

Functional alpha-1B adrenergic receptors on human epicardial coronary artery endothelial cells

Brian C. Jensen · Philip M. Swigart ·
Megan D. Montgomery · Paul C. Simpson

Received: 29 July 2010 / Accepted: 30 August 2010 / Published online: 22 September 2010
© The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract Alpha-1-adrenergic receptors (α 1-ARs) regulate coronary arterial blood flow by binding catecholamines, norepinephrine (NE), and epinephrine (EPI), causing vasoconstriction when the endothelium is disrupted. Among the three α 1-AR subtypes (α 1A, α 1B, and α 1D), the α 1D subtype predominates in human epicardial coronary arteries and is functional in human coronary smooth muscle cells (SMCs). However, the presence or function of α 1-ARs on human coronary endothelial cells (ECs) is unknown. Here we tested the hypothesis that human epicardial coronary ECs express functional α 1-ARs. Cultured human epicardial coronary artery ECs were studied using quantitative real-time reverse transcription polymerase chain reaction, radioligand binding, immuno-

blot, and ^3H -thymidine incorporation. The α 1B-subtype messenger ribonucleic acid (mRNA) was predominant in cultured human epicardial coronary ECs (90–95% of total α 1-AR mRNA), and total α 1-AR binding density in ECs was twice that in coronary SMCs. Functionally, NE and EPI through the α 1B subtype activated extracellular signal-regulated kinase (ERK) in ECs, stimulated phosphorylation of EC endothelial nitric oxide synthase (eNOS), and increased deoxyribonucleic acid (DNA) synthesis. These results are the first to demonstrate α 1-ARs on human coronary ECs and indicate that the α 1B subtype is predominant. Our findings provide another potential mechanism for adverse cardiac effects of drug antagonists that nonselectively inhibit all three α 1-AR subtypes.

Keywords Human heart · Endothelial cell · Coronary artery · Alpha-1-adrenergic receptor · Alpha-adrenergic antagonists

B. C. Jensen · P. M. Swigart
Cardiology Division, VA Medical Center;
and Department of Medicine,
University of California, San Francisco,
San Francisco, CA, USA

M. D. Montgomery · P. C. Simpson
Cardiology Division, VA Medical Center; and Cardiovascular
Research Institute and Department of Medicine,
University of California, San Francisco,
San Francisco, CA, USA

P. C. Simpson (✉)
VA Medical Center (111-C-8),
4150 Clement St.,
San Francisco, CA 94121, USA
e-mail: paul.simpson@ucsf.edu

Present Address:

B. C. Jensen
Cardiology Division, University of North Carolina,
160 Dental Circle,
Chapel Hill, NC 27599-7075, USA

Introduction

Alpha-1-adrenergic receptors (α 1-ARs) regulate coronary artery blood flow in response to the catecholamines norepinephrine (NE) and epinephrine (EPI). α 1-ARs on vascular smooth muscle cells (SMCs) mediate contraction. Interestingly, however, α 1-AR stimulation causes minimal constriction of normal coronary arteries, but markedly constricts coronary arteries with disrupted endothelium (Baumgart et al. 1999; Heusch et al. 2000). A likely mechanism is loss of endothelial nitric oxide synthase (eNOS) activation and vasodilator NO when endothelial cells (ECs) are lost (Zembowicz et al. 1991; Jones et al. 1993). However, nothing is known about α 1-ARs on human coronary endothelial cells (ECs).

$\alpha 1$ -ARs exist as three molecular subtypes, $\alpha 1A$, $\alpha 1B$, and $\alpha 1D$. All three subtypes are activated by NE and EPI, but differ in amino acid sequence, tissue expression, and signaling (Graham et al. 1996). Little is known about $\alpha 1$ -AR subtypes in ECs of any vascular bed or species. A few earlier studies suggested that the $\alpha 1D$ subtype was present in ECs from different vascular beds in animals (Filippi et al. 2001; de Andrade et al. 2006; Mendez et al. 2006) and in human umbilical vein ECs (Vinci et al. 2007). However, $\alpha 1$ -ARs in epicardial coronary artery ECs have never been studied, in animals or humans.

Recently, we showed that the $\alpha 1D$ is the predominant $\alpha 1$ -AR subtype in human epicardial coronary arteries, which have low levels of the $\alpha 1B$ subtype (25% or less of total $\alpha 1$ -ARs) and little or no $\alpha 1A$ (Jensen et al. 2009b). The $\alpha 1D$ subtype is also expressed and functional on human coronary SMCs (Jensen et al. 2009b) and causes constriction of mouse coronary arteries (Tumbull et al. 2003; Chalothorn et al. 2003). In striking contrast with coronary arteries, the human and mouse myocardium and cardiac myocytes express near-equal levels of the $\alpha 1A$ and $\alpha 1B$ subtypes, with little or no $\alpha 1D$ (Jensen et al. 2009a; O'Connell et al. 2003).

