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Transgenic α_{1A}-adrenergic activation limits post-infarct ventricular remodeling and dysfunction and improves survival

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Abstract

Objective: Myocardial contractility is enhanced in transgenic (TG) mice with cardiac-restricted overexpression of the α_{1A}-adrenergic receptors (α_{1A}-AR). We tested the hypothesis that this enhanced inotropy protects against dysfunction and remodeling after myocardial infarction (MI).
Methods: We subjected α_{1A}-TG and non-TG mice (NTG) to MI and determined changes in left ventricular (LV) function and diastolic dimension (LVDd) by echocardiography prior to and at 1, 3, 7, 12 and 15 weeks thereafter.
Results: Although infarct size was similar in the NTG and α_{1A}-TG groups (32±2 vs. 29±2% of LV, P=NS), mortality due to heart failure was lower after MI in the α_{1A}-TG (37%, n=39) than that in the NTG animals (63%, n=56, P=0.026). NTG and α_{1A}-TG mice showed similar reductions in LV fractional shortening (FS) and increases in LVDd at week-1 after MI. However, whereas NTG mice showed continuous deterioration over a 15-week period after MI in FS (fell by 40%, from 30±2 to 18±1%, P<0.01) and LVDd (increased by 24%, from 4.2±0.1 to 5.2±0.1 mm, P<0.01), the changes in both FS (fell by 14%, from 42±2 to 36±2%) and LVDd (increased by 8%, from 3.8±0.1 to 4.1±0.1 mm, both changes P<0.01 vs. NTG) were significantly less severe in the α_{1A}-TG mice and did not progress after 3 weeks. At 15 weeks after MI, LV catheterization revealed better preservation of dP/dt_{max} in the α_{1A}-TG vs. NTG mice (7270±324, vs. 5938±372 mmHg/s, P<0.05).
Conclusion: Enhanced inotropy resulting from transgenic overexpression of α_{1A}-AR is well maintained chronically after MI and limits echocardiography-determined LV remodeling, preserves function, and reduces acute heart failure death.

Keywords: α_{1A}-adrenergic receptor; Heart failure; Ventricular remodeling

1. Introduction

The myocardial α_{1A}-adrenergic receptor (AR) has long been regarded as one of key mediators of myocardial hypertrophy [1,2], but, in the normal heart are thought to contribute little to contractile function relative to β-ARs [3]. We showed previously that overexpression of the α_{1A}-AR (α_{1A}-TG) in mouse hearts by up to 170-fold leads to enhanced inotropy but not lusitropy [3]. Unlike studies using cultured rat cardiac myocytes, activation of α_{1A}-ARs in this model does not lead to cardiac hypertrophy, even at advanced ages [4]. Given that β-AR signaling is down-regulated in the hypertrophied and failing heart, whereas α_1-AR signaling is largely preserved [5], this model provides a useful tool for evaluating the contribution of α_{1A}-ARs to cardiac function under pathological conditions.

Recently we have shown that activated α_{1A}-AR signaling protects against LV dysfunction in α_{1A}-TG mice despite comparable degrees of pressure-overload hypertrophy to that

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in NTG controls [6]. However, it is possible that this beneficial effect of α_{1A}-AR activation is etiology-dependent. Indeed, we have also demonstrated that enhanced myocardial contractility due to cardiac overexpression of β_2-AR has opposing effects when evaluated in animals with pressure-overload as compared to myocardial infarction (MI) [7,8].

Infarction of a substantial mass of working myocardium results in ventricular dysfunction and remodeling [9,10]. This involves infarct-segment expansion, hypertrophy and fibrosis of non-infarcted myocardium, and dilatation of the LV cavity, a remodeling process that contributes to the development and worsening of heart failure (HF) [9,11]. Recent animal studies using genetically engineered mice, or mice subjected to adenoviral gene transfer, have shown that interventions that enhance myocardial contractility not only preserve function but also limit the degree of remodeling after MI [12,13]. Enhanced contractility also prevents remodeling in a genetic model of dilated cardiomyopathy [14].

Here we examined if activation of myocardial α_{1A}-ARs is beneficial after MI. Serial echocardiography was performed in α_{1A}-TG and NTG mice over a 15-week period after MI to evaluate the extent of LV remodeling and dysfunction. To address the long-term impact of α_{1A}-AR overexpression, we also examined survival and cardiac function in α_{1A}-TG mice at 12-months of age.

2. Methods

2.1. Animals and surgery

The A1A2 α_{1A}-TG line with 66-fold overexpression of the rat α_{1A}-AR and their NTG littermates were studied [3]. The functional phenotype of this α_{1A}-TG line has been previously described in detail [3,6]. Mice were 3–4 months of age and on a FVB/N genetic background. Both male and female mice were used and each group was matched for gender ratio. Animals were housed under standard conditions and were inspected at least twice daily during the study period. Experimental procedures were approved by our institutional Animal Ethics Committee in accordance with the NIH guidelines.

Animals (73 NTG and 56 α_{1A}-TG) were randomly assigned to either MI or sham-operation. MI was induced by open-chest surgery to occlude the left coronary artery, as we previously described in detail [7,15]. All mice that died during the study period were subjected to post-mortem examination to determine if the cause of death was HF. Our criteria for HF were signs of chest fluid accumulation, lung congestion and, in chronic HF, the presence of organized thrombus in the left atrium, as described previously [7,16].

Another cohort of α_{1A}-TG and NTG mice ($n=25$ per genotype with gender matching) were monitored for survival from ages 4 to 12 months, at which time cardiac function was evaluated by echocardiography and catheterization.

