JPET Fast Forward. Published on March 20, 2003 as DOI:10.1124/jpet.102.048553

JPET #48553

DIFFERENTIAL CARDIOVASCULAR REGULATORY ACTIVITIES OF THE ALPHA$_{1B}$- AND ALPHA$_{1D}$-ADRENOCEPTOR SUBTYPES

Dan Chalothorn*, Dan F. McCune*, Stephanie E. Edelmann, Kimimasa Tobita, Bradley B. Keller, Robert D. Lasley, Dianne M. Perez, Akito Tanoue, Gozoh Tsujimoto, Ginell R. Post, and Michael T. Piascik

The Department of Molecular and Biomedical Pharmacology, The University of Kentucky College of Medicine, Lexington, Kentucky (DC, DFM, SEE, MTP), The Cardiovascular Development Research Program, Department of Pediatrics, University of Kentucky (KT, BBK), Department of Cardiothoracic Surgery, The University of Kentucky, College of Medicine, Lexington, Kentucky (RDL), The Department of Molecular Cardiology, The Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH (DMP), Department of Molecular, Cell Pharmacology, National Center for Child Health and Development Research Institute, Tokyo, Japan (AK,GT), and The Division of Pharmaceutical Sciences, The University of Kentucky College of Pharmacy, Lexington, Kentucky (GRP).

Running Title: Differential Cardiovascular Regulatory Activities of α_1-ARs

Corresponding Author:

Michael T. Piascik, Ph.D.

Professor

Department of Molecular and Biomedical Pharmacology

The University of Kentucky College of Medicine

800 Rose Street

UKMC MS 305

Lexington, Kentucky 40536-0084

Phone: 859-323-5107

Fax: 859-323-1981

E-mail: mtp@uky.edu

text pages: 21

tables: 2

figures: 7

references: 29

number of words in Abstract: 238

number of words in Introduction: 449

number of words in Discussion: 1444

Abbreviations:

AR, Adrenergic receptor

ERK, Extracellular Signal-Regulated Kinase

GPCR, G-Protein-Coupled Receptor

JNK, c-*Jun* N-terminal Kinase

MAPK, Mitogen-Activated Protein Kinase

ABSTRACT

The regulation of cardiac and vascular function by the α_{1B}- and α_{1D}-adrenoceptors (ARs) has been assessed in two lines of transgenic mice, one over-expressing a constitutively active α_{1B}-AR mutation (α_{1B}-AR_{C128F}) and the other an α_{1D}-AR knockout line. The advantage of using mice expressing a constitutively active α_{1B}-AR is that the receptor is tonically active, thus avoiding the use of non-selective agonists that can activate all subtypes. In hearts from animals expressing the α_{1B}-AR_{C128F}, the activities of the mitogen-activated protein kinases, extracellular signal-regulated kinase and c-Jun N-terminal kinase, were significantly elevated compared to non-transgenic control animals. Mice over-expressing the α_{1B}-AR_{C128F} had echocardiographic evidence of contractile dysfunction and increases in chamber dimensions. In isolated-perfused hearts or left ventricular slices from α_{1B}-AR_{C128F} expressing animals, the ability of isoproterenol to increase contractile force or increase cAMP levels was significantly decreased. In contrast to the prominent effects on the heart, constitutive activation of the α_{1B}-AR had little effect on the ability of phenylephrine to induce vascular smooth muscle contraction in the isolated aorta. The ability of phenylephrine to stimulate coronary vasoconstriction was diminished in α_{1D}-AR knockout mice. In α_{1D}-AR knockout animals, no negative effects on cardiac contractile function were noted. These results show that the α_1-ARs regulate distinctly different physiologic processes. The α_{1B}-AR appears to be involved in the regulation of cardiac growth and contractile function while the α_{1D}-AR is coupled to smooth muscle contraction and the regulation of systemic arterial blood pressure.

G-protein-coupled receptors (GPCR) comprise about 1% of the human genome and perform vital and diverse roles in the regulation of physiologic processes. One of the members of the GPCR family is the α_1-adrenergic receptor (α_1-AR). Three subtypes, the α_{1A}-, α_{1B}-, and α_{1D}-ARs, have been isolated, cloned, and characterized. These receptors are intimately involved in the regulation of peripheral vascular resistance, cardiac function, and vascular and myocardial cell growth (for recent reviews on all aspects of the α_1-ARs see García-Sáinz *et al.,* 1999; Varma and Deng, 2000; Piascik and Perez, 2001).

