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The Alpha-1D Is the Predominant Alpha-1-Adrenergic Receptor Subtype in Human Epicardial Coronary Arteries

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Abstract

Objectives—The goal was to identify alpha-1-adrenergic receptor (α1-AR) subtypes in human coronary arteries.

Background—The α1-ARs regulate human coronary blood flow. α1-ARs exist as three molecular subtypes, α1A, α1B, and α1D, and the α1D subtype mediates coronary vasoconstriction in the mouse. However, the α1A is thought to be the only subtype in human coronary arteries.

Methods—We obtained human epicardial coronary arteries and left ventricular (LV) myocardium from 19 transplant recipients and 6 unused donors (age 19–70 years; 68% male; 32% with coronary artery disease). We cultured coronary rings and human coronary smooth muscle cells. We assayed α1- and β-AR subtype mRNAs by quantitative real-time reverse transcription PCR; and subtype proteins, by radioligand binding and ERK activation.

Results—The α1D subtype was 85% of total coronary α1-AR mRNA and 75% of total α1-AR protein, and α1D stimulation activated ERK. In contrast, the α1D was low in LV myocardium. Total coronary α1-AR levels were one-third of β-ARs, which were 99% the β2 subtype.

Conclusions—The α1D subtype is predominant and functional in human epicardial coronary arteries, whereas the α1A and α1B are present at very low levels. This distribution is similar to the mouse, where myocardial α1A and α1B-ARs mediate beneficial functional responses, and coronary α1Ds mediate vasoconstriction. Thus, α1D-selective antagonists might mediate coronary vasodilation, without the negative cardiac effects of non-selective α1-AR antagonists in current use. Furthermore, it could be possible to selectively activate beneficial myocardial α1A and/or α1B-AR signaling without causing coronary vasoconstriction.

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Keywords

receptors, adrenergic, alpha and beta; arteries; coronary disease

INTRODUCTION

Adrenergic receptors (ARs) play an important role in coronary arterial blood flow regulation. Coronary alpha-ARs (α-ARs) cause vasoconstriction, whereas beta-ARs (β-AR) cause vasodilation. α1-ARs constrict primarily epicardial coronary arteries and large arterioles, whereas α2-ARs act mostly on the coronary microcirculation (1,2). Stimulation of α1-ARs by endogenous catecholamines produces little constriction of normal coronary arteries (2–4), but causes pronounced vasoconstriction in coronary arteries with atherosclerotic endothelium (1, 2,4).

α1-ARs exist as three molecular subtypes, α1A, α1B, and α1D. All three subtypes are activated by norepinephrine (NE) and epinephrine, but differ in amino acid sequence, tissue expression, and signaling (5). In the mouse heart, cardiac myocytes express the α1A and α1B subtypes, whereas the α1D subtype is functional in coronary arteries (6–8). However, very few data exist on α1-AR subtypes in the human heart. A single small study of post-mortem tissue identified the α1A as the predominant α1-subtype in epicardial coronaries (9). The α1A is also thought to be the predominant α1-AR subtype in the human myocardium, based on mRNA assay (10). Taken together, these previous results suggest that α1-AR subtype expression is different in the human heart than the mouse heart.

The distribution of cardiac α1-AR subtypes has significant physiological impact in the mouse, where the myocardial α1A and α1B mediate adaptive and beneficial effects, including positive inotropy, physiological hypertrophy, and protection from myocyte death (6,11–13). The coronary α1D mediates vasoconstriction (7,8). In humans, nonselective blockade of all α1-subtypes can be associated with heart failure (14,15). These results and others raise the possibility that the human heart α1A and α1B subtypes should not be blocked, and might even be targets for selective agonists to treat myocardial disease (12,16). Thus, it could be significant clinically if human coronary arteries express predominantly the α1D subtype, as in the mouse (7,8), rather than the α1A subtype, as reported previously (9).

In this study, we re-examined the α1-AR subtypes in human epicardial coronary arteries, and measured β-ARs for comparison. Our results show that the α1D is the predominant and functional coronary α1-AR subtype, whereas the α1A and α1B are expressed at very low levels. We contrast this finding with the minimal expression of the α1D in human ventricular myocardium. We also find that α1-AR levels in coronary arteries are about one-third the level of β-ARs, most of which are the β2 subtype.

METHODS

Patients

With the approval of the UCSF Committee for Human Research, and with full informed consent, we obtained heart tissue from transplant recipients or unused donors provided by the California Transplant Donors Network (CTDN).

Tissue collection

The heart was explanted after cold cardioplegia, under anesthesia and analgesia with fentanyl, midazolam, rocuronium, and isoflurane at UCSF, and with varied agents at the CTDN

hospitals. The explanted heart was placed immediately in ice-cold physiologic solution, cleaned rapidly of fat, flash frozen in liquid nitrogen, and stored at −80°C.

RNA preparation

Coronaries were pulverized in a liquid nitrogen-cooled mortar and homogenized (Polytron) in TRIzol reagent (Invitrogen, Gibco BRL). Myocardium was homogenized directly in TRIzol. RNA was extracted using chloroform and isopropanol, purified on Qiagen Mini-Prep columns, and treated with DNase (Turbo DNAfree, Ambion). We found no significant RNA degradation (Agilent 2100 BioAnalyzer).

Quantitative real-time reverse transcription PCR (qRT-PCR)

RT reactions used 1 μg RNA, SuperScript III Reverse Transcriptase (Invitrogen), random hexamers (Invitrogen), and oligo-dT (Roche). qRT-PCR was done in triplicate in an ABI PRISM 7900HT Sequence Detection System with 5% of the RT product, primers at 125 nM and SYBR Green Master (Roche) with ROX reference dye. Data were analyzed with SDS software version 2.3 (Applied Biosystems).

Relative quantitation of PCR products used the ΔΔCT method, where Arbitrary Units (AU) was $2^{-\Delta\Delta CT} \times 1000$, CT=cycles to threshold, and ΔΔCT=[(mean target gene CT)–(mean CT of two reference genes, β-actin and TATA-binding protein (TBP), for improved accuracy)].

