Alpha-1-Adrenergic Receptor Subtypes in Non-Failing and Failing Human Myocardium

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ABSTRACT

Background: Alpha-1-adrenergic receptors (α1-ARs) play adaptive roles in the heart and protect against the development of heart failure (HF). The three α1-AR subtypes, α1A, α1B, and α1D, have distinct physiological roles in mouse heart, but very little is known about α1-subtypes in human heart. Here we test the hypothesis that the α1A and α1B subtypes are present in human myocardium, similar to the mouse, and are not down-regulated in heart failure.

Methods and Results: Hearts from transplant recipients and unused donors were failing (n = 12; mean EF 24%) or non-failing (n = 9; mean EF 59%), and similar in age (~44 years) and sex (~70% male). We measured the α1-AR subtypes in multiple regions of both ventricles by quantitative real-time reverse transcription PCR and radioligand binding. All three α1-AR subtype mRNAs were present, and α1A mRNA was most abundant (~65% of total α1-AR mRNA). However, only α1A and α1B binding were present, and the α1B was most abundant (60% of total). In failing hearts, α1A and α1B binding were not down-regulated, in contrast with β1-ARs.

Conclusions: Our data show for the first time that the α1A and α1B subtypes are both present in human myocardium, but α1D binding is not, and that the α1-subtypes are not down-regulated in HF. Since α1-subtypes in the human heart are similar to mouse, where adaptive and protective effects of α1-subtypes are most convincing, it might become feasible to treat HF with a drug targeting the α1A and/or α1B.

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INTRODUCTION

The myocardium contains adrenergic receptors (ARs) of two main classes, β and α1. Myocardial β-ARs are studied extensively, and blocking β-ARs in heart failure (HF), when the AR agonist norepinephrine (NE) is elevated, is now a cornerstone of therapy. Much less is known about myocardial α1-ARs. However, animal and human data suggest that activation of myocardial α1-ARs in HF is adaptive and protective, in contrast with the toxic effects of chronic β-AR stimulation.

α1-ARs exist as three molecular subtypes, α1A, α1B, and α1D. Knockout (KO) mouse models have provided the most convincing evidence for the beneficial effects of α1-AR stimulation, and have begun to reveal the distinct physiological roles of the cardiac α1-subtypes.1 KO of the two main myocardial α1-AR subtypes, the α1A and α1B, impairs normal post-natal cardiac growth, and causes severe dilated cardiomyopathy and death after pressure overload.2-4 In KO models, the role of the α1B subtype appears to be physiological cardiac hypertrophy,2,4,5 whereas the α1A is cardio-protective.3,6 Although all three subtypes can constrict peripheral arteries,4,7,8 the cardiac α1D stimulates coronary vasoconstriction.9,10

α1-AR gain-of-function in cardiac transgenic models generally supports the KO results, although phenotypes vary greatly with receptor level, promoter, and absence or presence of an activating mutation. Thus, cardiac α1B overexpression can cause hypertrophy, but this can be associated with β-AR down-regulation and late cardiomyopathy.11-14 Cardiac α1A overexpression can stimulate contractility and cause cardio-protection,15-18 but there can be late fibrosis and sudden death.19 Recently, we
found that modest augmentation of cardiac α1A signaling with a subpressor dose of an α1A-selective agonist can prevent doxorubicin-induced cardiomyopathy in mice.\textsuperscript{20}

In the human heart, α1-subtype roles have not been studied. However, in human clinical trials, non-selective blockade of all three α1-subtypes caused a two-fold increase in HF in the ALLHAT trial,\textsuperscript{21} and a trend toward increased mortality in the V-HeFT trial.\textsuperscript{22} Non-selective activation of all α1-subtypes in vitro has a robust positive inotropic effect in failing human myocardium.\textsuperscript{23, 24} Non-selective α1-activation in vitro also protects against ischemia.\textsuperscript{25-27} These beneficial effects of α1-AR activation suggest a novel interpretation of the harmful results of excessive NE reduction in clinical trials (MOXSE, MOXCON, and BEST),\textsuperscript{28-30} specifically, that some degree of α1-activation is essential in HF. Further consistent with a beneficial or compensatory role for human myocardial α1-ARs, chronic therapy with the cardioprotective β-blocker carvedilol potentiates α1-AR effects,\textsuperscript{31} and total α1-ARs are not down-regulated in HF, in contrast with β-ARs that are down-regulated.\textsuperscript{32-34}

The distinct and important roles of the α1A, α1B, and α1D in mouse heart, and, in human heart, the contrasting harmful effects of non-selective α1-blockade and beneficial effects of non-selective α1-stimulation, emphasize the need to define expression of the α1-subtypes in the human heart. Recently, we found that the α1D is the predominant subtype in human coronary arteries.\textsuperscript{35} However, little is known about the α1-subtypes in human myocardium. In fact, prior limited mRNA studies have concluded that the heart expresses only the α1A,\textsuperscript{36-39} and that mouse models of
α1-AR biology might not be relevant to human heart disease. The α1-subtype proteins in human myocardium have never been measured.