Which $\alpha 1$ -subtypes are present on human coronary ECs is potentially very important clinically. Subtype-nonspecific $\alpha 1$ -adrenergic antagonists, such as doxazosin and prazosin, are in widespread clinical use, especially to treat prostate disease in men and hypertension. However, large clinical trials detected adverse cardiac effects of nonspecific $\alpha 1$ -antagonists (ALLHAT CRG 2000; Cohn 1993). We suggested recently that adaptive signaling through the $\alpha 1A$ and $\alpha 1B$ subtypes in myocardium and myocytes might explain these adverse effects in part (Jensen et al. 2009a; O'Connell et al. 2006). Furthermore, inhibiting an $\alpha 1$ -AR subtype in epicardial coronary ECs might disturb endothelial-dependent vasodilation. Thus, the presence of $\alpha 1$ -ARs on epicardial coronary ECs would suggest another possible explanation for cardiac side effects of nonspecific alpha-blockers. Here, we tested the hypothesis that human epicardial coronary ECs express functional $\alpha 1$ -ARs.

Materials and methods

Human epicardial coronary artery EC culture

Clonetics normal human coronary artery ECs were from Lonza (Walkersville, MD, #CC2585, Lots 7F3649, 7F3667, and 7F4280). All cells were cultured in Clonetics EGM-2 medium [including human recombinant epidermal growth factor, fibroblast growth factor, insulin-like growth factor-1, vascular endothelial growth factor, heparin, and 2% fetal bovine serum (FBS)] and were used between passages 3 and 10, at 80–100% confluence.

Primary human coronary artery EC cultures came from heart transplants at the University of California, San Francisco (UCSF), with the approval of the UCSF Committee for Human Research and with full informed consent. The heart was perfused with cold cardioplegia in situ and explanted immediately into ice-cold physiologic solution. Epicardial coronary arteries were dissected, cleaned rapidly of fat, transported in ice-cold physiologic solution, then opened longitudinally and digested 20 min in Hanks buffer with collagenase type II (1 mg/ml, Worthington). The endothelium was removed with gentle scraping; the vessel was washed three times; collagenase was inactivated by EGM-2; and the cellular fraction was centrifuged at 1,200 rpm for 5 min. Cells were plated and grown in EGM-2 as with Lonza ECs and assayed at ~80% confluence.

Human coronary artery SMC culture

Clonetics normal human coronary artery SMCs from Lonza (#CC2583) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS for 3–11 passages and harvested at ~70% confluence.

Quantitative real-time reverse transcription (RT) PCR (qPCR)

Cultured coronary artery ECs from one T75 flask were washed with phosphate buffered saline (PBS), and ribonucleic acid (RNA) was extracted, purified (Qiagen RNeasy Mini Kit), and treated with DNase (Turbo DNase, Ambion). The approach was validated previously (Jensen et al. 2009a).

RT reactions used 1 μ g RNA, SuperScript III Reverse Transcriptase (Invitrogen), random hexamers (Invitrogen), and oligo-dT (Roche). qPCR was done in triplicate in an ABI PRISM 7900HT Sequence Detection System (SDS) 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).

Primers were:

- β -actin forward (F) ATGGCAATGAGCGGTTTC, β -actin reverse (R) GGATGCCACAGGACTCCAT;
- TATA-binding protein (TBP) F GGGCACCCTC CACTGTATC, TBP R CTCATGATTACCGCAG CAAA;
- $\alpha 1A$ F GGCTCCTTCTACCTGCCTCT, $\alpha 1A$ R AGGGCTTGAAATCAGGGGAAG;
- $\alpha 1B$ F CCTGAGGATCCATTCCAAGA, $\alpha 1B$ R GGTTGAGGCAGCTGTTGAAG;
- $\alpha 1D$ F TCTGCTGGTTCCCTTTCTTC, $\alpha 1D$ R CACGCAGCTGTTGAAGTAGC;

- β 3-AR F ATGCCAATTCTGCCTTCAAC, β 3-AR R CTTGGGGAGTTTCTTAGGCC;
- Smooth muscle myosin heavy chain (sm-MHC) F GGAGGCCCTAAAGACAGAGC, sm-MHC R TCTTCAGCACCGTCACCTC; and
- Platelet/endothelial cell adhesion molecule-1 (PECAM-1) F AGGTTCTGAGGGTGAAGGTG, PECAM-1 R ACAGCACATTGCAGCACAA

Relative quantitation of PCR products used the $\Delta\Delta$ CT method, with normalization to two reference genes for improved accuracy, where arbitrary units (AU) were $2^{-\Delta\Delta CT} \times 1,000$ (CT = cycles to threshold, and $\Delta\Delta CT = [(\text{mean target gene CT}) - (\text{mean CT of } \beta\text{-actin} + \text{TBP})]$).

Radioligand binding

ECs or SMCs (12×100 mm dishes or 16×T75 flasks) were rinsed, scraped directly into binding buffer (5 mM Tris-HCl, pH 7.4, 5 mM EDTA), and sonicated. α 1-AR saturation binding used 85–95 μ g cell protein in 1 ml/tube with six concentrations (0.04–1.2 nM) in triplicate of ^3H -prazosin (Perkin-Elmer); phentolamine (10 μ M, Sigma #P7561) defined nonspecific binding. All incubations were 60 min at 30°C. Prism 4.0a (GraphPad Software, San Diego, CA) analyzed binding data.