Radioligand binding (RLB)

Multiple protocols for membrane preparation from single arteries did not yield sufficient protein for reliable binding. Therefore, 15 epicardial coronaries totaling 10.2 g wet weight were pooled from 11 patients, pulverized in a liquid nitrogen-cooled mortar, homogenized in lysis buffer (5 mM Tris-HCl, 5 mM EDTA, 250 mM sucrose pH 7.4 plus PMSF), and centrifuged at 1000x*g* for 15 min. The supernatant was saved, and the pellet containing insoluble material was washed in lysis buffer and recentrifuged. The combined supernatants were centrifuged at 100,000x*g* for 1 h, and the resulting pellet was homogenized in lysis buffer and centrifuged at 100,000x*g* for 1 h. The resulting final membrane pellet, containing both plasma and intracellular membranes, was resuspended (50 mM Tris pH 7.4, 1 mM EDTA) and used for α1- and β-AR binding.

α1-AR saturation binding used 200 μg membrane protein in 1 ml per tube with ^{3}H-prazosin (0.04–1.2 nM, Perkin Elmer); phentolamine (10 μM, Sigma #P7561) defined non-specific binding. β-AR binding used 50 μg membrane protein per tube with ^{125}I-cyanopindolol (CYP, 0.04–1.0 nM, NEN Life Sciences); L-propranolol (1 μM, Sigma #P8688) defined non-specific binding (17). α1-AR subtype proteins were assayed using competition for ^{3}H-prazosin binding (0.5 nM) by BMY-7378 (0.05 nM-500 μM, Sigma #B134), an α1D-selective antagonist (18). All incubations were 60 minutes at 30°C. Binding data were analyzed by Prism 4.0b (GraphPad Software Inc., San Diego, CA).

Coronary artery smooth muscle cell (SMC) culture and immunoblots

Human coronary arteries were digested 20 min in Hanks buffer with collagenase Type II (1 mg/ml, Worthington) and elastase (0.5 mg/ml, Worthington), and intima and adventitia were removed mechanically. Rings of media (~2 mm) were cut free hand and cultured. Other rings were minced and digested for 2 h in collagenase and elastase; enzymes were inhibited with serum; and SMCs grew out of the minces (19). Clonetics normal human coronary artery SMCs were from Lonza (#CC2583, Walkersville, MD). All cells were cultured in DMEM with 10% fetal bovine serum for 8–48 h (rings) or 3–11 passages (cells). For experiments, cultures were incubated in DMEM without serum with 5 mg/ml BSA (Sigma #A7030) for at least 12 h (rings)

or 48 h (cells), pretreated without or with α1-antagonists (10 nM BMY-7378; 1 μM prazosin, Sigma #P7791) for 90 min (rings) or 15 min (cells), and treated with α1-agonists (1–200 nM L-NE, Sigma #N5785; 10 nM A61603, Tocris #1052, Ellisville, MO), in the presence of the β-AR antagonist L-propranolol (1 μM). After 30 min (rings) or 15 min (cells), homogenates were made in RIPA buffer with protease and phosphatase inhibitors. Ring homogenates were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant (rings) or total lysates (cells) were used (10–20 μg protein per lane) for immunoblotting with antibodies from Cell Signaling for total-ERK1/2 (rabbit pAb #9102) and phospho-ERK1/2 (rabbit mAb Thr202/Tyr204 #4370), and antibodies for phospho-S19/20-myosin light chain 2 (MLC), including Sigma #M6068, Cell Signaling #3671, and abcam #ab2480.

Data analysis

Results are mean ± SEM. Significant differences ($p<0.05$) were tested using analysis of variance (ANOVA) and Tukey's multiple comparison for more than two groups, and Student's unpaired t-test for two groups. The F test was used to compare goodness-of-fit for competition binding analysis (GraphPad Prism v4.0). Multivariable linear regression (Stata v.9, StataCorp, College Station, TX) was used to determine whether clinical variables were independently associated with α1- and β-AR density. Multivariate model assumptions were checked for all regression analyses.

RESULTS

Patients

To test α1-AR subtype expression in human coronary arteries, we collected hearts from 19 transplant recipients and 6 unused donors. Average age was 46 years (19–70), and 68% were male (Table 1). Coronary artery disease (CAD) was present in 32%, who were older ($p<0.005$) and had higher ejection fractions (EFs) ($p<0.05$, Table 1).

α1-AR subtype mRNA levels

To quantify α1-subtype mRNAs, we validated a qRT-PCR approach, using primers that span the single intron in each α1-AR subtype gene. Primer pairs were designed using Primer3 (v0.4.0) and BLAST and chosen for comparable reaction efficiencies (20). Specificity of the α1-subtype primers was confirmed using (a) PCR with human α1-AR cDNAs, (b) a dissociation step in all PCR reactions, and (c) sequencing of the PCR products. Amplification of genomic DNA was excluded by (a) use of intron-spanning primers, (b) DNase treatment of RNA, and (c) end-point PCR reactions using no-RT templates as negative controls.

In human epicardial coronary arteries, the α1D was 85% of total α1-mRNA (Figure 1A, Table 2). The α1B (11%) and the α1A (4%) were markedly less abundant than the α1D ($p<0.001$ for each). In LV myocardium, by contrast, the α1D was only 21% of the total α1-mRNA, and the α1A (63%) was the most abundant (Figure 1B). The absolute level of α1D mRNA in coronary arteries was almost twice that in LV (Table 2, $p=0.01$), whereas the absolute level of the α1A was 30-fold higher in LV than in coronary arteries (Table 2). As a control, there was no difference between coronary arteries and myocardium in the qRT-PCR cycles-to-threshold for the reference genes, β-actin and TBP (Table 2). Levels of α1-subtype mRNAs were the same in 4 right and 4 left anterior descending coronaries (data not shown). There were no differences in α1-subtype mRNA levels in coronaries collected at UCSF versus the CTDN hospitals, suggesting no important effects due to anesthetic and analgesic agents (data not shown).

In summary, the α1D is 85% of total α1-AR subtype mRNA in human epicardial coronary arteries, but is significantly less abundant in human LV myocardium.

α1-AR subtype protein levels

To test α1-AR subtype protein levels we used RLB with ^{3}H-prazosin. We could not use immunohistochemistry or immunoblot, because none of the 10 α1-AR antibodies that we tested is specific for α1-ARs (21).

We used pooled membranes from 11 patients for binding. Patient characteristics were similar in these 11 patients and the entire patient population (Table 1), and the α1-subtype mRNA levels in the pooled samples were similar to the levels of the entire group (data not shown). Saturation binding identified 8.7 fmol/mg protein of total α1-ARs in coronary artery membranes, with a Kd 0.03 nM, and specific binding 70% of total at the ^{3}H-prazosin Kd (Figure 2, Table 2). The level of α1-AR binding in coronaries was roughly twice that in LV myocardium (20).