2.2. Echocardiography and hemodynamic determination

Echocardiography was performed before surgery (week-0) and at 1, 3, 7, 12 and 15 weeks after MI, using a Hewlett–Packard Sonos 5500 ultrasound machine and a 15 MHz linear transducer. Animals were lightly anesthetized (ketamine, xylazine and atropine at 50, 10 and 0.6 mg/kg, i.p., respectively) for these evaluations. 2-D guided M-mode tracings were derived from the short-axis loop of the LV. The following parameters were determined from the M-mode tracings: heart rate (HR), LV dimensions at end-systole and end-diastole (LVDs, LVDd, respectively), external LV dimension at end-diastole (ExLVDd), and posterior (non-infarcted) wall thickness at end-diastole and end-systole. Fractional shortening [FS%=(LVDd−LVDs)/LVDd], and contractile increments in wall thickness were calculated. To ensure that differences observed between the genotypes were independent of the anesthetic used, we also performed echocardiography at 14 weeks after surgery, on mice anesthetized with avertin (at 250 mg/kg. i.p.). To analyze echocardiographic images, a coding system was used to ensure that the data remained blinded.

At the end of the 15-week study period, animals were anesthetized (ketamine, xylazine and atropine at 100, 20 and 1.2 mg/kg, i.p., respectively) and a 1.4 F Millar catheter inserted via the right carotid artery into the LV. Aortic blood pressure, LV pressure and the maximal rates of rise and fall of LV pressure (dP/dt_{max} and dP/dt_{min}, respectively) were determined, as described previously [6,7,15].

2.3. Organ weights and infarct size

After removal of atria and the right ventricle, with the aid of a surgical microscope, the LV was cut open and pinned down to flatten the entire LV wall, with the endocardial surface exposed. The LV was photographed using a digital camera. A chronic infarct in mouse hearts was easy to be identified by its pale color and clear demarcation from non-infarcted myocardium (Fig. 1A). The infarcted area and the entire LV surface area were measured digitally using Optima's image analysis system. Infarct size was calculated and expressed as a percentage of the entire LV surface area [17]. Non-infarcted LVs were then separated microscopically and frozen for biochemical assays.

2.4. Quantitative real-time PCR and hydroxyproline assays

Total RNA was extracted from non-infarcted LV with TRIzol. After DNAase treatment, 1 μg RNA was reverse transcribed using random primers and Superscript III RNase transcriptase. Using real-time PCR SYBR Green Master Mix with the ABI PRISM 7700 sequence detection system, we then determined, in duplicate, the mRNA levels of atrial natriuretic peptide (ANP), α-skeletal actin (α-SkA), β- or α-myosin heavy chain (β-MHC, α-MHC), sarcoendoplasmic

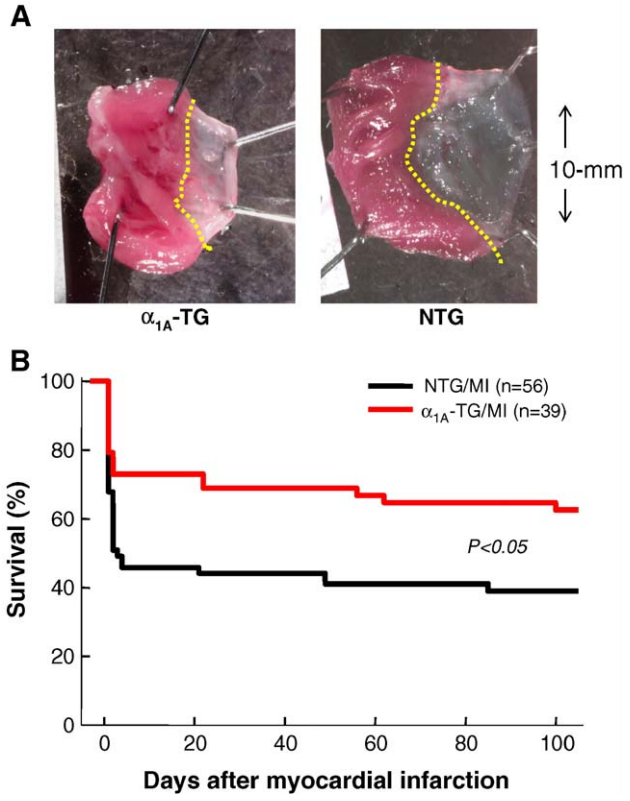

Fig. 1. A, Photos showing infarcted mouse left ventricle (LV, the right ventricle and atria were trimmed off). The LV was cut open and pinned in such a way that the entire LV wall was flat. From digital images, the endocardial surface areas of the infarcted (pale) and non-infarcted (pink) zones were determined using image analysis software. Infarct size was expressed as % entire LV surface area. Dilatation of the infarcted NTG LV was evident by an increase in LV size. B, Survival of the NTG and α_{1A}-TG mice over 15 weeks post-MI by Kaplan–Meier method. Only mice that fully recovered from surgery were included in this survival analysis. There was no loss of sham-operated mice during the study period (curve not shown).

reticulum Ca^{2+}-ATPase 2a (SERCA2a), procollagen (types 1 and 3), matrix metalloprotenases (MMP types 2, 9 and 13), connective tissue growth factor (CTGF) and fibronectin. Expression of a reference gene, 18S, was used to normalize the mRNA levels. For each sample, a single amplified product was confirmed by disassociation curve analysis.

The content of collagen in the LV myocardium was determined by measuring the concentration of hydroxyproline, as previously described [18].