Pharmacology and immunoblots

For experiments, EC cultures in 35 mm culture dishes (Falcon) were serum-starved for 2 h in DMEM with 5 mg/ml bovine serum albumin (BSA), then were pre-incubated for 15 min without or with α 1-AR-antagonists including BMY-7378 (8-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride, Sigma #B134) or prazosin (Sigma #P7791). The nonselective β -AR antagonist L-propranolol (Sigma #P8688) was also present throughout. Agonists or vehicle (100 μ M ascorbic acid) were then added including L-NE-bitartrate (Sigma #N5785), L-EPI-bitartrate (Sigma #E4375), A61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulfonamide hydrobromide, Tocris #1052, Ellisville, MO), or UK-14,304 [5-bromo-*N*-(2-imidazol-2-yl)-6-quinoxalinamine, 5-bromo-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-6-quinoxalinamine, Sigma #U104].

After agonist treatment, homogenates were made in radioimmunoprecipitation assay (RIPA) buffer with protease and phosphatase inhibitors. Total cell lysates were used in immunoblots with antibodies for total-ERK1/2 (Cell Signaling rabbit pAb #9102), phospho-ERK1/2 (Cell Signaling rabbit mAb Thr202/Tyr204 #4370), total-eNOS (BD Transduction Laboratories #610296), and phospho-

eNOS (Cell Signaling Ser-1177 #9571; BD Transduction Ser-633 #612665). Signals from enhanced chemiluminescence detection were quantified with a Bio-Rad ChemiDoc Molecular Imager (Hercules, CA).

Antibodies for cell phenotyping were PECAM-1 (R&D Systems #BBA7), smooth muscle actin (Zymed #08-0106), and sm-MHC (Biomedical Technologies #BT-562, Stoughton, MA).

^3H -Thymidine incorporation

DNA synthesis was estimated by measuring incorporation of ^3H -thymidine. ECs at 80–90% confluence in 24-well plates in EGM-2 were serum-starved and pre-incubated for 4 h in DMEM with BSA (5 mg/ml, Sigma #A7030) and L-propranolol 1 μ M, without or with prazosin 0.2 μ M. Cells were then incubated in the same medium with ^3H -thymidine, without or with NE or EPI. After 24 h, cells were extracted with 10% trichloroacetic acid for 12 h, washed, dissolved in 1% SDS for 1 h, and counted in mini-vials with 5 ml Opti-fluor (Perkin-Elmer).

Data analysis

Results are mean \pm SEM. Significant differences ($p < 0.05$) were tested using Student's unpaired *t*-test for two groups, and analysis of variance (ANOVA) and Tukey's multiple comparison for more than two groups. Concentration–response curves were fit with Prism 4.0a.

Results

Validation of human epicardial coronary EC cultures

Human coronary ECs from Lonza were from two females (ages 56 and 57) and one male (age 21). These ECs all expressed high levels of PECAM-1 mRNA and no sm-MHC, as expected (data not shown). Our primary cultures of human epicardial coronary ECs were from one male without coronary artery disease (age 46) and were validated by positive immunoblot for PECAM-1 and by negative immunoblot for smooth muscle actin and sm-MHC (data not shown).

α 1B-AR subtype mRNA predominates in human coronary ECs (Fig. 1)

We quantified α 1-AR subtype mRNAs using qPCR with primers validated previously that span the single intron in each α 1-AR subtype gene to eliminate artifacts from genomic DNA contamination (Jensen et al. 2009a). In the Lonza ECs, 92% of total α 1-AR mRNA

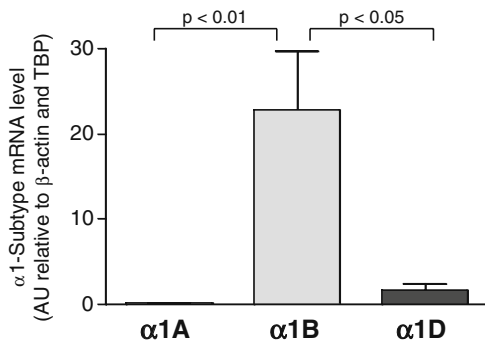
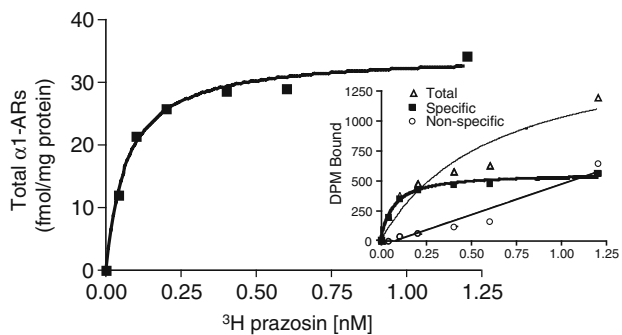
a Quantitative real-time reverse transcription PCR (N=4)**b** Saturation radioligand binding