Data from heterologous expression systems have shown that all three α_1-ARs can couple to a variety of G-proteins and second messenger systems. The α_1-ARs signal through both pertussis toxin sensitive G-proteins (Perez *et al*., 1993) as well as G-proteins of the G_q family (Wu *et al*., 1992). Studies in both transiently and stably transfected cells have demonstrated that all α_1-ARs activate phospholipases C and A_2 (Perez *et al*., 1993; Schwinn *et al*., 1991). In addition to mobilizing intracellular calcium (which would occur subsequent to activation of phospholipase C), the α_1-ARs have also been shown to activate calcium influx via voltage-dependent and -independent calcium channels (Minneman and Esbenshade, 1994; Lazou *et al*., 1994; Sayet *et al*., 1993).

While these studies have increased our understanding of α_1-AR regulatory biology, certain caveats must be established. Data from heterologous expression systems indicate the potential properties and regulatory activities of a given receptor. However, these data do not necessarily confirm that these regulatory events have a correlation in mammalian tissues that natively express these receptors. High density

expression of non-native receptors into cells could promote promiscuous coupling to pathways that may not normally be involved in *in vivo* receptor function.

Progress on the integrated regulatory activities of the α_1-ARs has been slowed by the availability of selective agonists and antagonists for these receptors. This is especially true for the α_{1B}-AR. In this report we have taken advantage of a unique line of transgenic mice systemically over-expressing a constitutively active α_{1B}-AR (see Zuscik *et al*., 2000, 2001), to examine the cardiovascular regulatory activities of the α_{1B}-AR. A constitutively active receptor is tonically active, thus eliminating the need for agonists that non-selectively activate all α_1-ARs. We have also examined regulatory activities in an α_{1D}-AR knockout line of mice (see Tanoue *et al*., 2002). Transgenic mouse models also have inherent shortcomings (see Discussion). Nonetheless, we can still use these models to propose and test hypotheses. In this communication, we test the hypothesis that the α_{1B}- and α_{1D}-ARs perform distinctly different regulatory activities. We postulate that the α_{1B}-AR is involved in the regulation of cardiac function and that the α_{1D}-AR is responsible for regulating systemic arterial blood pressure.

MATERIALS AND METHODS

ANIMAL USE AND CARE

All animal protocols were reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee. Tissues from two transgenic mouse lines were used in all aspects of this work. In one line, mice over-expressed a constitutively active mutation of the α_{1B}-AR, α_{1B}-AR_{C128F}. The over-expression of the constitutively active α_{1B}-AR was driven by the endogenous promoter, and the initial characterization of this mouse line has been described (Zuscik *et al.,* 2000, 2001). The other mouse line was a recently described α_{1D}-AR knockout (Tanoue *et al.,* 2002).

ASSESSMENT OF MAP KINASE ACTIVITY

Tissue Preparation: Transgenic mouse hearts were removed, quick frozen, and stored in liquid nitrogen. The frozen tissue was homogenized (Dremel, Racine, WI) and incubated on ice for 1 hr in 400 μl of the lysis buffer (20 mM Tris-HCl, 250 mM NaCl, 2.5 mM EDTA, 3 mM EGTA, 20 mM β-glycerophosphate, 0.5% NP-40, 100 μM Na_3VO_4, 5 μM AEBSF, 1.5 nM aprotinin, 10 nM E-64, 10 nM leupeptin, pH 7.4). Following the 1 hr incubation, the lysate was centrifuged for 15 min at 15,000 g at 4°C. The total protein content in the supernatant was determined by Lowry assay.

