephrine (10 μM). (A) Recorded changes in various cardiac parameters of a normal mouse heart are obtained using the Powerlab data acquisition system. Cardiac parameters are measured off-line at the end of the experiment at steady-state levels. Propranolol (1 μM) and rauwolscine (0.1 μM) were administered 20 min before the administration of phenylephrine. Phenylephrine (10 μM) was infused for a period of 10 min. LVP, left ventricular pressure; CPP, coronary perfusion pressure; dP/dT, rise and fall in ventricular pressure; BPM, beats per minute; CF, coronary flow. (B) Changes in LVP observed in normal (top) and transgenic (bottom) after phenylephrine infusion. The transient negative inotropy was similar in both samples.

Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (n = 6)</th>
<th>Transgenic (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>23 ± 2%</td>
<td>10 ± 3%*</td>
</tr>
<tr>
<td>DP (mm Hg)</td>
<td>45 ± 10%</td>
<td>14 ± 5%*</td>
</tr>
<tr>
<td>+dP/dT (mm Hg ms⁻¹)</td>
<td>66 ± 14%</td>
<td>22 ± 7%*</td>
</tr>
<tr>
<td>-dP/dT (mm Hg ms⁻¹)</td>
<td>74 ± 18%</td>
<td>19 ± 8%*</td>
</tr>
<tr>
<td>CF (ml min⁻¹)</td>
<td>45 ± 12%</td>
<td>59 ± 32%</td>
</tr>
</tbody>
</table>

Values are mean % change from its own baseline ± S.E.M.; HR, heart rate; DP, developed pressure; ±dP/dT, maximal rates of the rise and fall in ventricular pressures; CF, coronary flow.  
*P < 0.05 vs. normal.
decrease then a sustained increase. The rapid decrease falls below baseline pressure of about 10–20 mm Hg but is transient, only lasting about 2–3 s, and is not different in normal vs. transgenic (Fig. 1B). The sustained increase in pressure is taken as the point of comparison for inotropic responses.

Comparisons of the phenylephrine response between normal and transgenic are shown in Table 3. There are significant differences between the transgenic and normal hearts in all cardiac parameters except coronary flow with addition of phenylephrine (10 μM). Although all parameters increase due to phenylephrine stimulation in the transgenic, the values are below those normally seen in the control mice. Heart rate increases 23% in the normal hearts whereas there is only a 10% increase in the transgenic hearts. Indices of contractility such as developed pressure and +dP/dT show greater increases in the normal hearts (45% and 66%, respectively) compared to transgenic hearts (14% and 22%, respectively). Myocardial relaxation as assessed by −dP/dT followed the same trend with a 74% increase in the normal hearts compared with 19% in the hearts from transgenic animals. Coronary flow tended to increase in the transgenic heart but this was not significant.

Since the transgenic mice have a reduced inotropy but also a reduced heart rate, as a control, we also performed experiments where we paced both normal and transgenics hearts at their intrinsic heart rate and re-measured cardiac parameters after administration of phenylephrine. We found that the transgenic hearts still had reduced inotropy (Table 4), suggesting that changes in the heart rate was not responsible for the reduced cardiac parameters.

3.3. α₁-AR subtype mediation of inotropy

To determine the α₁-AR subtype responsible for the observed phenylephrine effects, we used 5-methylurapidil at a dose (1 nM), which is about 100-fold selective against the α₁A-AR subtype. Fig. 2 shows the phenylephrine response in both control and transgenic hearts in the presence of 5-methylurapidil (1 nM). There are no significant differences between the transgenic and normal hearts in any of the cardiac parameters with this treatment, suggesting that the α₁A-AR selective antagonist equalized the two systems. Although the % values in Fig. 2 are higher than the values in Table 3, there are no significant differences.