Fig. 1 $\alpha 1$ -ARs in human epicardial coronary ECs. $\alpha 1$ -AR subtype mRNAs were quantified by qPCR in Lonza cultured ECs ($N=4$, **a**), and total $\alpha 1$ -ARs were measured by saturation radioligand binding (**b**)

was $\alpha 1B$, with the remainder small amounts of $\alpha 1D$, and little or no $\alpha 1A$ ($N=4$ independent cultures from three subjects, Fig. 1a). There was no detectable $\beta 3$ -AR mRNA (data not shown). In our primary human epicardial ECs, 90% of total $\alpha 1$ -AR mRNA was $\alpha 1B$ ($N=2$ cultures from one subject).

$\alpha 1$ -AR binding is higher in ECs than in SMCs (Fig. 1)

To test $\alpha 1$ -AR protein levels, we used saturation radioligand binding with 3H -prazosin. We could not use immunohistochemistry or immunoblot, because there are no antibodies specific for $\alpha 1$ -ARs, among 10 antibodies that we tested (Jensen et al. 2009c), or three antibodies tested by others (Pradidarcheep et al. 2009).

Saturation binding identified 34 fmol/mg protein of total $\alpha 1$ -ARs in coronary ECs, with a K_d 0.07 nM, and specific binding 90% of total at the 3H -prazosin K_d (Fig. 1b). For comparison, in parallel experiments, we found that Lonza human coronary SMCs contained 17 fmol/mg protein of total $\alpha 1$ -ARs. We did not perform competition radioligand binding on ECs given the marked predominance of $\alpha 1B$ mRNA in our qPCR assays.

$\alpha 1B$ subtype activates ERK in coronary artery ECs (Fig. 2)

To test for functional $\alpha 1$ -ARs in human coronary ECs and to determine the $\alpha 1$ -AR subtype at the protein level, we used immunoblot to quantify dual Thr202/Tyr204 phosphorylation (activation) of ERK. ERK is a key target for $\alpha 1$ -ARs in cardiac myocytes (Huang et al. 2007) and is a key element of important signaling pathways in ECs that promote angiogenesis (Chung et al. 2008), and EC migration, survival, and proliferation (Lian et al. 2001;

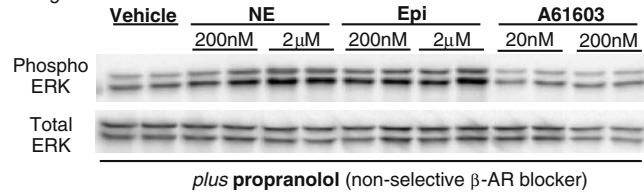
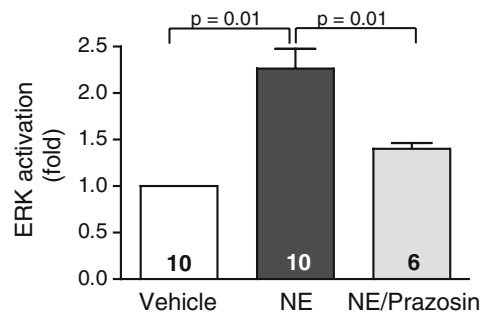
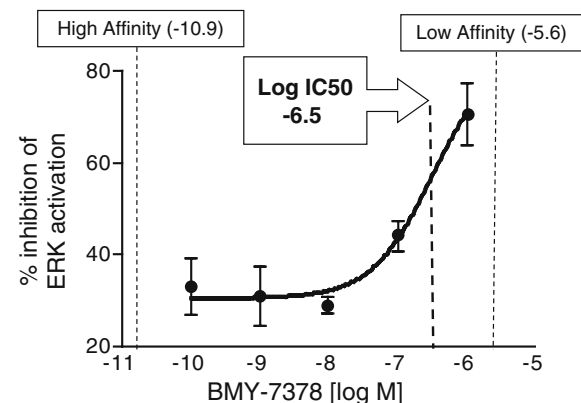
a Agonist doses at 15 min stimulation**b** Summary of NE-mediated ERK activation (all with propranolol)**c** BMY-7378 ($\alpha 1D$ -selective) inhibition of NE-mediated ERK activation

Fig. 2 The $\alpha 1B$ -AR subtype activates ERK in coronary ECs. Cultured ECs were treated for 15 min with the drugs indicated, and total and phosphorylated ERK were quantified by immunoblot. The nonselective β -AR antagonist propranolol (1 μM) was present throughout in all groups. **a** Representative blot with NE, EPI, and the $\alpha 1A$ -selective agonist A61603. **b** ERK activation by NE (mean 1 μM , range 0.2–2 μM) is inhibited by the nonselective $\alpha 1$ -antagonist prazosin (1 μM , $N=6$ –10). **c** The $\alpha 1D$ -selective antagonist, BMY-7378, inhibited NE (1 μM)-stimulated phospho-ERK with a log IC_{50} of -6.5 ($N=5$). This value is much closer to the known low-affinity BMY-7378 site (log IC_{50} -5.6) than the high-affinity site (-10.9)

Mavria et al. 2006; Nakagami et al. 2001; Secchiero et al. 2003).