Here we test the hypothesis that the α1A and α1B subtypes are both present in human myocardium, similar to the mouse, and are not down-regulated in HF. This is the first report of α1-AR subtype proteins in human heart, and the first characterization of α1-subtypes in non-failing and failing heart.
METHODS

Patients

With the approval of the University of California, San Francisco (UCSF) Committee for Human Research, we obtained tissue from hearts removed at the time of transplant at UCSF, or from organ donors whose hearts were not transplanted for technical reasons. Full informed consent was obtained from all UCSF transplant recipients prior to surgery. The California Transplant Donor Network (CTDN) provided the unused donor hearts and obtained informed consent from the donor's next of kin.

Tissue collection

Cold cardioplegia was perfused antegrade prior to cardiectomy, and the explanted heart was placed immediately in ice-cold physiologic solution. Full-thickness samples from multiple regions of the LV and RV were cleaned rapidly of all epicardial fat, flash frozen in liquid nitrogen, and stored at -80°C.

RNA preparation

Tissue was homogenized in TRIzol reagent (Invitrogen, Gibco BRL), using a rotor-stator homogenizer (Polytron) at speed 7 out of 10. RNA was extracted in chloroform and isopropyl alcohol, purified on Qiagen Mini-Prep columns, treated with DNase (Turbo DNasefree, Ambion), and quantified using spectrophotometry (BioRad SmartSpec 3000). Selected RNA samples were analyzed to confirm the absence of significant degradation (Agilent 2100 BioAnalyzer).
Quantitative real-time reverse transcription PCR (qRT-PCR)

Primer3 (v0.4.0) and BLAST were used to design multiple potential primer pairs for each target and reference gene. α1-AR subtype primers spanned the 25 kb intron at the end of the 6th transmembrane domain, and final primer pairs (Online Figure 1) were chosen for comparable reaction efficiencies as measured by serial dilution. Specificity of amplification was confirmed by (1) sequencing, (2) PCR with human α1-AR cDNAs, and (3) a dissociation step in all qRT-PCR reactions. Amplification of genomic DNA was excluded by (1) use of intron-spanning primers, (2) DNase treatment of RNA, and (3) PCR run on agarose gels using no-RT templates as negative controls.

For qRT-PCR, one µg of RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Invitrogen) with both random hexamers (Invitrogen) and oligo-dT (Roche). qRT-PCR reactions contained 5% of the cDNA product, primers at 125 nM per reaction, and SYBR Green Master (Roche) with ROX reference dye. All reactions were performed in triplicate in an ABI PRISM 7900HT Sequence Detection System. Data were analyzed with SDS software version 2.3 (Applied Biosystems).

Relative quantitation of PCR products used the ΔΔCt method.ΔΔCt Values for each mRNA are arbitrary units (AU) relative to two reference genes, β-actin and TATA-binding protein (TBP), for improved accuracy, as

\[
AU = 2^{-\Delta\Delta CT} \times 1000, \quad \Delta\Delta CT = [(\text{mean target gene } C_T) - (\text{mean reference genes } C_R)]
\]

Radioligand binding

Approximately 120 mg wet weight of tissue was homogenized (5 mM Tris-HCl, 5 mM EDTA, 250 M Sucrose pH 7.4 plus 0.1 mM PMSF), and centrifuged at 100,000 x g
for 1 h. The pellet was resuspended in homogenization buffer and centrifuged as before. The resulting final membrane pellet was resuspended in assay buffer (α1 binding: 50 mM Tris pH 7.4, 1 mM EDTA; β-AR binding: 154 mM NaCl, 5 mM MgCl₂, 20 mM Tris pH 7.4), and used for saturation and competition radioligand binding.

α1-AR saturation binding was at 30°C for 60 min with 200 μg membrane protein per tube (~2.5 mg tissue), 6 concentrations (0.04 - 1.2 nM) in triplicate of ³H-prazosin (Perkin Elmer), and phentolamine (10 μM) to define non-specific binding. β-AR binding was at 25°C for 90 min with 50 μg membrane protein per tube, 6 concentrations (0.04 - 1.0 nM) in triplicate of ¹²⁵I-cyanopindolol (CYP, NEN Life Sciences), and L-propranolol (1 μM) to define non-specific binding.