2.5. Statistics

Results are presented as means±SEM. Statistical analyses were performed using Sigma Stat 2.03 software with one-or two-way ANOVA for repeated measures, followed by the Neuman–Kuel test. Fisher exact test was used for comparison of events between groups. The least-square method was used for correlation analyses. Survival was analyzed using the Kaplan–Meier method. Statistical significance was accepted at a value of $P<0.05$.

3. Results

3.1. Post-infarct survival

Surgery-related deaths were comparable in the α_{1A}-TG and NTG mice (about 10%). Four mice (2 in each group) were excluded, as their infarct size was less than 15%, as determined at the end of the study. All mice that fully recovered from anesthesia for at least 8 h were counted in the survival analysis. NTG mice with MI had a higher mortality that occurred during the first few days after surgery. Typical autopsy findings in these mice included severe pulmonary edema, pleural effusions and the presence of a recent infarct, indicating acute left heart failure, rather than fatal arrhythmias, as the likely cause of death. There was no gender-bias in the prevalence of such acute deaths. Rupture of the LV free wall, another cause of death in mice within the first week after MI [17], was rare (1 NTG and 2 α_{1A}-TG). In α_{1A}-TG mice, acute HF deaths were significantly lower than that in NTG (28.2% vs. 53.7%, $P<0.05$, Fig. 1B). Lung wet weights were determined at autopsy as a measure of pulmonary edema in about half of the mice that died acutely, and found to be significantly greater in the α_{1A}-TG mice than in their NTG littermates (415±15 mg, $n=10$ vs. 337±14 mg, $n=16$, $P<0.01$), which suggest that the α_{1A}-TGs had tolerated a more severe degree of HF before death than the NTGs. Deaths during the chronic phase of MI were all due to HF and there was no difference in their incidence in the two groups (Fig. 1B). Thus, overall post-infarct survival was better in α_{1A}-TG than NTG mice ($P=0.026$), and this was entirely due to a lower incidence of acute death in α_{1A}-TG group.

3.2. Serial echocardiography

Consistent with the α_{1A}-TG mice displaying enhanced inotropy [3,6], echocardiography prior to surgery revealed markedly higher FS (Fig. 2C) in the α_{1A}-TG than in the NTG animals, which was associated with a smaller LVDs, as observed previously [3,6]. Such differences were maintained throughout the study in both sham-operated groups (Fig. 2B,C).

To monitor time-dependent changes in LV remodeling and dysfunction, serial echocardiography was performed during the 15-weeks after MI. HR determined by echocardiography with the animals lightly anesthesized, was similar in the α_{1A}-TG and NTG animals at all time-points studied (Table 1). Compared with the week-0 value or with the respective sham-operated group, MI led to significant increases in both LVDd and LVDs by week-1 (Table 1, Fig. 2B). The absolute degree of this acute remodeling was comparable in the two groups. From 1 to 15 weeks post-MI, the NTG mice showed a progressive increase in diastolic LV cavity, measured as LVDd (Fig. 2B, D). In comparison, the net increase in the LVDd of α_{1A}-TG mice during this period was less marked (Fig. 2D). Changes in the LVDs during 1 to 15 weeks showed a similar pattern, with more marked