![Fig. 2. Changes in cardiac parameters after the addition of phenylephrine (10 μM) in the presence of 5-methylurapidil (1 nM). Propranolol (1 μM), rauwolscine (0.1 μM) and 5-methylurapidil (1 nM) were administered 20 min before the administration of phenylephrine. Phenylephrine (10 μM) was infused for a period of 10 min. Light bars, normal mice; dark bars, transgenic mice; DP, developed pressure; ± dP/dT, the maximum rise and fall in ventricular pressure; HR, heart rate. Results are mean % change ± S.E.M. (n = 6 in both groups).]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (n = 3)</th>
<th>Transgenic (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP (mm Hg)</td>
<td>47 ± 8%</td>
<td>13 ± 6%*</td>
</tr>
<tr>
<td>+dP/dT (mm Hg ms⁻¹)</td>
<td>82 ± 12%</td>
<td>21 ± 13%*</td>
</tr>
<tr>
<td>−dP/dT (mm Hg ms⁻¹)</td>
<td>59 ± 7%</td>
<td>12 ± 9%*</td>
</tr>
<tr>
<td>CF (ml min⁻¹)</td>
<td>20 ± 7%</td>
<td>9 ± 3%</td>
</tr>
</tbody>
</table>

Values are mean % change from its own baseline ± S.E.M.; hearts were paced at their intrinsic heart rate; DP, developed pressure; ±dP/dT, maximal rates of the rise and fall in ventricular pressures; CF, coronary flow. *P < 0.02 vs. normal.
3.4. Phenylephrine response in isolated myocytes

To determine if a decrease in the drug-induced inotropic response was also apparent in isolated myocytes, we measured changes in myocyte cell-length as an index of contractility (Fig. 3). Increasing amounts of phenylephrine resulted in a concentration-dependent increase in shortening (≈ 65%) in the normal myocytes, whereas there was no increase and even a significant decrease in shortening in the transgenic myocytes. This suggests that

Fig. 3. Changes in myocyte cell-length with increasing phenylephrine concentrations. Changes in cell-length were quantified by an edge-motion detection with a video dimension analyzer. The effect of increasing concentrations of phenylephrine (1 nM to 10 µM) on the changes in cell-length was analyzed in normal ($n=24$ myocytes from 3 hearts) and transgenic ($n=25$ from 4 hearts) mice. Results are mean % change ± S.E.M.; *$P<0.05$ vs. normal.

Fig. 4. Changes in myocyte cell-length with increasing isoproterenol concentrations. Changes in cell-length were quantified by an edge-motion detection with a video dimension analyzer. The effect of increasing concentrations of isoproterenol (1 nM to 10 µM) on changes in cell-length was analyzed in normal ($n=24$ myocytes from 3 hearts) and transgenic ($n=25$ from 4 hearts) mice. Results are mean % change ± S.E.M.; *$P<0.05$ vs. normal.

Fig. 5. $\alpha_1$-AR subtype density in normal and transgenic hearts. Total $\alpha_1$-AR density was determined by saturation binding experiments using the nonselective $\alpha_1$-AR-antagonist $^{125}$I-HEAT as the radioligand and phentolamine (100 µM) to determine non-specific binding. $\alpha_{1B}$-AR density was determined by performing the identical saturation study with the $\alpha_1$-AR selective blocker, 5-methylurapidil (1 nM). (A) Representative scatchard analysis from a normal mouse heart performed in the presence of 5-methylurapidil. (B) Compiled scatchard analysis. The $\alpha_{1A}$-AR population was determined by subtracting the $\alpha_{1B}$-AR density from the total $\alpha_1$-AR pool. $B_{\text{max}}$ was determined using the non-linear regression analysis of GraphPad Prism. $K_D$ values ranged from 128 ± 23 to 202 ± 26 pM and were not statistically significant from each other. Bars represent the mean $B_{\text{max}}$ ± S.E.M. ($n=6-8$). *$P<0.05$ vs. normal. Specific binding represents 30% of total bound.
the transgenic myocyte has no positive but a negative
inotropy. To assess possible changes in the β-AR re-
response, changes in myocyte length with increasing con-
centrations of isoproterenol can be seen in Fig. 4. As
isoproterenol concentration increases, there is an increase
in shortening (= 200%) in the normal myocytes with
similar results in the transgenics.