Human coronary ECs from Lonza were treated 15 min with the non-subtype-selective natural α 1-AR agonists NE and EPI, and the α 1A-selective agonist, A61603, in the presence of propranolol, a β -AR antagonist. NE increased phospho-ERK by 2.2-fold, and the general α 1-AR antagonist prazosin reduced this activation by 70% ($p=0.01$, $N=6$ –10 cultures; Fig. 2a–b). The α 1A agonist A61603 did not activate ERK ($N=3$; Fig. 2a). The subtype-nonselective α 2-AR agonist UK-14,304 (10 μ M) increased phospho-ERK by only 1.11-fold.

These data indicated that an α 1-AR subtype activated ERK in human coronary ECs and that the subtype was not the α 1A, because A61603 was inactive. To determine whether the α 1B and/or α 1D activated ERK, we used an α 1D-selective agent, because there are no reliable α 1B-selective agonists or antagonists. We quantified ERK phosphorylation after stimulation with NE (1 μ M) for 15 min, in the presence of propranolol to block β -ARs, and varying concentrations of BMY-7378 (1 nM–1 μ M), the α 1D-selective antagonist.

Previously, we found that BMY-7378 competed for 3 H-prazosin binding to human epicardial coronary artery α -ARs at two receptor sites, one with high affinity (log IC_{50} -10.9, the α 1D) and the other with low affinity (log IC_{50} -5.6, the α 1A or α 1B; Jensen et al. 2009b). In the present experiments, BMY-7378 inhibited NE-stimulated phospho-ERK with a log IC_{50} -6.5, very close to the low affinity log IC_{50} ($N=5$ cultures; Fig. 2c). These data showed that NE-induced ERK phosphorylation in human coronary ECs is not mediated by the α 1D, because BMY-7378 inhibited with low affinity, and therefore is mediated by the α 1B.

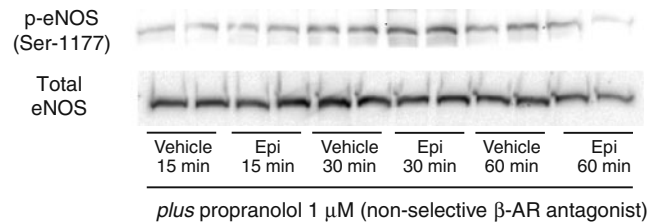
In summary, human coronary artery ECs expressed functional α 1B-ARs that activated the important intracellular mediator ERK in response to stimulation by modest concentrations of adrenergic agonists.

α 1-ARs activate eNOS in ECs (Fig. 3)

eNOS is a key regulator of EC function, including vasorelaxation and angiogenesis (Zembowicz et al. 1991; Murohara et al. 1998), and is activated by α 1-ARs in some systems (Hodges et al. 2005). To test whether α 1-ARs in human epicardial coronary ECs signal through eNOS, we treated with α 1-AR agonists and measured increased phosphorylation of eNOS at two common activation sites (Ser-1177 and Ser-633).

NE (1 μ M) and EPI (2 μ M) in the presence of propranolol each transiently increased phosphorylation of eNOS at Ser-1177, with a maximum at 30 min (Fig. 3a). In three independent cultures, α 1-AR stimulation increased phospho-eNOS (Ser-1177) by 1.85-fold over

a Time course of eNOS phosphorylation



b Summary of EPI-mediated eNOS activation at 30 min

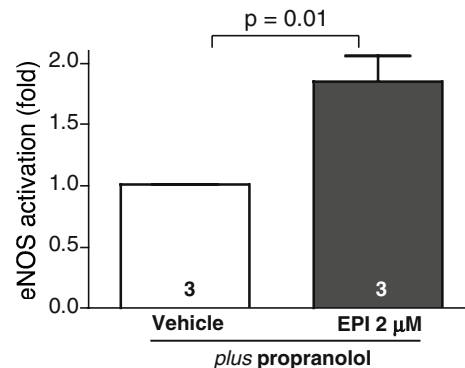


Fig. 3 α 1-AR stimulation activates eNOS in coronary ECs. Cultured ECs were treated for 15–60 min (a) with EPI (2 μ M) in the presence of propranolol (1 μ M), and phosphorylation of eNOS Ser-1177 was assayed by immunoblot. Maximum activation was at 30 min ($N=3$, b)

vehicle ($p=0.01$) but did not change phosphorylation at Ser-633. The α 1A-selective agonist, A61603, did not increase eNOS phosphorylation at either Ser-1177 or Ser-633 (data not shown).

These data indicated that an α 1-AR, most likely the α 1B, activated eNOS at Ser-1177 in human coronary artery ECs. However, we were unable to detect NO production in our cultures using the fluorophore DAF-2 μ M or the Griess reagent.