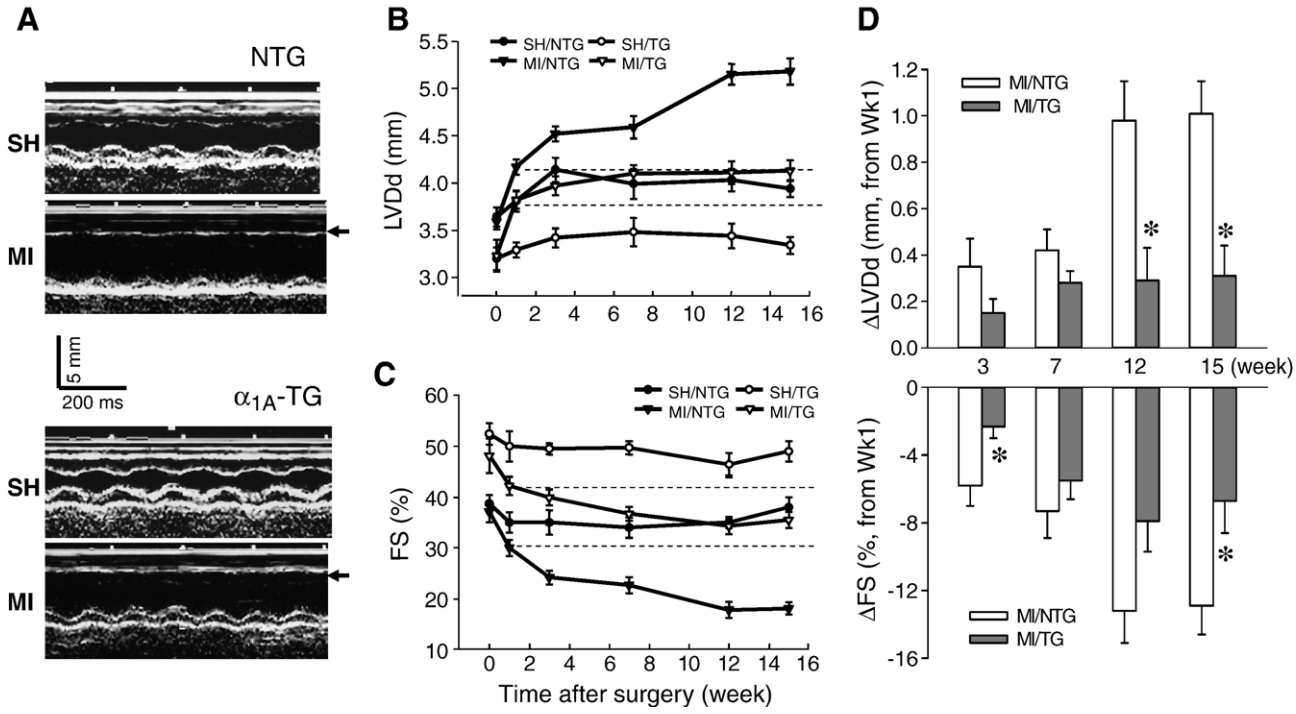

Fig. 2. A, Representative M-mode echocardiographic images obtained at week-15 from sham-operated (SH) and infarcted (MI) NTG and α_{1A}-TG mice. Note that the infarcted walls displayed akinesis (arrowheads). B and C, Time-dependent changes in the LV end-diastolic dimension (LVDd) and fractional shortening (FS) in the sham-operated (n=9–10/group) and infarcted (n=19–20/group) NTG (SH/NTG and MI/NTG, respectively, filled symbols) and α_{1A}-TG (SH/TG and MI/TG, respectively, open symbols) animals. Other echocardiographic parameters are given in Table 1. The dotted reference lines represent the week-1 levels for the infarcted groups. Both parameters differed significantly between NTG and α_{1A}-TG groups at all time-points studied over the 15 weeks post-infarction. The differences between SH/NTG vs. MI/NTG, SH/TG vs. MI/TG, SH/NTG vs. SH/TG or MI/NTG vs. MI/TG were highly significant (P<0.01 by two-way ANOVA). D, Temporal changes in LVDd and FS from week-1 to week-15 in both infarcted groups. Results were expressed as the net change from the week-1 values. *P<0.05 vs. MI/NTG by 2-way ANOVA for repeated measures.

increases being observed in NTG (+1.31 mm) than in the α_{1A}-TG group (+0.44 mm, P<0.01, Table 1).

LV contractile function was assessed by FS and systolic thickening of the non-infarcted posterior wall. Following a similar decline in FS at week-1 after MI in both groups, the α_{1A}-TG mice showed a less pronounced fall thereafter and FS was maintained at equivalent level of the NTG sham-operated controls (Fig. 2C, D). Throughout the study period, contractile thickening of the posterior wall in α_{1A}-TG mice with MI remained greater than that in NTG group (Table 1), further evidence for preservation of enhanced inotropy in α_{1A}-TGs. Similar measures were undertaken at week-14 post-MI in all animals anesthetized with avertin. This revealed between-group differences in the NTG and α_{1A}-TG mice, that were comparable to those observed with ketamine/xylazine/atropine anesthesia in LVDd (3.96±0.11 vs. 4.59±0.22 mm), LVDs (2.37±0.12 vs. 3.50±0.34 mm) and FS (41±2 vs. 27±4%, all P<0.05). In both groups with MI, HR was also comparable under avertin-anesthesia (α_{1A}-TG, 471±12 vs. NTG, 485±23 beats/min).

3.3. Hemodynamics

Micromanometry data was available for all sham-operated mice and for 11 NTG and 12 α_{1A}-TG animals with MI. At week-15 post-MI, there was no significant difference between the α_{1A}-TG and NTG groups in heart rate, blood pressure, LV pressure or dP/dt. NTG mice with MI had significant reductions in LV dP/dt_{max} and LV dP/dt_{min} (Table 2). A similar trend was observed in infarcted α_{1A}-TG vs. sham-operated α_{1A}-TGs. However, dP/dt_{max}, but not dP/dt_{min}, was higher in the infarcted α_{1A}-TG than NTG mice, and as a result, the ratio of dP/dt_{max}:dP/dt_{min} was also higher in the α_{1A}-TG animals (Table 2).

3.4. Organ weights and infarct size

All sham-operated mice (n=9/group) and 20 NTG and 19 α_{1A}-TG mice with MI survived to the end of the study. Body weight was not significantly different among the groups (Table 3). In mice with MI, infarct size ranged from 15% to 46% and was not significantly different in the NTG and α_{1A}-TG groups (Table 3). Also both groups had similar increase in the LV weight and heart weight compared to their respective sham-operated controls, and a similar incidence of chest fluid accumulation or chronic atrial thrombus-formation.

In both groups, infarct size correlated negatively with FS (r=−0.774 in α_{1A}-TG; r=−0.812 in NTG, both P<0.01) and positively with LVDd (r=0.816 in α_{1A}-TG, r=0.778 in

Table 1
Echocardiographic parameters obtained prior to (week-0) and at different time-points after sham-operation (SH) or myocardial infarction (MI) in α_{1A}-AR transgenic (α_{1A}-TG) and non-transgenic (NTG) mice