To determine if there were changes in the α₁-AR subtype protein population, as suggested by the data,
we first attempted to perform competition ligand binding
studies to determine the percentage of high and low
affinity sites. There are no high avidity antibodies avail-
able to determine protein expression. However, since the
total α₁-AR population in the mouse heart is very low
(86 fmol/mg membrane protein, Fig. 5B), this was not
feasible to simultaneously discriminate accurately two
receptor populations. We then decided to perform differ-
ential saturation binding studies using phentolamine at a
concentration of 100 µM, which would block all the α₁-
AR subtypes. This experiment would determine the total
α₁-AR population. The experiment is then repeated with
a concentration of the α₁A-selective blocker, 5-methylur-
apidil, used at the same concentration as in the functional
studies (1 nM). The specific binding would then deter-
mine the α₁B-AR population. Since it has been previously
shown that the α₁D-AR is not present in the heart or is at
very low numbers, as determined by binding [3], the
difference between the total and α₁B-AR densities would
determine the amount of α₁A-AR present. Although 1 nM
of 5-methylurapidil will only block 50% of the α₁A-AR
binding sites, we used this concentration to be sure of
only blocking the α₁A-AR and not any contribution from
the α₁B- or α₁D-ARs. A representative scatchard analysis
is shown in Fig. 5A. The low density of α₁-ARs precluded the use of only 4–5 points per curve. Percent
specific binding was 30% of total bound. Normal and
transgenic hearts had total α₁-AR B_max values of 86 ± 11
and 99 ± 9 fmol/mg membrane protein, respectively (Fig.
5B). This difference is not significant. The affinities of
the radioligand were also not significantly different be-
tween groups. However, the α₁B-AR density increased in
the transgenic 72.5 ± 13 vs. 40.3 ± 3.5 fmol/mg. This
leaves the α₁A-AR population in the transgenic at 26
fmol/mg compared to 46 fmol in normal hearts. This
represents about a 70:30 ratio of the α₁B/α₁A populations
in the transgenics, while the normal mouse has a 50:50
ratio.

4. Discussion

Our results indicate that the systemic overexpression of a
constitutive active mutant (CAM) α₁B-AR in the murine
myocardium leads to a functional change in the inotropic,
chronotropic and lusitropic response to phenylephrine com-
pared to normal hearts. These differences could be elimi-
nated by the α₁A-AR antagonist 5-methylurapidil and
supported by decreases in α₁A-AR density by saturation
binding. These results suggest that the overexpression of
the α₁B-AR leads to a down-regulation of the α₁A-AR, ulti-
mately resulting in the observed functional and decreased
changes in response to phenylephrine.

The inotropic response to α₁-AR stimulation has been
studied in a variety of animal species and preparations.
However, the responses have considerable variation (for
review, see Ref. [13]). Positive inotropy to α₁-AR agonists
have been found in whole heart and muscle strips from
various species. However, α₁-AR-mediated negative in-
otropy has recently been described in mouse muscle strips
and isolated myocytes [14–17]. The predominant subtypes
in the myocardium are the α₁A- and α₁B-AR subtypes with
densities varying according to species [18], cardiac region
[19] and developmental stage [20]. The mouse heart has
been documented to contain the lowest levels of α₁-ARs.
While we report α₁-AR density levels of approximately 80–
90 fmol/mg, previous reports are in the 5–10 fmol/mg range
[3,7]. This difference may be due to our use of radioactive
iodine, which has a higher specific activity and greater
sensitivity than tritium and our purer membrane preparation,
which removes most of the contaminating myosin with the
KCl wash.