To test whether the α1D subtype protein was also predominant in coronaries, as with mRNA, we did competition binding with the α1D-selective antagonist BMY-7378. BMY-7378 competition yielded a two-site binding curve ($p=0.002$ vs. one-site model), with 75% high affinity sites (Kd 13 pM) and 25% low-affinity (2.6 μM) (Figure 3A, Table 2). In ventricular myocardium, BMY competition gave a one-site curve with low BMY-7378 affinity, indicating minimal or no α1D binding (Figure 3B).

In summary, the α1D is 75% of total α1-AR subtype protein in human epicardial coronary arteries, but is much less abundant in myocardium. The coronary levels of α1D mRNA and protein agree very well (Table 2).

α1-AR signaling in coronary SMCs

To test whether the α1D was functional in human coronary SMCs, we used immunoblot to quantify phosphorylation (activation) of ERK, which is a target for the α1D in rat aortic SMCs (22), and is involved in activation of MLC kinase in smooth muscle (23). We used SMCs from Lonza, coronary media rings, and primary isolates from the coronary medial SMC layer (Figure 4 legend). qRT-PCR for α-smooth muscle actin and smooth muscle myosin heavy chain confirmed SMC identity, and α1D mRNA was the predominant α1-subtype, with less α1B and no α1A (data not shown). Low concentrations of NE (mean 27 nM), in the presence of propranolol to block β-ARs, induced a 1.8-fold increase in phospho-ERK, and activation was abrogated to an equal extent by a low concentration of BMY-7378 (10 nM), the α1D-selective antagonist, and prazosin, the non-selective α1-antagonist (Figure 4). The α1A-selective agonist, A61603 (10–100 nM), did not activate ERK (data not shown). Phospho-MLC was barely detectable with 3 different antibodies, and was not useful as a read-out (data not shown).

We conclude that the α1D is functional in human epicardial coronary SMCs, whereas there is no evidence for the α1A.

β-AR mRNA and protein levels

We measured β-AR mRNAs and protein in the same coronary arteries, to compare with α1-ARs. By qRT-PCR (20) the β2 was the predominant β-subtype mRNA (99% of total β-AR mRNA, Table 2). Total α1-AR mRNA was 37% of total β-AR mRNA (Table 2, $p<0.0001$).

β-AR proteins were quantified by saturation binding, because the available β-AR antibodies are not specific in our hands (12). Saturation binding using ^{125}I-CYP, a non-selective β-AR antagonist, identified 25.2 fmol/mg protein of total β-ARs in coronary artery membranes, with a Kd 0.16 nM, and specific binding 41% of total at the ^{125}I-CYP Kd (Figure 2B). Given the preponderance of β2-mRNA in coronary tissue, β-AR competition binding was not done. Total

α1-AR binding was 35% of total β-AR binding, in excellent agreement with the mRNA values (Table 2).

In summary, the β2 was the predominant β-AR subtype in epicardial coronary arteries, and α1-AR mRNA and binding were only one-third of β-ARs.

Impact of clinical variables on coronary α1- and β-AR mRNA levels

The qRT-PCR results were analyzed to determine whether clinical variables affected the expression of AR subtypes in human coronaries. Human non-coronary arterial and prostate α1-ARs are said to increase with age (9,24), and α1-mediated vasoconstriction is more prominent in CAD (1–4). However, we found that age, EF, β-agonist exposure, CAD (Figure 5), and sex (data not shown) had no effect on coronary artery α1-subtype mRNA levels. Interestingly, α1D and total α1-mRNA levels were 35% lower in coronary arteries of patients using β-blockers ($p=0.04$). This association persisted after adjusting for age, sex, coronary artery disease, and ejection fraction ($p=0.03$, Figure 5B). Among β-blockers, α1D mRNA levels appeared similar among patients taking metoprolol (1 patient), nadolol (1 patient), or carvedilol (8 patients) (Figure 5B).

The levels of coronary β-AR mRNAs, which were almost entirely β2, did not vary with age, EF, β-blocker or β-agonist use, or with sex (data not shown).

In summary, age, sex, CAD, EF, and β-agonists had no significant effect on α1- or β-subtype mRNA levels in coronary arteries. β-Blocker use was associated with a significant decrease in α1D and total α1-mRNA levels.

DISCUSSION

This study reports that the α1D subtype is the predominant α1-AR in human epicardial coronary arteries, comprising about 80% of total α1-AR mRNA and protein. These data are also reveal that coronary α1-AR levels are only one-third of β-ARs. This is the most extensive characterization of α1-AR subtypes in coronary tissue in any species.

Our results disagree with those of a previous investigation that identified the α1A as the predominant α1-subtype in human coronary arteries (9). In our study, the α1A in the coronaries was only 4% of total α1-mRNA in coronaries, and was absent in isolated SMCs, and the combined α1A and α1B were only 25% of total α1-binding. The discrepancy might be explained by the prior study's small sample size (5 arteries from an unspecified number of patients), the use of post-mortem tissue, or a qualitative RNA assay (RNase protection) (9).

Of particular importance is our finding that the α1-AR subtype profiles in the human coronary arteries and ventricular myocardium were quite different. Previous limited evidence suggests that α1A subtype mRNA is predominant in both coronary arteries and myocardium (9,10). In contrast, we found that the α1D was predominant in the coronary arteries, but was much less abundant in the myocardium, where α1A mRNA was predominant. In the mouse heart, myocardium has the α1A and α1B subtypes (6), whereas the α1D subtype is functional in coronary arteries (7,8). Thus, rodents and human might have similar α1-AR subtype expression in coronary arteries and myocardium (7,8,17,20,25–27) (Table 3), contrary to prior claims (9), and studies done in mouse models might therefore be applicable to human cardiac α1-AR biology.

Technical aspects of this study warrant emphasis. We studied coronaries from a large number of patients of both sexes without and with CAD or heart failure, and assessed the effects of these variables on α1-AR expression. We took extensive measures to validate our qRT-PCR

approach. We quantified α1-subtype proteins by competition binding, because binding is for now the only accurate method to measure α1-AR proteins (21).