Parameters	Week-0	Week-1	Week-3	Week-7	Week-12	Week-15
Heart rate, beats/min						
NTG/SH	379±18	365±14	351±11	409±18	359±14	386±10
α_{1A}-TG/SH	387±13	402±10	405±11	387±17	385±10	398±13
NTG/MI	370±17	328±18	355±7	367±10	397±13	417±13
α_{1A}-TG/MI	402±9	364±17	372±11	359±9	379±8	376±7
Ext LVDd, mm						
NTG/SH	5.04±0.08	5.20±0.08	5.31±0.09	5.26±0.14	5.45±0.14	5.31±0.08
α_{1A}-TG/SH	4.62±0.07*	4.58±0.11	4.73±0.08	4.82±0.09*	4.86±0.12*	4.92±0.10
NTG/MI	5.07±0.06	5.53±0.09	5.85±0.10	5.89±0.12	6.48±0.10	6.58±0.12
α_{1A}-TG/MI	4.78±0.13	5.35±0.08	5.57±0.11	5.65±0.10*	5.67±0.11*	5.67±0.11*
LVDs, mm						
NTG/SH	2.24±0.09	2.48±0.11	2.71±0.15	2.65±0.17	2.64±0.13	2.47±0.10
α_{1A}-TG/SH	1.54±0.10*	1.64±0.13*	1.73±0.07*	1.75±0.08*	1.86±0.13*	1.72±0.08*
NTG/MI	2.33±0.08	2.95±0.11	3.43±0.10	3.57±0.14	4.26±0.16	4.26±0.14
α_{1A}-TG/MI	1.70±0.18*	2.24±0.11*	2.40±0.10*	2.61±0.10*	2.72±0.13*	2.68±0.13*
Systolic wall thickening, mm						
NTG/SH	0.36±0.03	0.37±0.03	0.38±0.02	0.38±0.03	0.40±0.03	0.35±0.03
α_{1A}-TG/SH	0.60±0.03*	0.48±0.08	0.56±0.06*	0.63±0.08*	0.51±0.06*	0.54±0.01*
NTG/MI	0.48±0.03	0.36±0.03	0.32±0.03	0.34±0.03	0.29±0.03	0.26±0.04
α_{1A}-TG/MI	0.61±0.03*	0.52±0.03*	0.50±0.03*	0.51±0.03*	0.48±0.02*	0.47±0.03*

Ext LVDd, external LV diameter at end-diastole; LVDs, LV diameter at end-systole. *$P<0.05$ vs. respective NTG group by two-way ANOVA for repeated measures.

NTG, both $P<0.01$, Fig. 3). FS and LVDd were better preserved in the α_{1A}-TGs, irrespective of infarct size.

3.5. Gene expression and collagen content in the non-infarcted LV myocardium

In response to chronic MI (15-weeks), the non-infarcted LV myocardium of the NTG mice displayed significant increase in the expression of ANP (~6-fold) and β-MHC (~4-fold), and a lesser increase in α-SkA expression as compared to the LV myocardium of the sham-operated NTG controls (Fig. 4). Expression of ANP and α-SkA was elevated in the sham-operated α_{1A}-TG hearts compared to their NTG counterparts, with no further increase in these genes in the α_{1A}-TG hearts post-MI. α-MHC and SERCA2a expression were not different in the hearts of the NTG and α_{1A}-TG mice (Fig. 4).

Collagen content in the LV was comparable between α_{1A}-TG and NTG sham-operated groups. In the NTG animals, MI was associated with a 40% rise in collagen level in the non-infarcted LV. This increase was, however, more marked in the α_{1A}-TG hearts (~3-fold, $P<0.05$ vs. NTG, Fig. 4). Compared with sham-operated controls, Procollagen-1 and 3 transcripts in NTG hearts were significantly increased in the

Table 2
Hemodynamic determination by catheterization from mice that survived to week-15 after surgery

Parameters	NTG/SH	α_{1A}-TG/SH	NTG/MI	α_{1A}-TG/MI
Group size	9	9	11	12
HR, beats/min	274±18	325±11	316±9	310±14
SAP, mmHg	105±6	90±5†	97±3	99±3
DAP, mmHg	64±4	57±4	64±2	67±3
LVEDP, mmHg	10±1	5.4±0.7	7±1	5±1
LVSP, mmHg	110±6	92±5	98±3	102±3
dP/dt_{max}, mmHg/s	7708±442	8148±585	5938±372†	7270±324*
dP/dt_{min}, mmHg/s	5296±235	4311±249	3980±341†	3676±235
dP/dt_{max}:dP/dt_{min}	1.42±0.07	1.90±0.11*	1.47±0.04	1.98±0.05*

HR, heart rate; SAP or DAP, systolic or diastolic arterial pressure; LVEDP: LV end-diastolic pressure; LVSP, LV systolic pressure. *$P<0.05$ vs. respective NTG group; †$P<0.05$ vs. respective SH group by one-way ANOVA.

Table 3
Body and organ weights determined at autopsy at the end of the 15-week study period

Parameters	NTG/SH	α_{1A}-TG/SH	NTG/MI	α_{1A}-TG/MI
Group size	9	9	20	19
Body weight (BW), g	28±1	26±1	29±1	29±1
Infarct size, %LV	–	–	32.1±2.2	29.4±1.7
LV/BW, mg/g	3.52±0.2	3.32±0.1	3.70±0.1*	3.66±0.1*
RV/BW, mg/g	0.85±0.05	0.86±0.05	0.92±0.06*	0.88±0.08
Atria/BW, mg/g	0.47±0.03	0.47±0.03	0.74±0.10*	0.86±0.09*
Heart/BW, mg/g	4.83±0.2	4.65±0.2	5.36±0.2*	5.39±0.3*
Lung/BW, mg/g	5.4±0.3	5.6±0.3	6.6±0.6*	7.6±0.8*
Chest fluid, %	0	0	40*	33
Atrial thrombus, %	0	0	30	33

LV, left ventricle; RV: right ventricle. *$P<0.05$ vs. respective sham-operated group by one-way ANOVA.