Assay of Extracellular Signal-Regulated Kinase Activity: Extracellular signal-regulated kinase (ERK) activity was determined using an in-gel kinase assay. Equal amounts of protein were resolved on 10% SDS-polyacrylamide gels containing 0.5 mg/ml myelin basic protein (MBP) substrate that is polymerized together with

acryalamide thereby immobilizing it in the gel. Activated ERK kinase (Calbiochem) was used as a positive control. After electrophoresis, gels were washed with 20% 2-propanol in 50 mM HEPES, pH 7.6 and then with 5 mM β-mercaptoethanol in HEPES buffer. Proteins were denatured by washing the gels in 6 M Urea and then renatured with an overnight incubation in HEPES buffer containing 0.05% (v/v) Tween-20 (renaturation buffer) at 4°C. Following incubation in renaturation buffer, gels were pre-incubated in 25 ml cold kinase buffer (20 mM HEPES, 20 mM $MgCl_2$, 2 mM DTT, 5 mM β-glycerophosphate, 0.1 mM Na_3VO_4) for 30 min. Phosphorylation of MBP was performed *in situ* by incubating the gels in kinase buffer containing 20 μM ATP and 150-160 μCi [γ^{32}P]ATP for 90-120 min at 30°C. Gels were washed extensively in 5% trichloroacetic acid/1% sodium pyrophosphate to remove unbound ATP, dried and exposed to a phosphor screen. Incorporation of [^{32}P] into MBP was quantified with a phosphoimager (Molecular Dynamics), using ImageQuant software. Enzyme activity from each sample was normalized to the total amount of ERK present. This value was determined from immunoblotting as described below. Activity is reported as integrated optical density units, and is normalized to a percentage of enzyme activity detected in untreated tissues.

Assay for c-Jun N-Terminal Kinase Activity: c-Jun N-terminal kinase (JNK) activity was determined using an in-gel kinase assay as described above. In this case, protein was resolved on 10% SDS-polyacrylamide gels containing 0.1 mg/ml GST-cJun(1-135). Anisomycin is a known activator of the stress activated MAPKs; therefore, C6 Anisomycin extracts (Cell Signaling, Beverly, MA) were used as a positive control.

Immunoblotting: Equal amounts of protein samples were resolved on 10% SDS-polyacrylamide gels and transferred to PVDF (polyvinylidene fluoride) membranes (Biorad). The amount of total ERK was detected by immunoblotting using a 1:1,000 dilution of Goat (c-16) anti-ERK polyclonal IgG (Santa Cruz Biotech, Santa Cruz, CA) with horseradish peroxidase conjugated anti-goat IgG at 1:10,000 (Jackson ImmunoResearch Labs, West Grove, PA). The total JNK was detected by immunoblotting using a 1:1,000 dilution of Rabbit (c-17) anti-JNK1 polyclonal IgG (Santa Cruz Biotech, Santa Cruz, CA) with horseradish peroxidase conjugated donkey anti-rabbit IgG at 1:2,000 (Amersham, Buckinghamshire, U.K.). Following exposure of the membranes to ECL + reagent (Amersham, Buckinghamshire, U.K.), the chemiluminescent signal was detected with a phosphoimager (Molecular Dynamics). Quantitation was performed using ImageQuant software.

EXPERIMENTS IN THE ISOLATED-PERFUSED HEART

The Isolated-Perfused Heart Preparation: Mice were heparinized (200 U) and anesthetized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg). The chest cavity was opened and the heart was quickly excised and submersed in ice-cold saline. The aorta was dissected and the ascending aortic stump was cannulated with a 22-guage plastic cannula primed with ice cold modified-Krebs-Hensleit buffer (KHB) (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 11 mM Dextrose, 1.5 mM $CaCl_2$, and 1 mM pyruvate). The aorta was sutured into position and the cannula placed on a perfusion apparatus (Radnoti, Monrovia, CA). Retrograde (Langendorff) perfusion was immediately performed with oxygenated (95% O_2 and 5%

CO_2) modified KHB at 37.5°C. The hearts were allowed to beat spontaneously. The perfusion pressure was monitored with a pressure transducer (COBE, Lakewood, CO) connected to a Grass polygraph (Grass Instruments, Quincy, MA), and the coronary perfusion pressure was maintained at 75 mm Hg by adjusting the flow of the perfusion pump that was calibrated before each experiment by measuring volume perfused per min (Control Company, Friendswood, TX). A fluid-filled balloon catheter was inserted into the left ventricle, and the balloon was filled to attain a diastolic pressure of 5-10 mm Hg. The balloon catheter line was connected to a second pressure transducer and an amplifier module designated to measure the developed pressure, which was linked to a differentiator. The parameters measured were heart rate, left ventricular (LV) systolic and end diastolic pressure, and the rise and the fall in LV developed pressure as a function of time (+*dP/dt* and -*dP/dt*, respectively).