α 1-ARs increase DNA synthesis in human coronary artery ECs (Fig. 4)

Increased DNA synthesis in ECs is a marker for cellular proliferation and angiogenesis. To test whether stimulation of α 1-ARs on human coronary ECs increases DNA synthesis, we measured 3 H-thymidine incorporation after treatment with varying concentrations of NE (1 nM–20 μ M) in the presence of propranolol. NE increased 3 H-thymidine incorporation by a modest but significant extent (maximum 1.3-fold, $p=0.04$ by ANOVA, $N=3$ –5), with a low EC_{50} (39 nM) (Fig. 4). Prazosin (0.2 μ M) eliminated stimulation by NE (data not shown), indicating that the effect was mediated by α 1-ARs. By comparison, 5% FBS increased 3 H-thymidine incorporation by 2.6-fold.

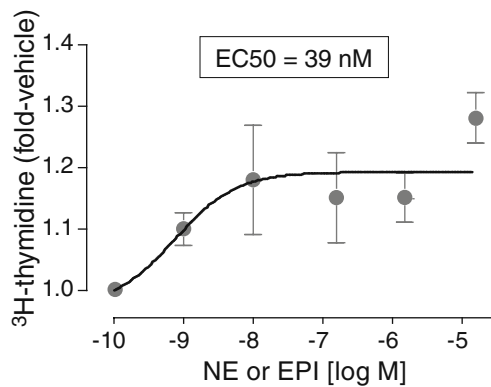


Fig. 4 α 1-AR stimulation increases DNA synthesis in coronary ECs. EC cultures were treated for 24 h with NE or EPI (1 nM–20 μ M) in the presence of propranolol, and DNA synthesis was estimated by 3 H-thymidine incorporation

Discussion

This study provides the first characterization of α 1-ARs in human epicardial coronary ECs and identifies the α 1B as the predominant and functional α 1-AR subtype. The α 1B subtype is present at relatively high density, and stimulation of the α 1B with modest doses of agonist activates ERK and eNOS and increases DNA synthesis. These results raise the possibility that the α 1B-AR subtype might have a role in the important adaptive processes mediated by coronary ECs.

We used qPCR and pharmacology to identify the α 1B as the predominant and functional α 1-subtype, because α 1-AR antibodies are not specific for α 1-ARs or α 1-subtypes (Jensen et al. 2009c; Pradidarcheep et al. 2009). By qPCR with validated primers, human coronary ECs expressed >90% α 1B mRNA, with very little α 1D, and no detectable α 1A. In functional experiments, NE and EPI stimulated phosphorylation of ERK-Thr202/Tyr204 and eNOS-Ser-1177, and the nonselective α 1-antagonist prazosin blocked stimulation by 70%. The potent and selective α 1A agonist A61603 did not activate ERK, and the general α 2-agonist UK-14,304 had a minor effect. To distinguish the α 1B from the α 1D, we used the α 1D-selective antagonist, BMY-7378, because there are no drugs sufficiently selective for the α 1B. BMY-7378 inhibited α 1-stimulated ERK phosphorylation with low affinity, not the high-affinity characteristic of the α 1D. Thus, these data identified the α 1B-subtype as functional in human epicardial coronary ECs.

The high level of the α 1B in ECs points to an important difference in α 1-subtypes in human coronary ECs and SMCs. Recently, we used mRNA and functional assays to show that the α 1D subtype is predominant and functional in human coronary SMCs (Jensen et al. 2009b). The α 1D is also predominant when assayed by qPCR and radioligand binding in intact human epicardial coronary arteries (Jensen

et al. 2009b). However, when binding was done on isolated cells, as in the present study, the density of the α 1B in ECs was twice that of the α 1D in SMCs (34 vs. 17 fmol/mg). Thus, the much larger total mass of SMCs likely explains the α 1D predominance in intact coronaries, rather than a higher density of α 1-ARs on SMCs than on ECs. In fact, it is notable that a minor population of the α 1B is detectable in intact human epicardial coronaries (less than 25%; Jensen et al. 2009b).

As noted in the “Introduction,” α 1-stimulation causes minimal constriction of normal coronary arteries but leads to markedly augmented vasoconstriction in coronary arteries with disrupted endothelium. The α 1D is predominant in coronary SMCs (Jensen et al. 2009b); the α 1D can cause coronary constriction in the α 1A and α 1B double knockout mouse; and coronary constriction is blunted in the single α 1D knockout mouse (Chalothorn et al. 2003; Turnbull et al. 2003). Therefore, it is interesting to speculate whether loss of the coronary EC α 1B might be responsible for α 1-mediated constriction through the α 1D when the endothelium is disrupted, as in atherosclerosis. The coronary EC α 1B might mediate counter-regulatory vasodilation by stimulating eNOS, as shown here. The α 1B might also have a role in EC proliferation and angiogenesis, given the stimulation of 3 H-thymidine uptake found here. More sophisticated studies in knockout mice will be needed to test these hypotheses, because the α 1B and α 1D are very difficult to distinguish by pharmacology, especially in intact animals.