There have been a number of pharmacological studies
looking at the effects of the various subtypes on myocar-
dial function (reviewed in Ref. [4]). Using antagonists to
isolate the contributions of the subtypes, these studies
postulate that both the α₁A- and α₁B-AR subtypes con-
tribute to the positive inotropic effect of α₁-AR stimula-
tion. One possible drawback of these pharmacological
studies is the lack of subtype selectivity of these anti-
gonists for these receptors. We decided to use a genetic
approach in combination with a pharmacological one to
determine the contribution of the α₁B-AR subtype to
myocardial function. We took a unique approach in
creating transgenic mice using the isogenic mouse mouse-
myosin to drive systemic overexpression of the
α₁B-AR [5]. Overexpression is mild, with no significant changes in α₁-
AR density but resulted in a 50% increase in the α₁B-AR
population (Fig. 5B). This is distinctly different from the
heart-targeted transgenics of the α₁A- and α₁B-AR
[6,7,21], which display dramatic overexpression of the α₁B-AR
[5]. Baseline cardiac parameters (DP, ± dP/dT, CF) from
transgenic animals were comparable to those in normal
hearts suggesting that the systemic overexpression of the
α₁B-AR did not affect basal function. This confirms the
finding of previous studies [23] where even 100-fold heart-
targeted overexpression of wild-type α₁B-AR did not result
in any change in basal parameters. However, using the same
mouse model, the studies of Grupp et al. [6] did produce a lower basal LV function.

Stimulation of α1-ARs with phenylephrine resulted in an increase in all cardiac parameters in both transgenic and normal hearts. In both groups, phenylephrine elicited a triphasic response (brief positive inotropy followed by brief negative inotropy and then sustained positive inotropy). This is the first study to show this type of triphasic response in the whole murine heart. However, this triphasic response is consistent with previous findings found in isolated rat papillary muscle [24], mouse cardiac trabeculae [14] and now the isolated mouse heart [12]. The mechanism of the triphasic response has not been clearly established. It has been proposed in rat papillary that the negative inotropic effect is mediated by the α1A-AR subtype and the positive inotropic responses are mediated by both the α1A- and α1B-AR subtypes [25]. In the mouse trabeculae, both subtypes are thought to mediate the negative phase, but there is no positive inotropy but only a partial recovery from the negative inotropy, again mediated by both α1-AR subtypes [14]. This is contrasted with the α1A-AR transgenic, which showed dramatic increases in inotropy [7]. Interestingly, Nishimaru et al. [16] showed that, in isolated mouse right ventricle, phenylephrine stimulation results in a sustained negative inotropy mediated by enhanced Ca\textsuperscript{2+} efflux through the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. The most recent work of Turnbull et al. [12] utilized the α1A/B double knockout mice. Convincingly, they show that the α1A/B double knockout did not have any phenylephrine-induced positive inotropy but only a small sustained negative inotropy, which could be reversed with BMY7378, suggesting that the α1D-AR was responsible for the negative inotropy but via the coronary vasculature and the observed decreased coronary flow causing the negative inotropy.

We did performed similar isolated tissue experiments (data not shown) and found that in the isolated mouse right ventricle a sustained negative inotropic response is observed with phenylephrine stimulation. However, in the isolated myocyte, phenylephrine does induce a positive inotropic effect (Fig. 3). The whole heart mouse model of α1B-AR overexpression of Akhter et al. [23] and Grupp et al. [6] also show positive α1-AR-mediated inotropy, but isolated mouse myocytes have also been described to have negative inotropy [17]. The discrepancy between the two systems (isolated or intact heart and myocyte vs. isolated ventricular tissue) cannot be attributed to different perfusate solutions or temperatures since they were consistent between our two preparations. One possibility is that the frequency of stimulation may be responsible for the difference. It has been shown that frequency of pacing can affect the response of tissues to phenylephrine [26], and tissue/myocyte preparations are stimulated at significantly lower frequencies than the spontaneously beating heart. However, this does not account for our own differences between the isolated heart and tissue-bath studies. Nevertheless, repeatable and opposite results in α1-AR-mediated inotropy is observed depending upon the system used. Another likely and disturbing possibility is that the α1-AR subtype distribution may change upon the removal of the organ. This has precedence in guinea pig liver studies in which the α1-AR subtype switched (α1A\textsuperscript{A} to α1D\textsuperscript{A}) from the removal to the isolation of the hepatocytes [27]. The only consistent explanation is that whole or intact heart studies produce an α1-AR-mediated positive inotropy in the mouse [6,7,12,23], while tissue and/or myocyte preparations may produce the negative inotropy [14,16,17], suggesting that whole heart studies may be more physiologically relevant.