Drug-Induced Increases in Inotropy: In both lines of transgenic animals and their respective controls, hearts were perfused at a constant pressure of 75 mm Hg to assess the effects of α_1-AR modulation on β-AR-induced positive inotropy. Following a 25 min equilibration period, an isoproterenol dose-response curve was generated by infusing a stock solution of 100 nM at increasing rates (.037 to 2.9 ml/min) into the aortic cannula. Measurements of coronary flow, heart rate, and ventricular function were collected at baseline (0 min) and 1 min after drug administration.

Drug-Induced Coronary Vasoconstriction: The effects of phenylephrine on coronary perfusion pressure were determined in the myocardium. Once a perfusion pressure of 80-85 mm Hg was reached, experiments were performed at a constant flow. The

protocol was conducted in the presence of 100 nM of propranolol to limit the effect of β-AR stimulation on coronary perfusion pressure. Following a 25 min equilibration, a stock solution of 1 mM phenylephrine was infused via an infusion pump to attain a final concentration of 100 μM. The effect of phenylephrine on coronary pressure was recorded, and constriction was assessed by determining the relative change in the coronary perfusion pressures from baseline at specified time points following phenylephrine infusion.

ECHOCARDIOGRAPHY

Echocardiographic studies were performed on mice of 5-6 months of age (12 with the α_{1B}-AR_{C128F} and 11 NTs). Before determination of body weight, the mouse was anesthetized with 1.25% isoflurane, and the animal was placed on a custom-designed heated water-filled glass chamber that maintained an euthermic body temperature of 37°C. The thorax hair was shaved and warm ultrasonic coupling jelly was applied to cover the thorax. Transthoracic echocardiography was performed using the Acuson Sequoia C256 system with a 13 MHz linear ultrasonic transducer (15L8, Acuson, Mountain View, CA, USA) in a phased array format. This system offers 0.35 mm lateral resolution and 0.25 mm axial resolution and is capable of acquiring and storing real-time digital images simultaneously. M-mode measurements on the LV short axis view (papillary muscle level) was performed (see Gardin *et al*., 1995). The M-mode tracings were used to measure the end-diastolic and end-systolic LV internal chamber dimensions (LVID) as well as the posterior wall thickness (PWT). The maximum end-diastolic (ED) LV internal chamber dimensions (LVIDd) and PWTd were measured

when the LV chamber cavity reached end-diastole, and the LV end-systolic (ES) internal chamber dimensions (LVIDs) were measured at the time corresponding to maximum motion of the LV posterior wall. The cycle length (CL) and ejection time (ET) were measured from aortic flow waveforms. The LV fractional shortening (%FS), LV mass, and the heart rate corrected mean velocity of circumferential fiber shortening (mVcfc) were estimated as follows: $\%FS = [(LVIDd-LVIDs)/LVIDd]\cdot 100$; $LV\ mass = 1.055[(LVIDd + 2\cdot PWTd)^3 - LVIDd^3]$; and $mVcfc = [(LVIDd-LVIDs)/LVIDd]/(ET\cdot CL^{.5})$. The LV mass was calculated by using the uncorrected cube assumption (Pombo *et al*., 1971) without the use of the interventricular septal wall thickness since it was difficult to detect the endocardial border between the right ventricular cavity and the interventricular septum. Three beats were averaged for each measurement. The stroke volume (SV) was calculated from the dimensions as follows: SV = (ED volume - ES volume) and cardiac output (CO) was calculated from $SV\cdot HR$.