We also measured β-AR subtypes in the coronaries, and found that α1-AR levels were only one-third of β-ARs. However, these lower α1-AR levels do not negate the physiological significance of the α1D. The contractile response to NE in isolated human epicardial coronary arteries is constriction at low concentrations (nM) and relaxation at high concentrations (μM) (28). The α1D has the highest NE affinity of any subtype (29), and thus constriction at low NE concentrations is consistent with an α1D-response. The larger population of coronary ®-ARs could mediate relaxation with μM NE.

Indeed, our experiments in epicardial coronary SMCs revealed that the α1D mediated activation of ERK by low concentrations of NE (Figure 4). ERK is activated by the α1D in rat aortic SMCs (22), and ERK phosphorylation facilitates activation of MLC kinase in smooth muscle, thus contributing to the adrenergic contractile response (23). These findings suggest that the α1D is both abundant and functional in human epicardial coronary arteries.

We analyzed coronary α1- and β-AR subtype mRNA levels by age, sex, CAD, EF, β-blockers, and β-agonists (Figure 5). The only association we found was a decrease in the α1D and total α1-ARs in patients treated with β-blockers, possibly implying that α1D expression in coronary vascular cells is increased by β-stimulation. In fact, in human monocytes, β2-stimulation induces α1D mRNA and protein (30).

Coronary α1-subtype mRNA levels did not differ in patients with CAD versus without CAD. This was noteworthy, since α1-ARs cause pronounced vasoconstriction in atherosclerotic coronary arteries, but have little effect in normal coronaries (1–4). Thus, increased α1D levels in coronary vascular SMCs might not explain augmented α1-vasoconstriction in CAD. Instead, a small population of endothelial cell α1ARs mediating endothelium-dependent vasodilation (31) could be lost in CAD.

Clinical implications

Important clinical implications derive from the predominance of the α1D subtype in human coronary arteries. Cell and animal models show that the cardiac myocyte α1A and α1B subtypes have significant adaptive and protective roles (6,11–13,16). Clinical trials also suggest α1-mediated cardioprotection, since non-selective antagonism of all α1-subtypes was associated with a two-fold excess of heart failure in the doxazosin arm of the ALLHAT trial, and a trend towards increased mortality in the prazosin arm of the V-HeFT trial (14,15).

Despite the ALLHAT results, 13.4 million prescriptions were dispensed in 2002 for mostly non-selective α-blockers (32), primarily to treat symptoms from prostate hypertrophy. However, the α1D-selective antagonist, naftopidil, is effective in relieving prostate symptoms (33). In light of our results, it seems possible that more selective antagonism of the α1D subtype might relax both coronary and prostate smooth muscle, without blocking beneficial signaling mediated by the myocardial α1A and α1B subtypes. Furthermore, the adaptive and protective roles of the myocardial α1A and α1B raise the intriguing possibility of activating one or both of these subtypes selectively to treat myocardial disease (16). The low levels of the α1A and α1B in human coronary arteries would make α1A- or α1B-agonist-induced coronary vasoconstriction unlikely.

In summary, we present here the most extensive characterization of human coronary ARs to date. We show that the α1D is the predominant α1-AR subtype in human epicardial coronary arteries, not the α1A as reported previously (9), and that the α1D is low in myocardium. The α1A and α1B are present in coronaries at very low levels, and total α1-AR levels are one-third

the level of β-ARs, most of which are the β2 subtype. The tissue distribution of α1-subtypes in human heart is similar to the rodent heart (Table 3). These results are relevant clinically to the widespread use of α1-antagonists, and to the potential development of α1A- and/or α1B-selective agonists.

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ABBREVIATIONS

α1-ARs	Alpha-1-adrenergic receptors
β-ARs	beta-adrenergic receptors
CTDN	California Transplant Donors Network
CAD	coronary artery disease
EF	ejection fraction
KO	knockout
LV	left ventricle
MLC	myosin light chain
NE	norepinephrine
qRT-PCR	quantitative real-time reverse transcription PCR
RLB	radiologand binding
SMC	smooth muscle cell

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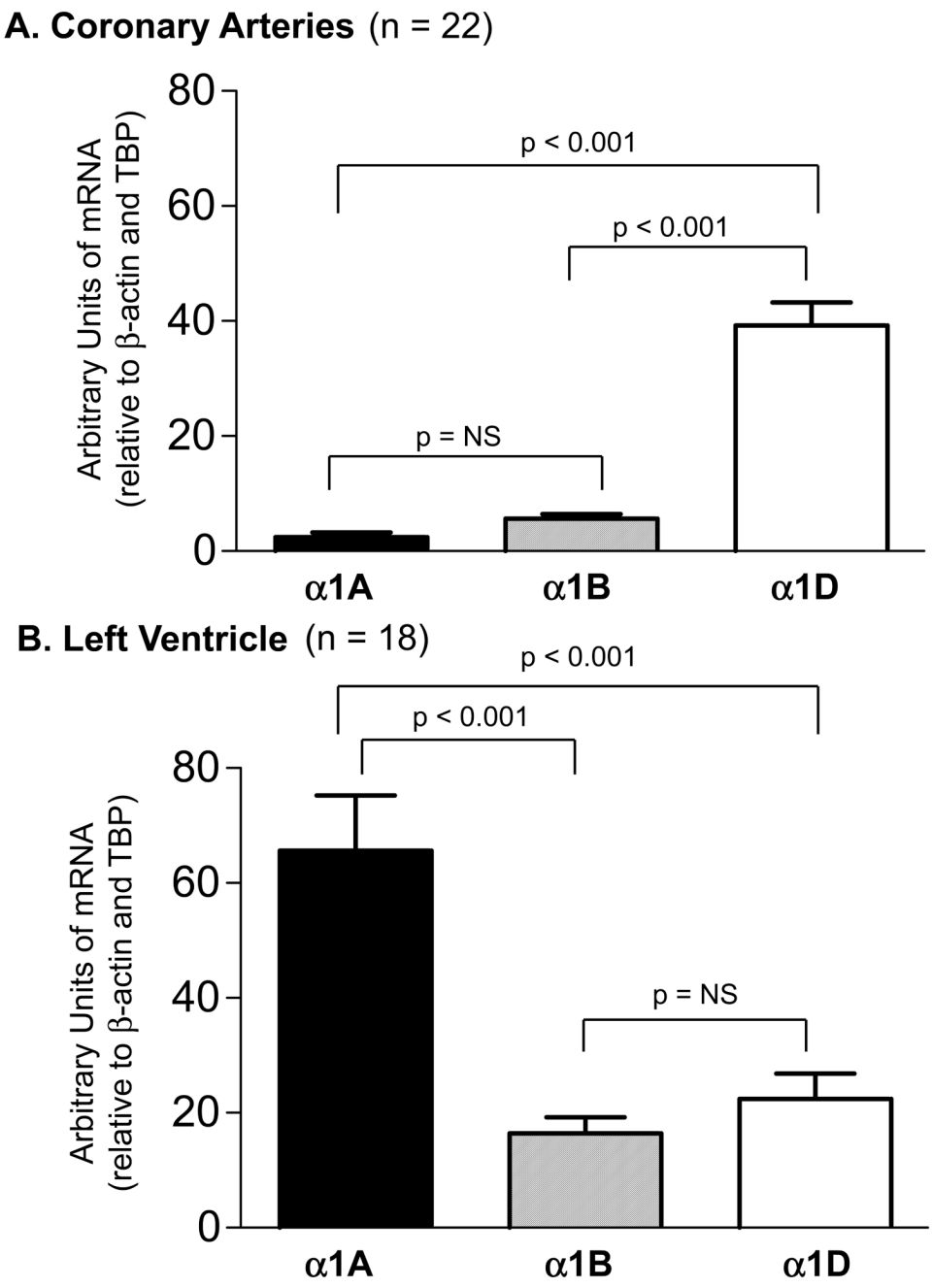