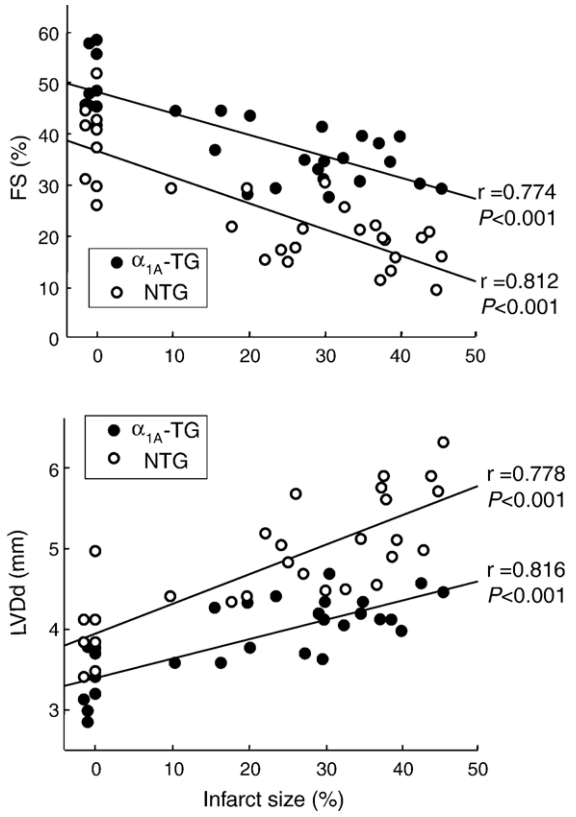

Fig. 3. Diagrams showing infarct size negatively correlated with echocardiography-derived fractional shortening (FS) and positively correlated with LV end-diastolic dimension (LVDd) as determined at 15-weeks after MI in NTG (n=31) and α_{1A}-TG mice (n=30). Irrespective of infarct size, the difference in FS and LVDd between the two groups is evident.

Table 4
Organ weights, cardiac dimensions and systemic hemodynamics in 12-month-old NTG and α_{1A}-TG mice

Parameters	NTG	α_{1A}-TG
Body weight, g	35±2	33±2
Tibia length, mm	18.2±0.1	18.2±0.1
LV, mg	99±5	95±4
Heart, mg	135±6	129±5
LVDd, mm	4.1±0.04	3.7±0.2
LVDs, mm	2.6±0.1	2.0±0.1*
FS, %	37±2	45±2*
HR, beats/min	408±18	412±10
SAP, mmHg	95±3	102±6
DAP, mmHg	61±3	66±4
LVEDP, mmHg	7.1±0.7	5.8±0.9
LVSP, mmHg	96±3	107±5*
dP/dt_{max}, mmHg/s	7349±447	8915±379*
dP/dt_{min}, mmHg/s	5525±265	5226±350
dP/dt_{max}:dP/dt_{min}	1.33±0.03	1.79±0.11*

Abbreviations see Tables 1–3. n=12 to 20 mice/group with gender matching. *P<0.05 vs. NTG by ANOVA.

viable LV myocardium after MI, whereas only procollagen-3 mRNA expression was significantly increased in infarcted α_{1A}-TG hearts (Fig. 4). Expression of MMP-2, 9 and 13 transcripts were not significantly different between NTG and α_{1A}-TG hearts with and without MI (data not shown). Expression of CTGF mRNA was significantly higher (~2.7-fold and ~1.6-fold, respectively) in α_{1A}-TG than in the NTG hearts of sham-operated and infarcted mice while fibronectin mRNA level was higher in α_{1A}-TG hearts with MI (Fig. 4).