In summary, our present and past results indicate that the α 1-AR subtypes are expressed heterogeneously in the human heart. The α 1B is in human epicardial coronary ECs; the α 1D is in human epicardial coronary SMCs (Jensen et al. 2009b); and the α 1A and α 1B are in human myocardium (Jensen et al. 2009a). Drugs used for prostate disease and hypertension that block all α 1-subtypes nonselectively have adverse cardiac effects in human clinical trials (Cohn 1993; ALLHAT CRG 2000; ALLHAT 2003), and mouse knockouts show that loss of cardiac α 1A and α 1B signaling could account for these adverse effects (O’Connell et al. 2003, 2006). The α 1A subtype has a protective role in cardiac myocytes (Huang et al. 2007; Chan et al. 2008), and the α 1B in epicardial coronary ECs might also have an adaptive role. Potential risks of blocking the α 1A and α 1B might be avoided with α 1D-selective drugs, such as naftopidil, to relax both prostate and coronary smooth muscle (Takei et al. 1999; Nishino et al. 2006; Kojima et al. 2009).

Acknowledgments PCS was supported by the Department of Veterans Affairs, and the National Heart Lung and Blood Institute at the National Institutes of Health, USA. BCJ was supported by the GlaxoSmithKline Research and Education Foundation for Cardiovascular Disease Award to Young Investigators, and the University of California, San Francisco, Foundation for Cardiac Research.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- ALLHAT (2003) Diuretic versus alpha-blocker as first-step antihypertensive therapy: final results from the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). *Hypertension* 42:239–246
- ALLHAT CRG (2000) Major cardiovascular events in hypertensive patients randomized to doxazosin vs chlorthalidone: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). [see comments]. *JAMA* 283:1967–1975
- Baumgart D, Haude M, Gorge G, Liu F, Ge J, Grosse-Eggebrecht C, Erbel R, Heusch G (1999) Augmented alpha-adrenergic constriction of atherosclerotic human coronary arteries. *Circulation* 99:2090–2097
- Chalothorn D, McCune DF, Edelmann SE, Tobita K, Keller BB, Lasley RD, Perez DM, Tanoue A, Tsujimoto G, Post GR, Piascik MT (2003) Differential cardiovascular regulatory activities of the alpha 1b- and alpha 1d-adrenoceptor subtypes. *J Pharmacol Exp Ther* 305:1045–1053
- Chan T, Dash R, Simpson PC (2008) An alpha-1a-adrenergic receptor subtype agonist prevents cardiomyopathy without increasing blood pressure (abstract). *Circulation* 118:S533
- Chung BH, Kim JD, Kim CK, Kim JH, Won MH, Lee HS, Dong MS, Ha KS, Kwon YG, Kim YM (2008) Icaritin stimulates angiogenesis by activating the mek/erk- and pi3k/akt/enos-dependent signal pathways in human endothelial cells. *Biochem Biophys Res Commun* 376:404–408
- Cohn JN (1993) The vasodilator-heart failure trials (V-HeFT). Mechanistic data from the va cooperative studies. Introduction. *Circulation* 87:VI1–VI4
- de Andrade CR, Fukada SY, Olivon VC, de Godoy MA, Haddad R, Eberlin MN, Cunha FQ, de Souza HP, Laurindo FR, de Oliveira AM (2006) Alpha1d-adrenoceptor-induced relaxation on rat carotid artery is impaired during the endothelial dysfunction evoked in the early stages of hyperhomocysteinemia. *Eur J Pharmacol* 543:83–91
- Filippi S, Parenti A, Donnini S, Granger HJ, Fazzini A, Ledda F (2001) Alpha(1d)-adrenoceptors cause endothelium-dependent vasodilatation in the rat mesenteric vascular bed. *J Pharmacol Exp Ther* 296:869–875
- Graham RM, Perez DM, Hwa J, Piascik MT (1996) Alpha 1-adrenergic receptor subtypes. Molecular structure, function, and signaling. *Circ Res* 78:737–749
- Heusch G, Baumgart D, Camici P, Chilian W, Gregorini L, Hess O, Indolfi C, Rimoldi O (2000) Alpha-adrenergic coronary vasoconstriction and myocardial ischemia in humans. *Circulation* 101:689–694
- Hodges RR, Shatos MA, Tarko RS, Vrouvlianis J, Gu J, Dartt DA (2005) Nitric oxide and cgmp mediate alpha1d-adrenergic receptor-stimulated protein secretion and p42/p44 mapk activation in rat lacrimal gland. *Invest Ophthalmol Vis Sci* 46:2781–2789
- Huang Y, Wright CD, Merkwang CL, Baye NL, Liang Q, Simpson PC, O'Connell TD (2007) An alpha1a-adrenergic-extracellular signal-regulated kinase survival signaling pathway in cardiac myocytes. *Circulation* 115:763–772