Figure 1. α1-AR Subtype mRNA Levels in Coronary Arteries and Left Ventricle
qRT-PCR of α1-AR subtypes in (**A**) human coronary arteries; (**B**) left ventricle free wall.

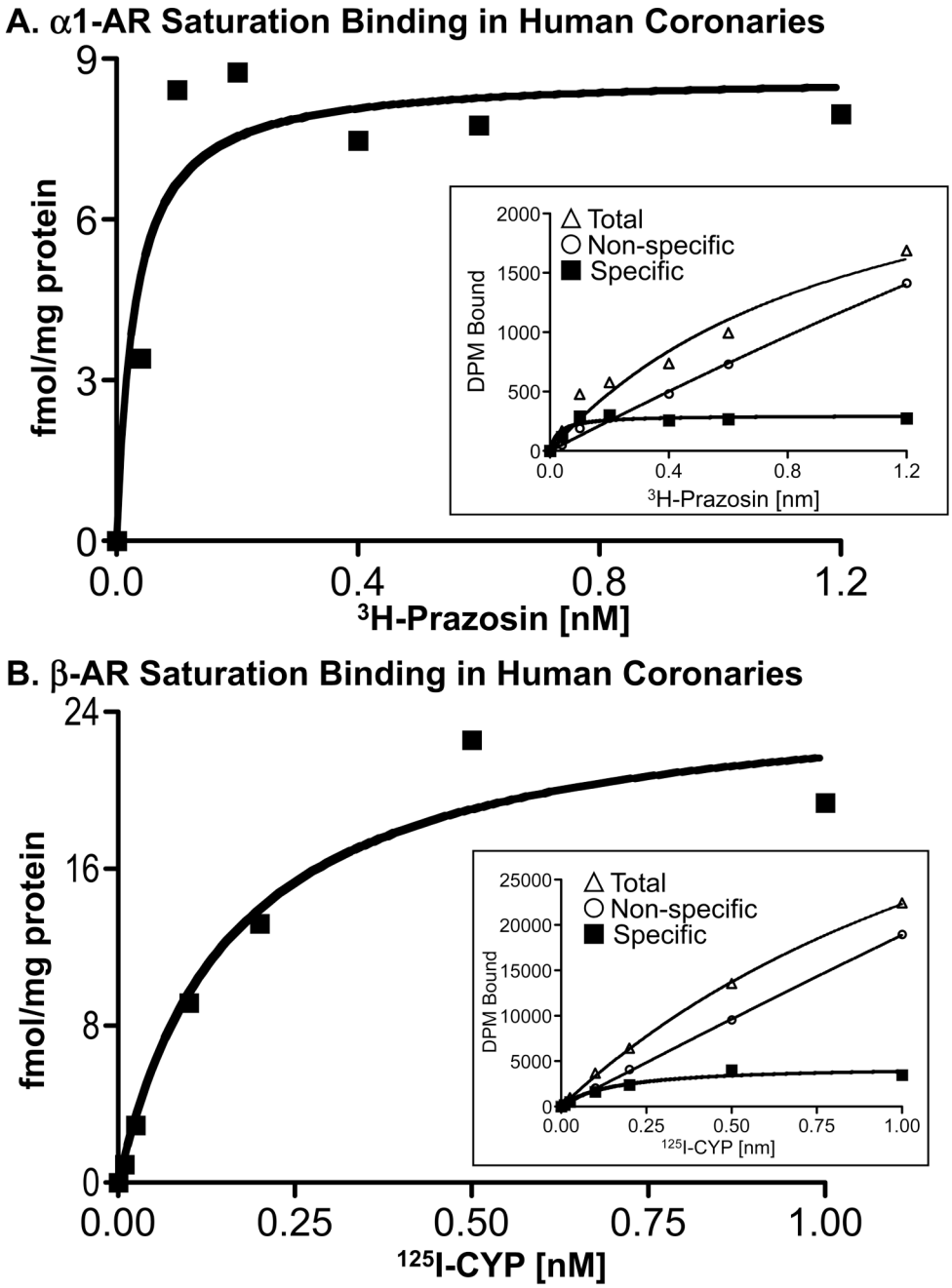

Figure 2. α1-AR and β-AR Protein Levels by Saturation Binding

Saturation RLB was done in membranes pooled from 15 epicardial coronary arteries of 11 patients. (**A**) Binding with ^{3}H-prazosin for total α1-ARs; (**B**) Binding with ^{125}I-CYP for total β-ARs.