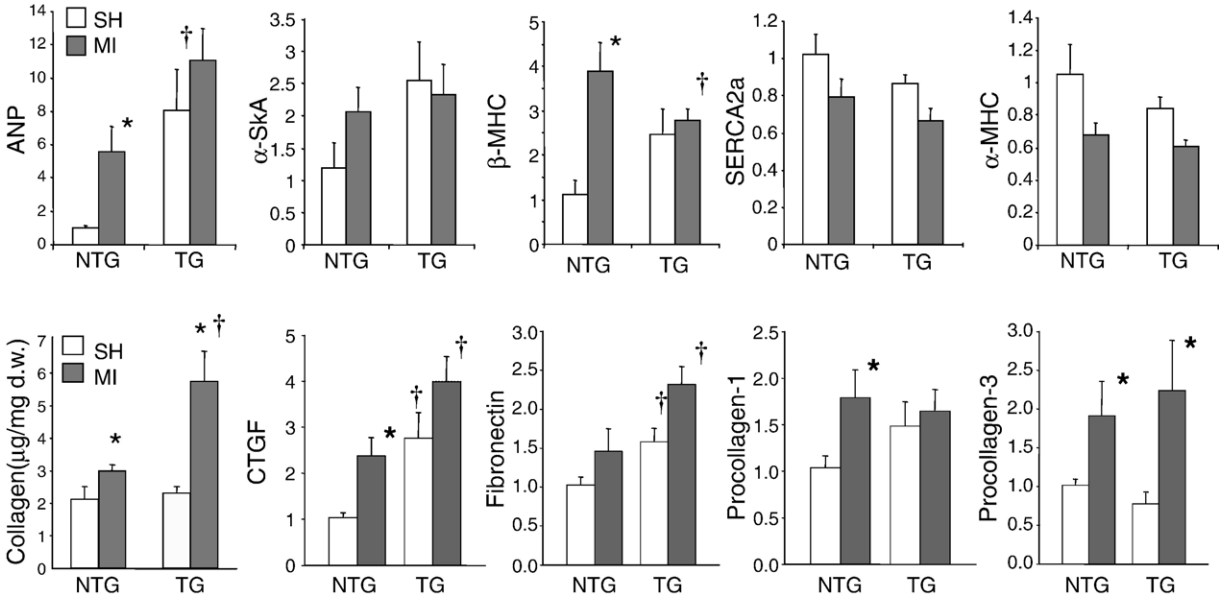

Fig. 4. Expression, in the non-infarcted LV myocardium of sham-operated (SH) and infarcted (MI) NTG and α_{1A}-TG mice, of atrial natriuretic peptide (ANP), α-skeletal actin (α-SkA), β-myosin heavy chain (β-MHC), α-myosin heavy chain (α-MHC) sarcoendoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a), connective tissue growth factor (CTGF), fibronectin, and procollagens-1 and 3, as determined by quantitative real-time PCR. Values shown are relative expression levels determined by normalizing the expression of the test genes to 18S rRNA as a reference gene. Also shown are collagen in LVs from SH and viable LV from infarcted mice (MI). d.w. = dry weight; *P<0.05 vs. respective SH group; and †P<0.05 vs. respective NTG group by two-way ANOVA.

3.6. Cardiac function in 12-month-old mice

A cohort of α_{1A}-TG and NTG mice ($n=25$/group) were monitored for up to 12-months of age. There was no death over this time in either genotype, and echocardiography and micromanometry studies at the end of this time revealed persistence of the hypercontractile phenotype in the α_{1A}-TGs mice (Table 4). At this age, LV and heart weights were comparable, but collagen content was higher in the LVs of α_{1A}-TG versus the NTG groups (2.69±0.04 vs. 2.03±0.11 μg/mg dry weight, $P<0.05$).

4. Discussion

We previously demonstrated that α_{1A}-TG mice have better preservation of cardiac function, despite similar degrees of hypertrophy, than their NTG counterpart, when subjected to thoracic aorta constriction-induced pressure-overload [6]. Importantly, preservation of function in the former was associated with reduced mortality from HF. Here we studied the response of these α_{1A}-TG mice to the hemodynamic stress of MI. Our results show that despite similar sized infarcts as compared to their NTG littermates, enhanced inotropy in the α_{1A}-TG mice was preserved over a 15-week period of observation, and was associated with fewer acute deaths that were due most likely to HF. Importantly, as determined by serial echocardiography, progressive ventricular dilatation observed in the NTG mice during the chronic phase of MI, was attenuated in α_{1A}-TG animals. We have also shown that the hypercontractile function persists in 12-month-old α_{1A}-TG animals and this is not associated with premature mortality over the observation period. Collectively, these findings indicate that cardiac-restricted overexpression of the α_{1A}-AR at the level studied, provides inotropic support to the infarcted ventricle and limits post-infarct remodeling. In keeping with our findings and those of our previous study [6], O'Connell et al. [19] recently demonstrated that dual inactivation of both α_{1A}- and α_{1B}-ARs increased interstitial fibrosis and apoptosis, and attenuated LV function under conditions of pressure-overload.

An acute functional decline and LV dilatation that were of a similar magnitude in both NTG and α_{1A}-TG mice were observed at week-1 after MI. These changes are the expected initial responses to the loss of a substantial amount of LV mass, but are also partly due to the insult of open-chest surgery *per se*, since sham-operated controls displayed similar but less marked functional decline. The functional benefit of enhanced contractility due to transgenic α_{1A}-AR activation become more evident in the chronic phase post-MI as the α_{1A}-TG animals did not display the continued progression of global ventricular dilatation observed in the NTG animals. We previously found that FVB/N mice have a high incidence of acute HF death following MI [17]. This was confirmed in the present study of the α_{1A}-TG on a FVB/N background. Interestingly, transgenic activation of the α_{1A}-AR in the heart of the A1A2 line studied here reduced acute HF deaths after MI. This finding suggests that the increased inotropy due to α_{1A}-AR overexpression, evidenced by higher FS, is compensatory in the acute post-MI period. Indeed, a greater lung wet weight in α_{1A}-TG than in the NTG mice that died acutely suggests a better tolerance of HF-associated pulmonary congestion in the former. However, no survival advantage was evident during the chronic phase of MI nor was there a difference in the incidence of chronic HF, as evidenced by the presence of chest fluid accumulation, lung congestion and atrial thrombus in the two groups of mice. This is likely due to the fact that we specially avoided creating large sized infarcts in this study because of the marked sensitivity of this mouse strain to acute HF after MI. Another factor that may have contributed to the lack of a difference in the incidence of sequel in the chronic phase post-MI, despite the progressive nature of LV remodeling in NTGs, is that the study period may not have been long enough to allow for progression to decompensated HF. In addition, some "side-effects" of α_{1A}-AR overexpression and inotropic phenotype, such as long-term higher energy expenditure and increased interstitial collagen in α_{1A}-TGs, offset in part the beneficial effects.

Unlike the α_{1A}-TG model studied here following MI, and previously in response to pressure-overload [6], the enhanced ventricular contractility observed in several other mouse models is lost when they are subjected to a disease-causing hemodynamic challenge [8,16,20]. For example, whereas a TG model of β_2-AR overexpression [21] shows preserved myocardial contractile augmentation, when subjected to chronic MI [7], pressure-overload-induced HF development was facilitated and exacerbated in this model and HF-related deaths were increased [8,16]. Likewise, functional benefit was not observed after pressure-overload in mice with a dramatic enhancement in myocardial contractility due to phospholamban deficiency [20]. Thus, the mechanisms mediating enhanced inotropy are likely to be distinct, with only that associated with enhanced α_{1A}-AR signaling being persistent long-term under different diseased conditions: a contention supported by the marked increase in the ratio of dP/dt_{max}:dP/dt_{min} in the α_{1A}-TG mice but no in other genetically engineered models.