- Jensen BC, Swigart PM, De Marco T, Hoopes C, Simpson PC (2009a) {alpha}1-adrenergic receptor subtypes in nonfailing and failing human myocardium. *Circ Heart Fail* 2:654–663
- Jensen BC, Swigart PM, Laden ME, DeMarco T, Hoopes C, Simpson PC (2009b) The alpha-1d is the predominant alpha-1-adrenergic receptor subtype in human epicardial coronary arteries. *J Am Coll Cardiol* 54:1137–1145
- Jensen BC, Swigart PM, Simpson PC (2009c) Ten commercial antibodies for alpha-1-adrenergic receptor subtypes are non-specific. *Naunyn-Schmiedebergs Arch Pharmacol* 379:409–412
- Jones CJ, DeFily DV, Patterson JL, Chilian WM (1993) Endothelium-dependent relaxation competes with alpha 1- and alpha 2-adrenergic constriction in the canine epicardial coronary microcirculation. *Circulation* 87:1264–1274
- Kojima Y, Sasaki S, Oda N, Koshimizu TA, Hayashi Y, Kiniwa M, Tsujimoto G, Kohri K (2009) Prostate growth inhibition by subtype-selective alpha(1)-adrenoceptor antagonist naftopidil in benign prostatic hyperplasia. *Prostate* 69:1521–1528
- Lian J, Marcinkiewicz C, Niewiarowski S, Beacham DA (2001) Extracellular signal-regulated kinase (erk) activation is required for gp 1b/alpha-dependent endothelial cell migration. *Thromb Haemost* 86:1555–1562
- Mavria G, Vercoulen Y, Yeo M, Paterson H, Karasarides M, Marais R, Bird D, Marshall CJ (2006) Erk-mapk signaling opposes rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. *Cancer Cell* 9:33–44
- Mendez E, Calzada C, Ocharan E, Sierra A, Castillo C, Ramirez I, Meaney E, Meaney A, Asbun J, Miliar A, Herrera J, Ceballos G (2006) Differential expression of alpha1-adrenergic receptor subtypes in coronary microvascular endothelial cells in culture. *Eur J Pharmacol* 546:127–133
- Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, Kearney M, Chen D, Symes JF, Fishman MC, Huang PL, Isner JM (1998) Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 101:2567–2578
- Nakagami H, Morishita R, Yamamoto K, Taniyama Y, Aoki M, Matsumoto K, Nakamura T, Kaneda Y, Horiuchi M, Ogihara T (2001) Mitogenic and antiapoptotic actions of hepatocyte growth factor through erk, stat3, and akt in endothelial cells. *Hypertension* 37:581–586
- Nishino Y, Masue T, Miwa K, Takahashi Y, Ishihara S, Deguchi T (2006) Comparison of two alpha1-adrenoceptor antagonists, naftopidil and tamsulosin hydrochloride, in the treatment of lower urinary tract symptoms with benign prostatic hyperplasia: a randomized crossover study. *BJU Int* 97:747–751, discussion 751
- O'Connell TD, Ishizaka S, Nakamura A, Swigart PM, Rodrigo MC, Simpson GL, Cotecchia S, Rokosh DG, Grossman W, Foster E, Simpson PC (2003) The alpha1c- and alpha1b-adrenergic receptors are required for physiological cardiac hypertrophy in the double-knockout mouse. *J Clin Invest* 111:1783–1791
- O'Connell TD, Swigart PM, Rodrigo MC, Ishizaka S, Joho S, Tumbull L, Tecott LH, Baker AJ, Foster E, Grossman W, Simpson PC (2006) Alpha1-adrenergic receptors prevent a maladaptive cardiac response to pressure overload. *J Clin Invest* 116:1005–1015
- Pradidarcheep W, Stallen J, Labruyere WT, Dabhoiwala NF, Michel MC, Lamers WH (2009) Lack of specificity of commercially available antisera against muscarinic and adrenergic receptors. *Naunyn-Schmiedebergs Arch Pharmacol* 379:397–402
- Secchiero P, Gonelli A, Carnevale E, Milani D, Pandolfi A, Zella D, Zauli G (2003) Trail promotes the survival and proliferation of primary human vascular endothelial cells by activating the akt and erk pathways. *Circulation* 107:2250–2256
- Takei R, Ikegaki I, Shibata K, Tsujimoto G, Asano T (1999) Naftopidil, a novel alpha1-adrenoceptor antagonist, displays

- selective inhibition of canine prostatic pressure and high affinity binding to cloned human alpha1-adrenoceptors. *Jpn J Pharmacol* 79:447–454
- Turnbull L, McCloskey DT, O'Connell TD, Simpson PC, Baker AJ (2003) Alpha 1-adrenergic receptor responses in alpha 1ab-AR knockout mouse hearts suggest the presence of alpha 1d-ar. *Am J Physiol Heart Circ Physiol* 284:H1104–H1109
- Vinci MC, Bellik L, Filippi S, Ledda F, Parenti A (2007) Trophic effects induced by alpha-1d adrenoceptors on endothelial cells are potentiated by hypoxia. *Am J Physiol Heart Circ Physiol*
- Zembowicz A, Hecker M, Macarthur H, Sessa WC, Vane JR (1991) Nitric oxide and another potent vasodilator are formed from NG-hydroxy-L-arginine by cultured endothelial cells. *Proc Natl Acad Sci USA* 88:11172–11176