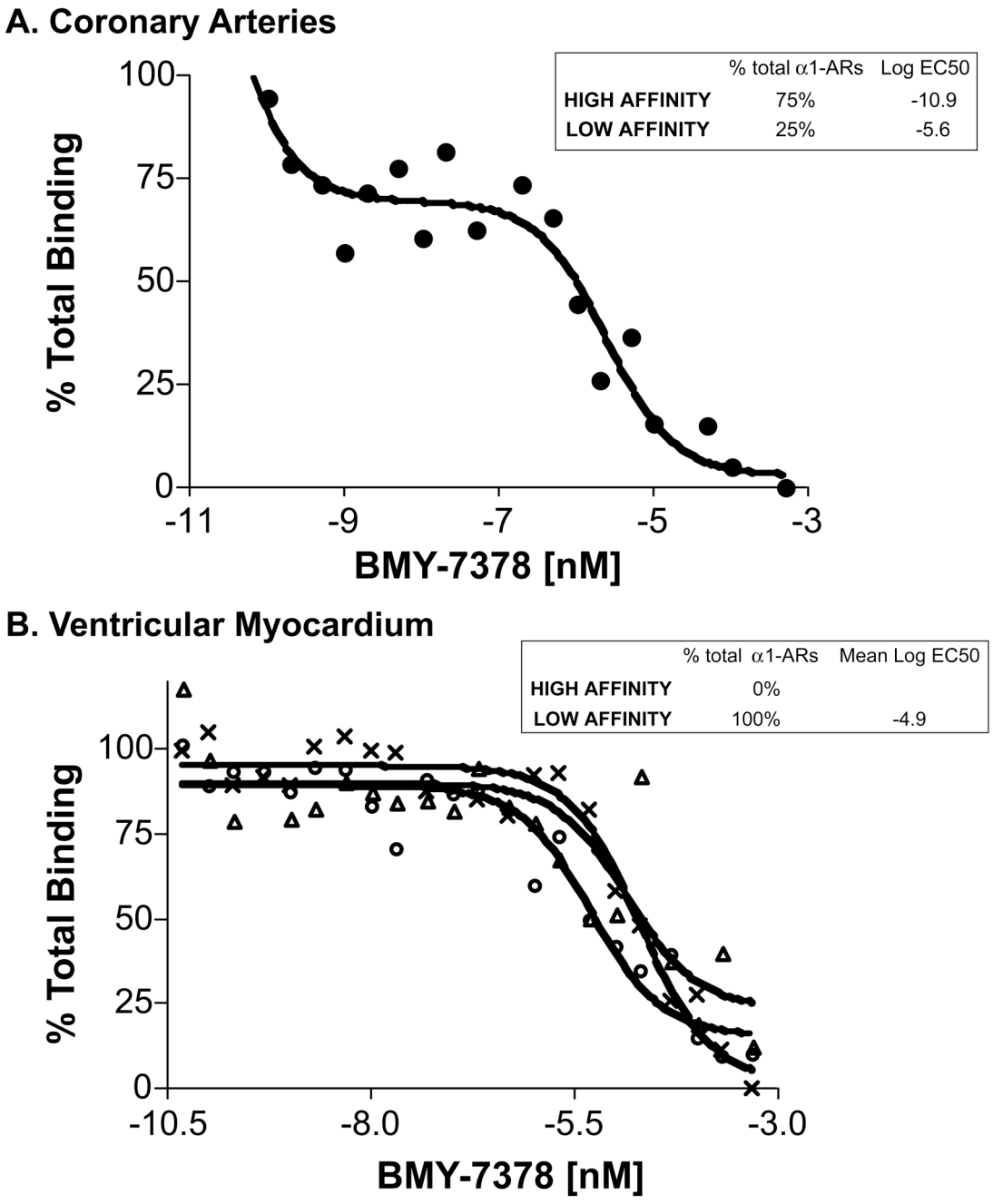

Figure 3. α1-AR Subtype Protein Levels by Competition Binding

Competition for ^{3}H-prazosin binding by the α1D-selective antagonist BMY-7378 yielded a two-site binding curve with (**A**) predominantly high-affinity sites in coronary artery membranes; (**B**) a one-site low affinity curve in ventricular myocardium from 3 patients.