ASSESSMENT OF AORTIC CONTRACTILE FUNCTION

Isolated blood vessels were prepared by techniques routinely used in our laboratory (Piascik *et al.*, 1994, 1995, 1997). Briefly, aortic segments were removed from transgenic mice and placed in cold physiologic salt solution (PSS). Stainless steel or platinum wires were threaded through the lumen of each vessel. One wire was connected to a fixed base and the other to a micrometer clamp to adjust the passive force on the tissue. The tissues were mounted in water-jacketed muscle baths filled with PSS maintained at 37°C under constant oxygenation (95% O_2, 5% CO_2; pH 7.4). A passive force of 1.0 g was placed on the aorta. Previous studies have shown that

this passive force gives optimal agonist responses. Changes in the force generation were recorded using Grass FT .03 force transducers connected to a Grass model 7 polygraph. The muscle rings were equilibrated in oxygenated PSS and then challenged with KCl at 80 mM for 1 min. The muscles were then washed with oxygenated PSS every 15 min until the contraction returned to baseline. Arterial segments were exposed to phenylephrine and the contractile effects were recorded. Contractile responses to phenylephrine were also measured following a 20 min incubation with 30 nM BMY 7378, a selective α_{1D}-AR antagonist. The equilibrium dissociation constant for BMY 7378 was calculated as described by Besse and Furchgott (1976).

CYCLIC AMP ASSAY IN THE MOUSE MYOCARDIUM

Tissue Preparation and Treatment: Mouse hearts were quickly removed and cleaned in non-supplemented DMEM. The ventricles were sliced and placed in a fresh non-supplemented DMEM with 100 μM 3-isobutyl-1-methylxanthine (IBMX, Sigma Chemicals, St. Louis, MO) in a 37°C incubator with a 5% CO_2 atmosphere. At the appropriate time, the tissue was treated with vehicle, isoproterenol alone or isoproterenol in the presence of propranolol. Forskolin was used as a positive control. Following drug treatment, the slices were quick frozen in liquid nitrogen and stored at -80°C. The tissue samples were powdered and incubated in 250 μl of lysis solution (0.1 M HCl) for 1 hr on ice. The lysate was centrifuged for 5 sec at 11,750 g. The supernatant was collected for the determination of cAMP levels and total protein content (determined by Lowry assay).

Assaying for cAMP levels: After the total protein content was adjusted to 100 μg/ml with 0.1 M HCl, the lysate was assayed for cAMP levels (non-acetylated) using a commercial enzyme immunoassay cAMP assay kit (BioMol, Plymouth Meeting, PA). Samples were performed in duplicates. The optical densities of the samples were read at 405 nm. The quality control parameters and the mean and the standard errors of the mean are listed below for four curves: Total activity (maximum colormetric enzymatic reaction with substrate) added = 11.02 ± 0.35 Optical Density; % Non-specific binding = 0.0008 ± 0.0003 %; % Maximum Binding/Total Activity = 2.92 ± 0.07 %. From cAMP standards, the curves for calculating cAMP concentrations of the unknowns had a 20 % Intercept = 35.00 ± 5.85 pmol/ml, 50 % Intercept = 7.65 ± 0.59 pmol/ml, and 80 % Intercept = 1.60 ± 0.28 pmol/ml. The line obtained had a slope of -32.85 ± 1.54 with a correlation coefficient of 0.942 ± 0.012.

STATISTICAL ANALYSIS

In all figures, the data are expressed as the mean and standard error of the mean (S.E.). When appropriate, statistical significance as assessed with either the unpaired two-tailed Student's *t* test or the two-way analysis of variance (ANOVA) followed by Student-Newman-Keuls analysis. A value of $P<0.05$ was considered statistically significant.

RESULTS

EXPERIMENTS IN MICE OVER-EXPRESSING THE α_{1B}-AR_{C128F}

Activation of Mouse Myocardial MAPKs

The hearts from α_{1B}-AR_{C128F} mice exhibited significantly elevated levels of ERK and JNK activity when compared to the non-transgenic controls (see Figure 1A and B). These results support the idea that the over-expressed α_{1B}-AR_{C128F} is functional and can couple to signaling pathways in the absence of agonists (however, see Discussion). ERK activity was not altered in hearts from α_{1D}-AR knockout mice (data not shown).

Echocardiographic Analysis

Activation of MAPKs has been proposed to link the α_1-ARs to growth responses. Echocardiography was performed as a non-invasive method of assessing the effect(s) of constitutive activation of the α_{1B}-AR on left ventricular (LV) dimensions and cardiac function (Table 1). The LV dimensions were normalized to the body weight. The transgenic animals showed significantly increased LV internal dimensions during either diastole or systole (Table 1) as well as an increase in chamber diameters. Chamber diameters were increased in the transgenic animals without a change in the wall thickness (this is indicated by no change in the posterior wall thickness in either diastole or systole in Table 1). The LV dimensional analysis reveals that there is a significant reduction in the percent fractional shortening in mice over-expressing the α_{1B}-AR_{C128F} when compared to the non-transgenic controls. The fractional shortening value, an index of contractile function, indicates poor cardiac performance in the transgenic line.