The subtype proteins were quantified by competition binding. For α1-ARs, ³H-prazosin binding (0.5 nM) was competed with 22 concentrations (0.05 nM - 500 μM) in duplicate of BMY-7378, an α1D-selective antagonist, ⁸⁻⁹ or 5-methylurapidil (5-MU) an α1A-selective antagonist. For β-ARs, ¹²⁵I-CYP binding (50 pM) was competed with 22 concentrations (50 pM - 500 μM) in duplicate of the β1-selective antagonist, CGP 20712A, or the β2-selective antagonist, ICI-118,551. Binding data were analyzed using GraphPad Prism 4.0b (GraphPad Software Inc., San Diego, CA). Subtype percents were calculated from fitting competition curves, and subtype levels in fmol/mg were calculated from total binding in saturation analysis, multiplied by percent from competition in the same preparation.
Data analysis

Results are presented as mean ± SEM. Significant differences ($p < 0.05$) were tested using one-way ANOVA and Tukey’s multiple comparison for more than two groups, or Student’s unpaired t-test for two groups, and a normal distribution was assumed for all continuous variables. Linear regression tested for association between mRNA abundance and clinical variables. The F test compared goodness-of-fit to one- or two-site models for competition binding analyses (GraphPad Prism v4.0).

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.
RESULTS

Patients (Table 1)

We tested α1-AR subtype expression in human myocardium from 21 explanted hearts of 14 transplant recipients and 7 unused donors. As shown in Table 1, there were 9 non-failing (NF) and 12 failing (F) patients, one-third of which had ischemic cardiomyopathy. NF and F were similar in age and sex, but the mean ejection fraction (EF) was 59 ± 3% in the NF group, and 24 ± 2% in the F group (p<0.0001).

Characterization of NF and F LV myocardium (Figure 1)

To characterize NF and F LV myocardium, we assayed select myocyte and non-myocyte mRNAs by qRT-PCR, and correlated these with EF. As EF decreased, LV β-MyHC levels did not change (p = 0.71), but α-MyHC decreased (p = 0.01), and Type I collagen increased (p = 0.02) (Figure 1A-C). α-MyHC mRNA in NF was 19% of total MyHC, but only 1% of total in F. Importantly, the levels of the reference genes, β-actin and TBP, were comparable in the NF and F groups (Figure 1D).

Competition binding for β-AR subtypes using ICI-118,551, an antagonist selective for the β2-subtype, showed that β1-subtype levels decreased markedly in F, whereas the β2-subtype was not changed (Figure 1E). Competition binding with CGP 20712A, a β1-selective antagonist, confirmed these values (data not shown).

In summary, we found in F LV the expected repression of α-MyHC, induction of collagen indicative of fibrosis, and down-regulation of the β1-AR subtype, showing a molecular phenotype similar to prior studies in human HF. 33, 46-48
**α1-AR subtype mRNAs in the NF myocardium (Figure 2, Table 2)**

To begin to test if all three α1-subtypes were present in human myocardium, we did qRT-PCR with validated primers spanning the long intron in each α1-subtype gene. We made RNA from transmural samples of myocardium from three regions, LV free wall (LVFW), LV septum (LVS, the LV side of the interventricular septum), and RVFW, all taken at the level of the papillary muscles. Figure 2 (left) shows that the α1A was the predominant α1-subtype mRNA in all NF myocardial regions. The α1B and α1D were present at much lower levels (p < 0.05), and also did not differ among the regions (Figure 2). In NF myocardial LVFW and LVS, the α1A was ~63% of total α1-AR mRNA, the α1B was 22%, and the α1D was 15% (Table 2).

**α1-AR subtype proteins in the NF myocardium (Figures 3-4, Table 2)**

We quantified α1-AR subtype protein levels using radioligand binding. Recently, we found that commercial α1-AR antibodies are not specific for α1-ARs, and thus we could not use immunoblot or immunohistochemistry to detect or quantify the α1-subtype proteins.

Saturation radioligand binding in myocardial membranes with ³H-prazosin, a non-selective α1-AR antagonist, produced typical curves (Figure 3A), with Bmax ~ 4 fmol/mg protein. Specific binding at the ³H-prazosin Kd (0.2 nM) averaged 40% of total ³H-prazosin bound. Specific total α1-binding reflecting all α1-subtypes in NF myocardium was about 10% of β-AR binding (Table 2), similar to prior studies. Total α1-binding was similar in NF LVFW, LVS, and RVFW (Figure 3B left).
To detect and quantify the $\alpha_1$-subtype proteins, we used competition for $\text{H}^3$-prazosin binding with subtype-selective antagonists. Competition with 5-MU, an $\alpha_1$A-selective antagonist,$^7$ produced classic two-site curves (Figure 4A left). In NF LVFW myocardium, there was a high affinity component (Ki $11 \pm 4$ nM, n = 6), representing the $\alpha_1$A subtype, and a low affinity component (Ki $99 \pm 53$ $\mu$M, n = 6), which could have been the $\alpha_1$B and/or $\alpha_1$D. To distinguish these, we did competition binding in the same preparations with BMY-7378, an $\alpha_1$D-selective antagonist.$^8, 45$ Competition binding with BMY-7378 yielded one-site binding curves (Figure 4A right), with only a low affinity component (Ki $11 \pm 6$ $\mu$M, n = 6), indicating no detectable $\alpha_1$D binding. Taken together, these results suggested that the $\alpha_1$A and $\alpha_1$B subtype proteins were both present in human NF LV myocardium, but the $\alpha_1$D was not. NF LV had $40 \pm 2\%$ $\alpha_1$A and $60 \pm 2\%$ $\alpha_1$B ($p < 0.001$, n = 6) (Figure 4B left, Table 2). Total $\alpha_1$-binding in NF RV myocardium was similar to LV (Figure 3B), but the NF RV contained relatively less $\alpha_1$A ($25 \pm 5\%$, n = 3, $p = 0.02$) (Figure 4B left).