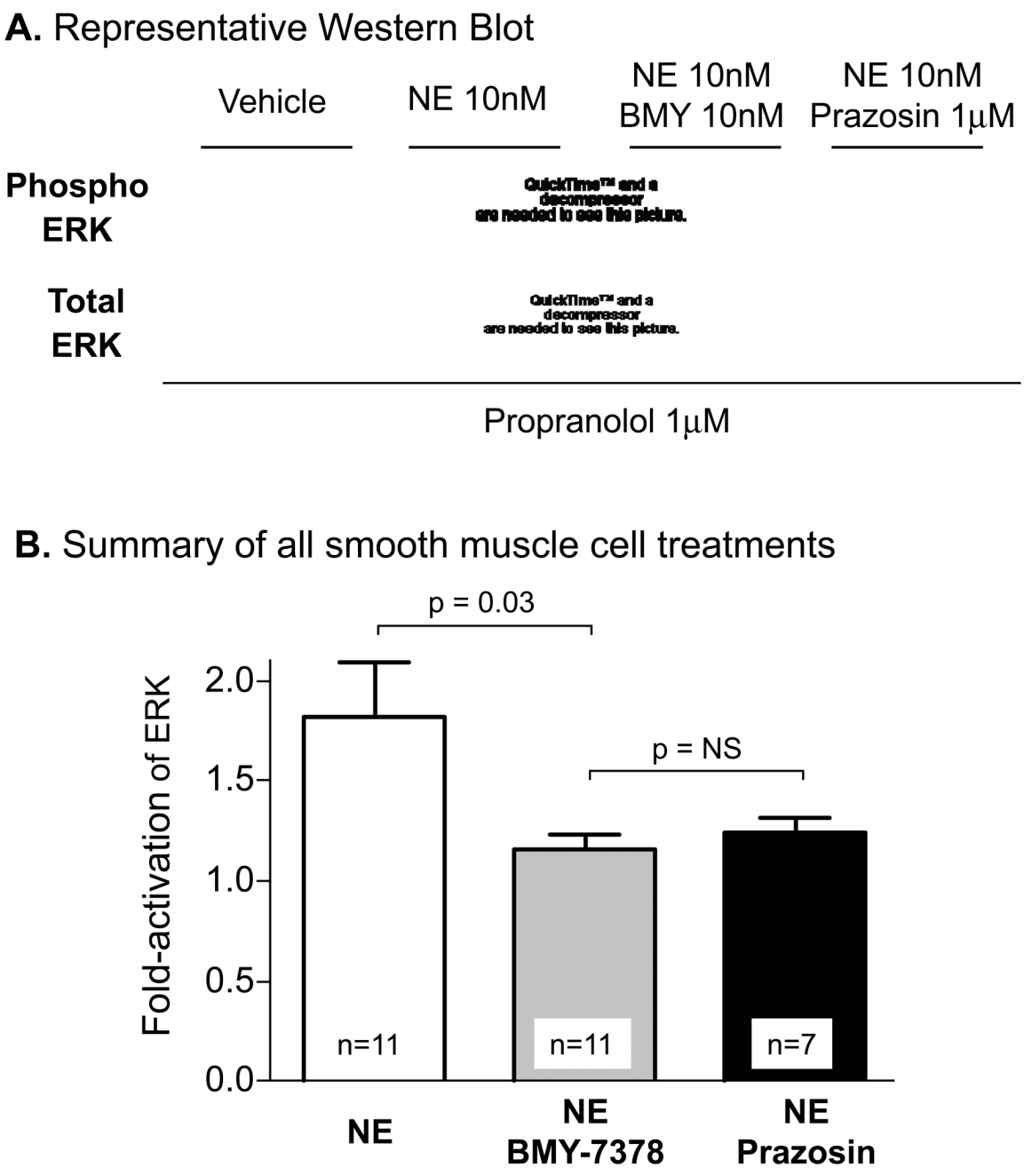

Figure 4. α1-AR-induced ERK Activation in Human Coronary Artery SMCs

Cultured human epicardial coronary SMCs and coronary media rings were treated for 15–30 min with low concentrations of NE (1–200 nM, mean 27 nM), and the nonselective β-AR antagonist propranolol (1μM), in the absence or presence of the α1D-selective antagonist, BMY-7378 (10nM), or the non-selective α1-antagonist, prazosin (1μM). (**A**) Western blot showing ERK activation in duplicate dishes from a Lonza SMC culture; (**B**) Summary data for 8 Lonza SMC preparations from 2 patients, a ring preparation from 1 patient, and 2 primary SMC cultures from 1 patient.

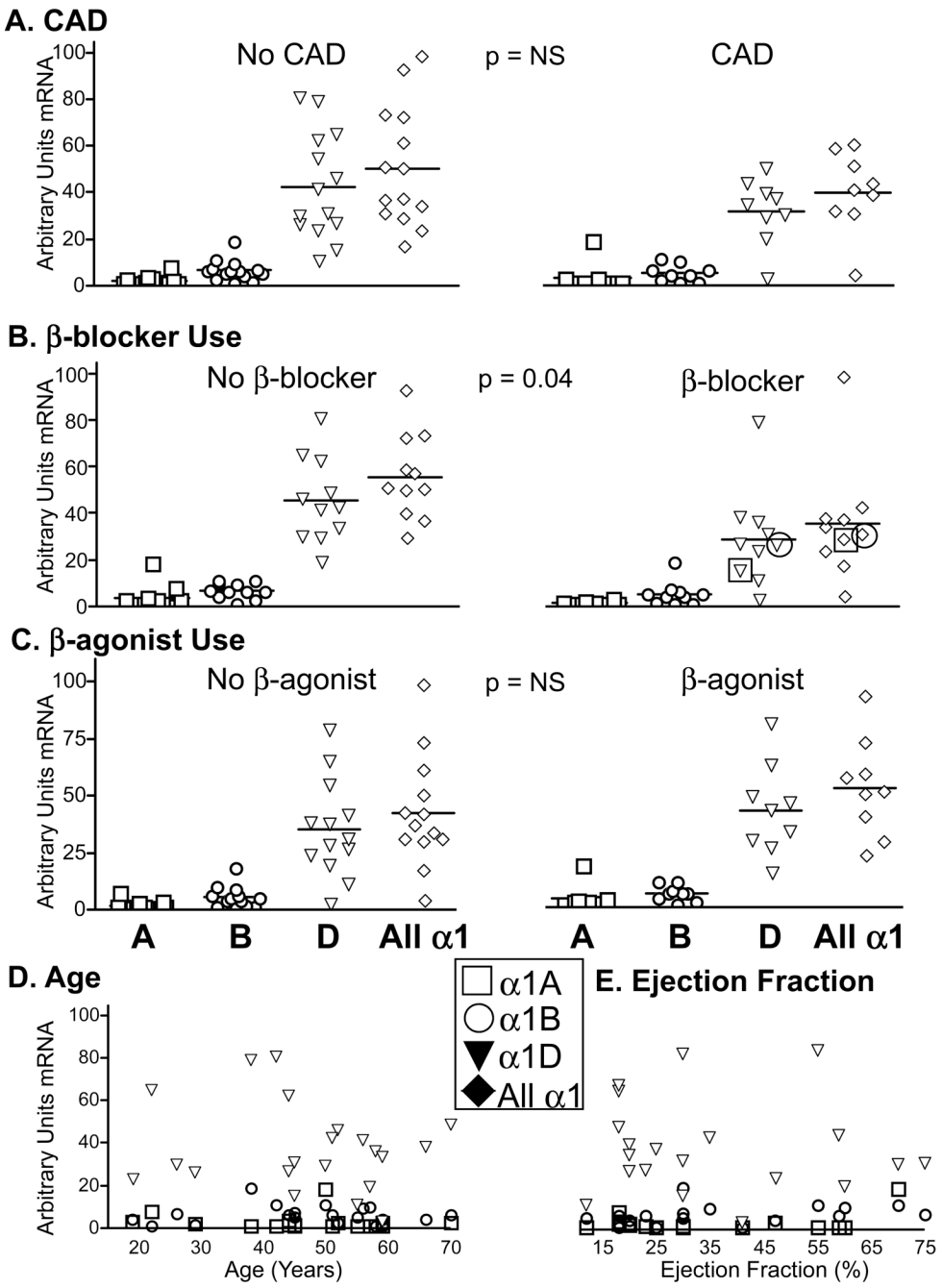

Figure 5. α1-Subtype mRNA Levels by Clinical Variables

qRT-PCR for α1-subtype mRNAs and all α1 mRNA are displayed according to (**A**) CAD, (**B**) β-blocker use, all carvedilol, except metoprolol (circled) and nadolol (squared), (**C**) β-agonist exposure, (**D**) age, and (**E**) EF. p values are for multivariate analysis.