In this study, we found that the sustained increase in inotropic response to phenylephrine in the transgenic hearts is significantly less than that found in the normal hearts. This result was unexpected since α1B-ARs have been implied to have a positive inotropic response to phenylephrine in whole heart. This decreased inotropy is not due to differences in heart rate as paced hearts show the same effect (Table 4). The decreased inotropy is also not due to decreased second messengers that may regulate contractility. In hearts from these same transgenic mice, the activities of the mitogen-activated protein kinases, extracellular signal-regulated kinase and c-Jun N-terminal kinase were significantly elevated compared with nontransgenic control animals [28]. However, our work is consistent to Grupp et al. [6] that demonstrated an impaired left ventricular contraction in the heart-targeted wild-type α1B-AR model. One possibility that could explain this phenomenon was that overexpression of the α1B-AR may have altered the expression levels of the other α1-AR subtypes. Indeed, there is some precedence in the literature that this is possible. Prolonged incubation with norepinephrine, which causes hypertrophy in normal rat myocytes also causes the α1B- and α1D-AR to decrease while the α1A-AR increases [29]. Deng et al. [30] have shown that there is crosstalk between α1A- and α1B-ARs in neonatal rat myocardium, whereby knocking out the α1B-AR with CEC caused a potentiation of the α1A-AR response. In our studies, the α1A-AR antagonist 5-methylurapidil eliminated the functional differences between the transgenic and normal hearts for the effects on cardiac contractility, suggesting that the α1A-AR was responsible. The results correlated to the loss of α1A-AR binding sites. These results suggest that α1A-AR levels are decreased in the transgenic hearts and that this subtype is responsible for the differences in cardiac responsiveness observed between the transgenic and normal hearts to phenylephrine. This result would be consistent to the heart-targeted transgenic α1A-AR results [7], which suggests that the α1A-AR is a potent mediator of positive inotropy in the mouse myocardium.

The α1-ARs have prominent effects on cardiac function. In this communication, we show that the major positive inotropic activity for this subtype family resides in the α1A-
AR. Other work has also implicated the α1B-AR as being involved in major positive inotropic effects. Our data do not support this but we cannot rule out minor contributions. Indeed, we demonstrate that overexpression of a constitutively active α1B-AR is associated with a negative effect on myocardial contraction. The mechanism underlying these effects does not appear to be a direct linkage of the α1B-AR to negative inotropic pathways. Rather, the α1B-AR appears to interfere with the activity of receptors, such as the α1A-AR that mediates positive inotropic responses. Thus, via mechanisms that remain to be elucidated, there is molecular cross talk between the α1B-AR and receptors coupled to positive inotropic responses that result in a decrease in contractile function. Thus, we propose that the role of the α1B-AR is to negatively modulate contractile activation through indirect mechanisms. Complicating mechanisms such as changes in afterload is not possible since the transgenic mice are hypotensive [8]. Reduced sympathetic input, a phenotype of the mice [8] would likely produce a supersensitivity to adrenergic stimulation, opposite to our phenotype.

The role of the α1B-AR in the myocardium is unlikely to be only a modifier of the α1A-AR. Distinct differences are present when constitutively active receptors of the α1A- and α1B-AR, respectively, were transfected into the cardiac murine myocyte cell line (HL-1) [21]. In this study, they demonstrate that the α1A-α1B-AR preferentially couples to different pathways with the α1B-AR being involved in mitogenic signals. We also published an oligonucleotide microarray study of the changes associated with α1B-AR-mediated gene expressions in the hearts of the same transgenic animals used this study [31] and found changes in the gene expressions for Src-related signals consistent with the role of this subtype in growth and development.

In conclusion, we have shown that overexpression of the α1B-AR does not lead to any basal functional changes in the isolated heart. However, with α1-AR stimulation, we see a depressed response from the transgenic hearts compared to controls. We attribute this difference to a decrease in α1A-AR expression levels in the transgenic hearts. Therefore, we suggest that the α1B-AR does not play a major role in modulating cardiac contraction directly but may be a negative modifier of inotropy.

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References