In summary, human NF myocardium expressed all three $\alpha_1$-subtype mRNAs, with a striking predominance of the $\alpha_1$A. However, binding assays detected only the $\alpha_1$A and the $\alpha_1$B, and the $\alpha_1$B was predominant.

$\alpha_1$-AR subtype mRNAs and binding in the F myocardium (Figures 2-4, Table 2)

The relative pattern of $\alpha_1$-subtypes mRNA expression within the F myocardium was identical to NF, with a predominance of $\alpha_1$A mRNA, and no differences between $\alpha_1$B and $\alpha_1$D in any region (Figure 2 right). Total $\alpha_1$-subtype mRNA levels were also
similar in F and NF myocardium, although $\alpha_1$A mRNA was increased in F LV ($p < 0.05$, Table 2), and tended to increase in F RV (Figure 2).

Total $\alpha_1$-binding was not reduced in any region of F myocardium (Figure 3B right). Similarly, binding levels of the $\alpha_1$A and $\alpha_1$B subtypes were not reduced in F myocardium (Figure 4B), and the $\alpha_1$D remained undetectable (data not shown). The relative levels of $\alpha_1$A and $\alpha_1$B binding also were unchanged in F LV (Table 2), but in RV there was a relative increase in $\alpha_1$A binding in F versus NF (44% versus 27%, $p = 0.04$) (Figure 4B).

In summary, the $\alpha_1$A and $\alpha_1$B were not repressed or down-regulated in the F myocardium. The $\alpha_1$A tended to increase.

Comparison of $\beta$-AR subtypes in F myocardium (Table 2, Figure 1)

We measured $\beta$-AR subtype mRNAs and binding, to test if the two AR families had distinct regulation in HF. Myocardium from F LV had a significant decrease versus NF in $\beta_2$-subtype mRNA and $\beta_1$-subtype binding (Table 2 and Figure 1E). Stable binding levels in HF of the $\alpha_1$A- and $\alpha_1$B-subtypes and down-regulation of the $\beta_1$-subtype caused a marked increase in the ratio of binding of $\alpha_1$-ARs to $\beta$-ARs, from about $\sim$10% in NF LV to $\sim$20-40% in F LV (Table 2; the 41% $\alpha_1$/$\beta$ ratio in the F LV in Table 2 is from four samples with $\alpha_1$- and $\beta$-binding on the same membranes).

Demographic and clinical variables and myocardial $\alpha_1$-AR mRNA levels (Figure 5)
The qRT-PCR results were analyzed to determine whether demographic or clinical factors affected the expression of α1-subtype mRNAs. We found that age, EF, sex, β-blocker use, and CAD had no effect on myocardial total or α1-subtype mRNAs (Figure 5A-D and data not shown). On the other hand, β-agonist use was associated with a decrease in both α1B (p = 0.04) and α1D (p < 0.01), but did not affect α1A levels (Figure 5E). We compared β-AR mRNAs in a similar analysis, and found that β1- and β2-subtype mRNA levels in myocardium did not change with age, EF, sex, β-blocker use, β-agonist use, or CAD (Online Figure 2 and data not shown).
DISCUSSION

We present here the first data on α1-AR subtype proteins in human heart, and the first comparison of α1-AR subtypes in NF and F human hearts. The main findings are that the α1A is the predominant α1-subtype mRNA, the α1A and α1B are both present by binding, with the α1B predominant, and the α1D is undetectable by binding. In HF, α1-subtype mRNAs are not repressed, and α1-binding is not down-regulated, in contrast with β-AR subtypes.

Prior studies of human myocardial α1-AR subtypes used semi-quantitative mRNA assays with a very limited number of undefined patients, and never measured α1-subtype proteins.\textsuperscript{36-39} These studies identified the α1A as the most abundant or only α1-subtype mRNA in myocardium, and a separate study concluded that the α1A was the only α1-subtype in human coronary arteries.\textsuperscript{40} These results prompted the conclusion that the human heart is exclusively α1A, and thus that mouse models are irrelevant to human cardiac α1-AR biology.\textsuperscript{40,41} On the contrary, the present and our recent studies show that α1-subtype expression is the same in the human and mouse heart, with the α1A and α1B subtypes in myocardium, and the α1D in coronary arteries. This is important, because it implies that findings in mouse genetic models, where α1-subtype functions can be studied with precision, are relevant to human cardiac α1-AR biology, in particular, the adaptive and protective effects of the α1A- and α1B-subtypes.