Table 1

Patient Characteristics

Patient #	Age (yrs)	Sex	Medical History	EF (%)	Medications
Without CAD (n = 17)					
062606	19	M	CPVT	47	Amio, BB, CCB,
070307	22	M	AS, HF	18	ACEI, Amio, BB, D, Hyd, Nit
062107 *(b)*	26	M	AF, RHD	20	BB, D, Hyd, Mil
D-062607	26	F	None	N/A	None
112806 *(b)*	29	F	AF, CoHD, VT	20	ACEI, BB, D, Db, Epi, Mil
D-100406	31	M	None	65	None
092106	38	F	CoHD, HF	30	ACEI, Amio, BB, D, Lev
D-101606	42	M	DM, HTN	55	ACEI, D, PE, Vaso
100906	44	M	HF, VTE	18	ACEI, Amio, D, Db, Mil
112206 *(b)*	44	M	CKD, HF	23	ACEI, Amio, BB, D, Lev, Mil, Sil
112306 *(b)*	45	F	CKD, HF, Sar, VTE	30	BB, D, Db, Dig, Hyd, Nit
032007 *(b)*	45	F	HF	30	ACEI, Amio, BB, D, Hyd, Nip
060508 *(c)*	46	M	HF, COPD	26	ARB, BB, D, Dig, Mil
032407 *(b)*	52	F	AF, CKD, HF, HTN	18	D, Db, Dig, Hyd, Nit
050207	55	M	CKD, HF	12	ACEI, Amio, BB, Hyd, Mil
070407	56	M	AF, CKD, HF, HTN	35	Amio, BB, D, Hyd, Mil, Nit
080607	70	F	HF, CA, CKD, COPD	9	BB, D, Db, Hyd, Nit
With CAD (n = 8)					
D-022807 *(b)*	50	M	CAD, PSA	70	DA, PE
D-053107 *(b)*	51	M	CAD, HTN, PSA	59	DA, PE
D-011607 *(b)*	57	F	CAD, DM, HTN	60	None
081607 *(b)*	58	M	CAD, CKD, DM, HF, HTN	25	Ins, VAD
113006	59	M	CAD, CKD, DM, HF, HTN	41	ARB, BB, D, Ins, Nit
021207	59	M	AF, CAD, CKD, HF, MI	20	Amio, Db, Dig, Nip, Mil
032507	61	M	AS, CAD, CKD, DM	68	ARB, BB, D, Ins, Nit

Patient #	Age (yrs)	Sex	Medical History	EF (%)	Medications
062007 *(b)*	66	M	CAD	20	BB, VAD
	Mean ± SE	% M		Mean ± SE	
All patients	46 ± 3	68		34 ± 4	
Without CAD	41 ± 3	59		29 ± 4	
With CAD	58 ± 2*	88		45 ± 8†	

*p < 0.005;

†p < 0.05 with versus without CAD. Tissue was from heart transplant recipients (n = 19) or unused donor hearts (D-) (n = 6).

*(b)*indicates tissue used in binding assay;

*(c)*indicates used for culture. Clinical data were from University of California, San Francisco computerized medical records or the California Transplant Donors Network chart.

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation/flutter; Amio = amiodarone; ARB = angiotensin receptor blocker; AS = aortic stenosis; BB = β-blocker; CA = cancer; CAD = coronary artery disease; CCB = calcium-channel blocker; CKD = chronic kidney disease; CoHD = congenital heart disease; COPD = chronic obstructive pulmonary disease; CPVT = catecholaminergic polymorphic ventricular tachycardia; D = diuretic; DA = dopamine; Db = dobutamine; Dig = digoxin; DM = diabetes mellitus; EF = ejection fraction; Epi = epinephrine; HF = heart failure; HTN = hypertension; Hyd = hydralazine; Ins = insulin; Lev = levothyroxine; 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.

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Table 2

α1-AR mRNAs and Proteins in Human Epicardial Coronary Arteries

	mRNA		Binding		
	AU	%	fmol/mg	%	Cycles to Threshold
Coronary artery					
α1-AR					
α1A	2 ± 1	4	2.2	25	
α1B	5 ± 1	11			
α1D	39 ± 4*	85	6.5	75	
Total α1	46 ± 4†	100	8.7	100	
β-AR					
β1	1 ± 0	1			
β2	124 ± 12	99			
Total β	125 ± 12	100	25.2	100	
Total α1- and β-AR	171		34		
Total α1/total β		37		35	
TBP and β-actin mRNAs					20.8 ± 0.6
LV free wall					
α1-AR					
α1A	66 ± 9	63			
α1B	17 ± 3	16			
α1D	22 ± 5	21			
Total α1	105 ± 11	100			
TBP and β-actin mRNAs					22.1 ± 0.4

The α1- and β-adrenergic receptor (AR) subtype messenger ribonucleic acid levels (mRNAs) were quantified by quantitative real-time reverse transcription polymerase chain reaction. The mRNA values are arbitrary units (AU) (mean ± SE) normalized to TATA-binding protein (TBP) and β-actin mRNAs; n = 22, α1 in coronaries; n = 20, β in coronaries; n = 18, α1 in left ventricular (LV) free wall. The α1- and β-AR binding were quantified by saturation analysis in membranes pooled from 15 arteries of 11 patients, indicated by (b) in Table 1, and % α1D subtype was estimated by competition with BMY-7378.

*p = 0.01 α1D in coronary versus in LV free wall;

†p < 0.0001 total α1 mRNA versus total β-AR mRNA.

Table 3

Cardiac α1-AR Subtypes in Human, Mouse, and Rat

	Human	Mouse	Rat
Coronary α1-AR subtype	α1D*	α1D†	Unknown
Evidence	mRNA, binding, pharmacology	Vasoconstriction, KO studies	
Myocardial α1-AR subtype	α1A and α1B‡	α1A and α1B§	α1A and α1B‖
mRNA	A (63%) > B (16%), D (21%)	A (47%) = B (49%) ≫ D (4%) *	A (65%) > B (27%) ≫ D (8%)
Binding	B (60%) > A (40%) ⋙ D (0%)	B (70%) > A (30%) ⋙ D (0%)	B (74-80%) > A (20%–26%) ⋙ D (0%)

*References: present study;

†Turnbull et al. (7), Chalothorn et al. (8);

‡Jensen et al. (20);

§Myagmar et al., unpublished data, 2009, and Simpson (27);

‖Rokosh et al. (17), Stewart et al. (25), and Michel et al. (26).

KO = knockout; other abbreviations as in Table 1 and Table 2.