Enhanced sympatho-adrenergic signaling observed with cardiac disease, is associated with adverse consequences [22]. For example, mice with transgenic overexpression of the β_1-AR [23] the β_2-AR [8,24], the stimulatory GTP-binding protein Gsα [25] or the α_{1B}-AR [26,27] all develop cardiac pathology, dysfunction and premature death, and the incidence of these events increases with age. The α_{1A}-TG model is no exception since we recently showed that with ageing mice of other α_{1A}-TG lines that overexpress the receptor at much higher levels (120- or 170-fold) than the A1A2 line (66-fold) studied here, develop progressive cardiac fibrosis, partial loss of the inotropic phenotype and sudden rather than HF deaths [4]. It is of interest, therefore, that in this study on A1A2 line, cardiac function at baseline

continued to be higher than that of their NTG littermates even at 12 months of age, and despite the fact that at this age collagen content in the LV myocardium was increased by 32%. This is in keeping with the view, as documented in β_2-AR TG lines by Liggett et al [24] that extremely high levels of AR overexpression are detrimental. However, unlike a range of other murine cardiomyopathy models, the α_{1A}-TG model does not develop hypertrophy even at an advanced age [4].

Post-infarct ventricular remodeling involves regional expansion, chamber dilatation, hypertrophy of non-infarcted myocardium and interstitial fibrosis [9,28]. Acute LV dilatation following MI is largely attributable to infarct wall thinning and regional expansion, whereas dilatation that occurs during the chronic post-MI phase is due to the development of eccentric hypertrophy [10,28]. In this study, NTG and α_{1A}-TG mice with MI had comparable increases in LV weight, despite loss of significant amounts of LV myocardium, indicating hypertrophy of the non-infarcted myocardium. Again, as concluded from studies of pressure-overloaded α_{1A}-TG mice [6], the ability of this model to develop hypertrophy in response to the hemodynamic stress of MI excludes the possibility that α_{1A}-AR overexpression modulates hypertrophic signaling — despite the observation that expression of hypertrophy-related genes, such as ANP and α-SkA, is already increased at baseline in these α_{1A}-TGs. The α_{1A}-TG mice did, however, show a greater increase (+150%) in myocardial collagen post-MI than in the NTGs (+30%).

In the NTG animals, expression of CTGF mRNA was increased chronically after MI in the non-infarcted myocardium as well as being increased in the LVs of sham-operated and infarcted α_{1A}-TG mice. Recent studies have shown that CTGF, in cooperation with transforming growth factor-β, contributes to fibrotic signaling in hearts subjected to MI [29,30]. This is in keeping with our recent studies that revealed the development of a fibrotic phenotype and upregulation of CTGF with ageing in the A1A1 line overexpressing the α_{1A}-AR by 170-fold [4]. There is good evidence that ANP signaling inhibits myocardial fibrosis [31]. However, collagen content was increased in the α_{1A}-TG hearts with or without MI, despite a markedly upregulated ANP expression. Although potentially detrimental, increased interstitial collagen might in part be a compensatory matricellular change in response to a prolonged hypercontractility, allowing maintenance of myocyte structural integrity and contractile-force transduction. Nevertheless, it is of interest that the more marked interstitial fibrosis observed in the infarcted α_{1A}-TG animals was not sufficient to compromise contractile function.

Whereas partial prevention and reversal of post-infarct ventricular remodeling have been reported with the use of angiotensin-converting enzymes inhibitors [32,33] or a LV assist device [34], other effective approaches remain to be developed. The exact mechanism for the suppressed remodeling in the α_{1A}-TG model remains undefined. It is likely, however, that the improved global LV function is expected to limit the increase in end-systolic volume and the resultant increase in wall stress, thereby, in part, preventing the development of chamber dilatation. Interestingly, inhibition of ventricular remodeling under conditions of dilated cardiomyopathy (due to deletion of muscle-LIM protein), or infarction, has been reported following genetic interventions that enhanced myocardial contractile function, including inhibition of phospholamban [13,14] or expression of the β-AR-kinase inhibitory peptide [35]. Based on these studies and our findings using the α_{1A}-TG mice, it is plausible to suggest that enhancing contractility of the viable myocardium is a promising approach to limit the extent of post-infarct ventricular remodeling — a response that carries deleterious consequences such as HF and arrhythmias.

Treatment of HF patients with conventional inotropic agents, like β-adrenergic agonists, phosphodiesterase (PDE) inhibitors or digitalis, has not been effective [36]. Likewise, calcium sensitizers possesses some unwanted actions including PDE-inhibition. Consistent with these clinical findings are recent research showing that transgenic enhancement of β-adrenergic signalling is detrimental [8,16,23–25]. The interpretation of these data could be that either the inotropic strategy is inappropriate or, alternatively, that approaches, which ultimately lead to increased cAMP, should be avoided. Our collective findings in the α_{1A}-TG model with MI or pressure-overload [6] appear to support the second possibility. Thus, cardiac-restricted activation of α_{1A}-AR signalling at a moderate level, via means such as gene delivery, form a potential therapeutic approach to limit global cardiac remodeling and to improve survival from HF, albeit that long-term CTGF-mediated fibrosis may be an unwanted effect. Indeed, the findings from the α_{1A}-TG model are consistent with the outcomes of the ALLHAT trial showing that treatment of hypertensive patients with the α_1-antagonist, doxazosin, increased incidence of HF by 80%, compared to treatment with the diuretic, chlorthalidone [37]. However, we cannot exclude the possibility that another as yet undefined signalling pathway, which is altered by α_{1A}-AR activation, contributes to the observed benefits.

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