Technical aspects of this study warrant emphasis. Although our patient population was modest in size, it was significantly larger and more thoroughly characterized than in prior studies. We quantified 1-subtype mRNAs in DNase-treated...
RNA, using qRT-PCR with carefully evaluated primer pairs that cross the large intron in all 1-AR genes, to eliminate contamination from genomic DNA. We quantified 1-subtype proteins by radioligand binding. Prior studies have used commercial antibodies to measure 1-subtypes in human non-cardiac tissues, but we find that ten different antibodies are not specific for 1-ARs, indicating that binding is currently the only valid method for detecting and quantifying 1-AR proteins. Our membrane preparation for binding was not "purified", i.e. we did not discard any low speed pellets. This meant that we did not discard the large number of receptors that are found in low speed pellets, and also that our denominator of mg protein was higher than in purified membranes.

A potential technical concern was the discordance between 1-subtype mRNA levels and binding levels. The 1A mRNA in human myocardium was by far most abundant, but 1A binding was less than 1B. The myocardial 1D mRNA was as abundant as 1B, but 1D binding was undetectable. Importantly, the same discordance between the levels of 1A and 1B mRNAs and binding is seen also in the mouse and rat heart, and mouse myocardium has 1D mRNA without 1D binding. Furthermore, we are able to detect 1D binding in human coronary arteries, where the 1D is 75% of total binding, ruling out a technical problem. In summary, these results together indicate that the 1-AR subtypes in myocardium have substantial post-transcriptional regulation in man and rodent.

A major finding was that 1-subtype mRNAs and 1-subtype binding were unchanged or increased in HF, in contrast with β-AR subtypes, where the β2 mRNA was repressed, and β1-binding, as shown before, was down-regulated. To simplify
characterization of the AR phenotype in F versus NF myocardium, we normalized all subtype binding, given in Table 2, to binding of the α1A in NF LV. Normalization provides the following binding ratios in human LV myocardium of α1A:α1B:β1:β2: in NF LV, 1:2:17:7, and in F LV, 1:2:6:7. Thus, the failing human myocardium has potentially much greater relative signaling through the α1A, α1B, and β2 subtypes. The F RV was especially notable, with a significant increase in relative α1A binding (Figure 4).

Clinical relevance. Loss and gain of function studies in the mouse heart show adaptive and protective effects of stimulation of the α1A and/or α1B subtypes (Introduction), raising the possibility of using α1-subtype-selective agonists as therapy for HF. We demonstrate here that the α1A and α1B are both present in the human myocardium and are not down-regulated in HF, indicating that this idea might be feasible. Furthermore, the fact that the α1-subtype in human coronary arteries is the α1D suggests that an agonist for the α1A or α1B would not cause coronary constriction. On the other hand, an α1D-selective antagonist might be safe and efficacious in prostate disease, without the potential harmful side effects of non-selective blockade of myocardial α1A and α1B subtypes.

Summary. In this study, we characterize the α1-AR subtypes in the non-failing and failing human heart. As in the mouse, the α1A and α1B are the predominant binding subtypes in human myocardium, and the α1D is absent. In contrast to β1-ARs, the α1A and α1B are not down-regulated in HF. Thus, α1-subtypes in the human heart appear to be similar to mouse heart, where adaptive and protective effects of α1-subtypes are
most convincing. Further studies will help determine whether it might be feasible to
treat HF with a drug targeting the α1A and/or α1B.
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DISCLOSURES

None
REFERENCES


12. Akhter SA, Milano CA, Shotwell KF, Cho MC, Rockman HA, Lefkowitz RJ, Koch WJ. Transgenic mice with cardiac overexpression of alpha1B-adrenergic


FIGURE LEGENDS

Figure 1. Characterization of NF and F LV Myocardium.
RNA from LV myocardium was used in qRT-PCR for (A) β-MyHC, (B) α-MyHC, and (C) Type I Collagen, and mRNA levels were related to EF by linear regression. (D) Levels of β-Actin and TBP, the reference mRNAs used for normalization, are shown as cycles to threshold in real-time PCR (p by t-tests). (E) The β1- and β2-subtype binding percents were measured by competition for \(^{125}\text{I}-\text{CYP}\) binding with ICI-118,551, a β2-selective antagonist, and the fmol/mg protein for each subtype was calculated as in Methods (p by t-test).

Figure 2. \(\alpha1\)-AR Subtype mRNA Levels in NF and F Myocardium.
Levels of \(\alpha1\)-subtype mRNAs by qRT-PCR in 3 regions of NF and F myocardium. Values are mean ± SE Arbitrary Units (AU) normalized to β-actin and TBP, n = 8 NF, 10 F (p by ANOVA and Tukey’s multiple comparison). LVFW, left ventricle free wall; LVS, LV septum; RVFW, right ventricle free wall.

Figure 3. Saturation Binding for Total \(\alpha1\)-ARs in Regions of NF and F LV and RV.
(A) Representative curves from a saturation binding assay with \(^3\text{H}-\text{prazosin}\) in myocardial membranes. (B) Total \(\alpha1\)-AR protein levels by saturation binding in LVFW, LVS, and RVFW of NF and F myocardium, n = 6 NF, n = 6 F (lines are means, p = NS by ANOVA).
Figure 4. Competition Binding for α1-AR Subtype Levels in NF and F LV and RV. (A) Antagonists for α1-subtypes compete for ³H-prazosin binding to myocardial membranes. On the left, competition with 5-MU, an α1A subtype antagonist, produces a distinctly 2-site curve; one site has high affinity for 5-MU (i.e., the α1A), and one has low affinity (the α1B and/or α1D). On the right, competition with BMY-7378, an α1D subtype antagonist, reveals a single low affinity site (i.e., not the α1D). These results together indicate that α1A and α1B binding are present, but α1D is not. (B) α1-Subtype proteins as fmol receptor bound per mg membrane protein, calculated from levels determined by saturation binding and percents measured from competition binding, n = 6 LV, n = 4 RV (p = NS by ANOVA). RV has a significant increase in relative α1A binding (F 44% versus NF 27%, p = 0.04 by t-test).

Figure 5. α1-Subtype mRNAs by Clinical and Demographic Variables. Total and α1-subtype mRNA levels by qRT-PCR are grouped by (A) Age, (B) EF, (C) Sex, (D) β-blocker use, and (E) β-agonist use (A-B; linear regressions; C-F, lines are means, p by t-tests). In panels C and D, p=NS for each subtype and total α1-ARs.
TABLE 1. Patient Characteristics

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Failing (n=12)

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<td>18</td>
<td>AS</td>
<td>ACE/Amio/BB/D/Hyd/Nit</td>
</tr>
<tr>
<td>062107-NI</td>
<td>26</td>
<td>M</td>
<td>20</td>
<td>AF/RHD</td>
<td>BB/D/Hyd/Mil</td>
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<tr>
<td>112806-NI</td>
<td>29</td>
<td>F</td>
<td>20</td>
<td>AF/CoHD/VT</td>
<td>ACE/BB/D/Db/Epi/Mil</td>
</tr>
<tr>
<td>092106-NI</td>
<td>38</td>
<td>F</td>
<td>30</td>
<td>CoHD</td>
<td>ACE/Amio/BB/D/Lev</td>
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<tr>
<td>100906-NI</td>
<td>44</td>
<td>M</td>
<td>18</td>
<td>VTE</td>
<td>ACE/Amio/Db/Mil</td>
</tr>
<tr>
<td>112206-NI</td>
<td>44</td>
<td>M</td>
<td>23</td>
<td>CKD</td>
<td>ACE/Amio/BB/Lev/Mil/Sil</td>
</tr>
<tr>
<td>112306-NI</td>
<td>45</td>
<td>F</td>
<td>30</td>
<td>CKD/Sar/VTE</td>
<td>BB/D/Db/Dig/Hyd/Nit</td>
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<tr>
<td>071006-I</td>
<td>45</td>
<td>M</td>
<td>31</td>
<td>CAD/HTN</td>
<td>ARB/BB/D/Dig/Hyd/Nit</td>
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<tr>
<td>070407-NI</td>
<td>56</td>
<td>M</td>
<td>35</td>
<td>AF/CKD/HTN</td>
<td>Amio/BB/D/Hyd/Mil/Nit</td>
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<tr>
<td>081607-I</td>
<td>58</td>
<td>M</td>
<td>25</td>
<td>CAD/CKD/DM/HTN</td>
<td>Ins/VAD</td>
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<td>021207-I</td>
<td>59</td>
<td>M</td>
<td>20</td>
<td>AF/CAD/CKD/MI</td>
<td>Amio/Db/Dig/Nip/Mil</td>
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<tr>
<td>062007-I</td>
<td>66</td>
<td>M</td>
<td>20</td>
<td>CAD</td>
<td>BB/VAD</td>
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<table>
<thead>
<tr>
<th>Av±SE</th>
<th>% M</th>
<th>Av±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>44±3</td>
<td>71%</td>
</tr>
<tr>
<td>Non-Failing</td>
<td>43±5</td>
<td>67%</td>
</tr>
<tr>
<td>Failing</td>
<td>44±4</td>
<td>75%</td>
</tr>
</tbody>
</table>

Patients were unused donors (DON) or transplant recipients (non-ischemic, NI; ischemic, I). ACE, ACE-inhibitor; AF, atrial fibrillation/flutter; Amio, amiodarone; ARB, angiotensin receptor blocker; AS, aortic stenosis; BB, β-blocker; CAD, coronary artery disease; CCB, calcium channel blocker; CKD, chronic kidney disease; CoHD, congenital heart disease; COPD, chronic obstructive pulmonary disease; CPVT, catecholaminergic polymorphic VT; D, diuretic; DA, dopamine; Db, dobutamine; Dig, digoxin; DM, diabetes mellitus; EF, ejection fraction; Epi, epinephrine; F, female; HTN, hypertension; Hyd, hydralazine; Ins, insulin; Lev, levothyroxine; M, male; MI, myocardial infarction; Mil, milrinone; Nip, nitroprusside; Nit, nitrates; PE, phenylephrine; PSA, polysubstance abuse; RHD, rheumatic heart disease; Sar, sarcoidosis; Sil, sildenafil; VAD, ventricular assist device; Vaso, vasopressin; VT, ventricular tachycardia; VTE, venous thromboembolic disease. A p<0.0001, Non-Failing vs. Failing.
<table>
<thead>
<tr>
<th></th>
<th>Non-Failing mRNA (LVFW &amp; Septum)</th>
<th>Non-Failing Binding (LVFW)</th>
<th>Failing mRNA (LVFW &amp; Septum)</th>
<th>Failing Binding (LVFW)</th>
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<tr>
<td></td>
<td>AU</td>
<td>%</td>
<td>CTT</td>
<td>fmol/mg</td>
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<tr>
<td>α1-AR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total α1</td>
<td>92±5 (8)</td>
<td>3.6±0.6 (6)</td>
<td>118±12 (10)</td>
<td>4.7±0.8 (6)</td>
</tr>
<tr>
<td>α1A</td>
<td>56±5^A</td>
<td>63±6</td>
<td>80±10^B</td>
<td>37±4 (4)</td>
</tr>
<tr>
<td>α1B</td>
<td>20±4</td>
<td>22±4</td>
<td>14±2</td>
<td>13±2</td>
</tr>
<tr>
<td>α1D</td>
<td>15±4</td>
<td>0%</td>
<td>24±6</td>
<td>20±4</td>
</tr>
<tr>
<td>β-AR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total β</td>
<td>887±90 (8)</td>
<td>38±6 (6)</td>
<td>698±83 (10)</td>
<td>19±7 (4)</td>
</tr>
<tr>
<td>β1</td>
<td>116±27</td>
<td>13±2^H</td>
<td>73±5 (4)</td>
<td>113±41</td>
</tr>
<tr>
<td>β2</td>
<td>770±73</td>
<td>87±2</td>
<td>754±57^C</td>
<td>87±4</td>
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<tr>
<td>TBP &amp; β-actin</td>
<td>22±0.3</td>
<td></td>
<td>22±0.3</td>
<td></td>
</tr>
<tr>
<td>α1- + β-AR</td>
<td>978±92 (8)</td>
<td>41.8±6 (6)</td>
<td>816±78 (10)</td>
<td>24.7±6 (4)</td>
</tr>
<tr>
<td>Mean α1/β</td>
<td>11±1</td>
<td></td>
<td>21±4^F</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>12±3 (6)</td>
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</table>

α1- and β-AR subtype mRNAs by qRT-PCR are arbitrary units (AU) normalized to TBP & β-actin; LV data are average Free Wall (FW) and Septum. Total α1- and β-AR binding in LVFW was by saturation analysis, and subtype percents by competition. The ratios of α1 to β were from the same samples. Values are mean±SE (n). CTT, cycles to threshold. ^A^, p<0.05 α1A mRNA v α1B and α1D in NF and F; ^B^, p<0.05 α1A mRNA in NF v F; ^C^, p<0.05 β2 mRNA in NF v F; ^D^, p<0.05 β1 binding% in NF v F; ^E^, p<0.05 β2 binding% in NF v F; ^F^, p = 0.05 α1/β mRNAs in NF v F; ^G^, p<0.05 α1/β binding in NF v F; ^H^, β1-mRNA levels are lower here than in prior studies, which also found decreased β1-mRNA in failing myocardium.
Figure 1. Characterization of NF and F LV Myocardium

A. β-MyHC mRNA

B. α-MyHC mRNA

C. Type I Collagen mRNA

D. Reference mRNAs

E. β-AR Subtypes (competition binding)
Figure 2. α1-AR Subtype mRNA Levels in NF and F Myocardium

<table>
<thead>
<tr>
<th></th>
<th>α1D</th>
<th>α1A</th>
<th>α1B</th>
<th>α1D</th>
<th>α1A</th>
<th>α1B</th>
</tr>
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<tbody>
<tr>
<td>LVFW</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>LVS</td>
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<td>RVFW</td>
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</tr>
<tr>
<td>LVFW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVFW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(N = 10, N = 8)

> 0.01
> 0.001
> 0.05
> 0.01
> 0.001
Figure 3. Saturation Binding for Total $\alpha_1$-ARs in Regions of NF and F LV and RV

A. Saturation Binding for Total $\alpha_1$-ARs in Myocardial Membranes

B. Total $\alpha_1$-AR Levels in Myocardial Regions ($n = 6$)
**Figure 4.** Competition Binding for $\alpha_1$-AR Subtype Levels in NF and F LV and RV

**A. Competition Binding for $\alpha_1$-Subtypes**

![Graph showing competition binding for $5$-methylurapidil (5-MU) and BMY-7378](image)

**B. $\alpha_1$-Subtype Proteins in NF and F LV (n = 6) and RV (n = 4)**

![Bar graphs showing $\alpha_1$-subtype protein levels in Non-Failing and Failing conditions](image)
Figure 5. $\alpha_1$-Subtype mRNAs by Clinical and Demographic Variables

A. Age

B. Ejection Fraction

C. Sex

D. β-blocker Use

E. β-agonist Use

\[ p = 0.53 \]
\[ p = 0.15 \]
\[ p = \text{NS} \]
\[ p = 0.04 \text{ for } \alpha_1B \]
\[ p < 0.01 \text{ for } \alpha_1D \]
Alpha-1-Adrenergic Receptor Subtypes in Non-Failing and Failing Human Myocardium
Brian C. Jensen, Philip M. Swigart, Teresa DeMarco, Charles Hoopes and Paul C. Simpson

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SUPPLEMENTAL MATERIAL
(Figures 1-2)
Online Figure 1. Human qRT-PCR Primers & Validation

A. Primer sequences & efficiencies.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Position</th>
<th>Tm</th>
<th>%GC</th>
<th>Intron-spanning</th>
<th>Efficiency</th>
</tr>
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<tbody>
<tr>
<td>α1A</td>
<td>Forward: GGCTCCTTCTACCTGCCTCT</td>
<td>1007-1026</td>
<td>60</td>
<td>60</td>
<td>Yes</td>
<td>1.78</td>
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<tr>
<td></td>
<td>Reverse: AGGGCTTGAATCAGGGAAG</td>
<td>1327-1346</td>
<td>60</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1B</td>
<td>Forward: CCTGAGGATCCATTCAAGA</td>
<td>929-948</td>
<td>60</td>
<td>50</td>
<td>Yes</td>
<td>1.81</td>
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<tr>
<td></td>
<td>Reverse: GGTTGAGGCGAGCTGTTGAAG</td>
<td>1187-1206</td>
<td>61</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1D</td>
<td>Forward: TCTGCTGTTCCCTTCTTC</td>
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<td>60</td>
<td>61</td>
<td>Yes</td>
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<td></td>
<td>Reverse: CACGCAGCTGTTGAAATG</td>
<td>1232-1250</td>
<td>60</td>
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<td>β1-AR</td>
<td>Forward: AGTGGCCTTGCTGATGTTCT</td>
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<td>β2-AR</td>
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<td>60</td>
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<td>α-MyHc</td>
<td>Forward: TGAAACCGAGAATGGGAAGA</td>
<td>237 - 256</td>
<td>60</td>
<td>45</td>
<td>Yes</td>
<td>not tested</td>
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<tr>
<td></td>
<td>Reverse: GGCCATGTCTCATAATTTGT</td>
<td>308 - 327</td>
<td>59</td>
<td>50</td>
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<tr>
<td>β-MyHc</td>
<td>Forward: CTGCTCTGAGGCCTTGG</td>
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<td>1.94</td>
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<td>Reverse: CCTGTTGCCCAAAAATGA</td>
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<td>Coll1</td>
<td>Forward: AACAGCCGCTTCACCTACAG</td>
<td>4333-4531</td>
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<td>Reverse: TGGGATGGAGGAGTTTACA</td>
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<td>58</td>
<td>50</td>
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<td>β-actin</td>
<td>Forward: ATTTGCAATGAGCGGTTC</td>
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<td></td>
<td>Reverse: GGATGCCACAGGACTCCAT</td>
<td>878 - 896</td>
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<tr>
<td>TBP</td>
<td>Forward: GGGCACCCTCCACTGTATC</td>
<td>631-650</td>
<td>60</td>
<td>60</td>
<td>Yes</td>
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<tr>
<td></td>
<td>Reverse: CTCATGATTACCAGCAAAA</td>
<td>821-840</td>
<td>60</td>
<td>45</td>
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</tr>
</tbody>
</table>

B. α1-AR primers flank the single long intron.

C. Any contaminating genomic DNA is not amplified.
Online Figure 2. β-Subtype mRNAs by Clinical and Demographic Variables

A. Age

B. Ejection Fraction

C. Sex

D. β-blocker Use

E. β-agonist Use

p = NS