The ejection time, heart rate, and mean velocity for circumferential fiber shortening corrected for heart rate were reduced in the animals with the α_{1B}-AR_{C128F} mutation. However these reductions were not statistically significant. Neither the stroke volume nor the cardiac output was found to be statistically different between groups. Therefore persistent, unregulated activation of the α_{1B}-AR results in a decrease in contractile function and chamber dilation.

Responses in the Isolated-Perfused Heart

To more completely assess the effect of constitutive activation of the α_{1B}-AR on contractile responses, experiments were performed in the isolated-perfused heart. Resting heart rates were 348 $\pm$ 18 bpm and 384 $\pm$ 12 bpm in control and transgenic mouse hearts, respectively. This difference was not statistically significant and is consistent with the echocardiographic analysis of heart rate. We did not observe any significant change in basal coronary flow rate in these hearts (data not shown). Isoproterenol infusion produced similar increases in heart rate in both groups (Figure 2A). The ability of isoproterenol (30 and 100 nM) to increase contractile force was significantly decreased in hearts from mice over-expressing the α_{1B}-AR_{C128F} mutation (LVDP and +dP/dt in Figures 2B & C). The -dP/dt curves were not significantly different (Figure 2D).

cAMP Production

The blunted isoproterenol-induced response prompted additional experiments to determine if there were changes in the β_1-AR signaling pathway that resulted from α_{1B}-

AR over-activity. We therefore assessed the ability of isoproterenol to increase cAMP levels in ventricular slices from control and transgenic animals. The positive control, sodium forskolin, produced similar increases in cAMP in both groups (Figure 3). In control ventricular slices, isoproterenol (1 and 10 μM) produced an increase in cAMP levels that was antagonized by 0.1 μM propranolol. In ventricular segments from α_{1B}-AR_{C128F} mice the cAMP response to either 1 or 10 μM isoproterenol was reduced. This difference was statistically significant at a concentration of 10 μM.

Contractile Responses in the Mouse Aorta

In the aortae from non-transgenic control mice, phenylephrine produced concentration-dependent increases in developed tension (Figure 4A). The dose-response curve was shifted to the right by 30 nM of the α_{1D}-AR selective antagonist BMY 7378. From these data we calculated the equilibrium dissociation constant for BMY to be 0.294 ± 0.149 nM. This value is in good agreement with that obtained from experiments with cloned α_{1D}-AR as well as the receptor expressed on rat blood vessels (2 nM, Piascik *et al*., 1995), indicating that, like the rat aorta, the phenylephrine contractile response in the mouse aorta is mediated by the α_{1D}-AR. Over-expression of a constitutively active α_{1B}-AR did not enhance the response of the mouse aortae to phenylephrine (see Figure 4B). BMY 7378 was also a potent antagonist in the aorta from α_{1B}-AR_{C128F} expressing mice with an estimated equilibrium dissociation constant of 0.385 ± 0.401 nM (see Table 2), indicating that the α_{1D}-AR still mediates contraction in this blood vessel. These data show that despite over-expression of a constitutively

active and signaling competent form of the α_{1B}-AR, the response of the aorta is unaffected and remains under the control of the α_{1D}-AR. Consistent with this lack of effect on vascular smooth muscle contraction, we did not observe any effect on the ability of phenylephrine to induce coronary vasconstriction in hearts from mice expressing the constitutively active α_{1B}-AR (data not shown).

EXPERIMENTS IN α_{1D}-AR KNOCKOUT MICE

Responses in the Isolated-Perfused Heart

The effects of α_{1D}-AR knockout on β-AR-induced responses were assessed in the isolated-perfused heart preparation. The ability of isoproterenol to induce positive chronotropy or inotropy was not significantly different between the control and the mice lacking the α_{1D}-AR (Figure 5A and B). (+) or (-) dP/dt curves were also not different in hearts from α_{1D}-AR deficient mice (Figure 5C and D). Echocardiographic analysis also showed no differences in cardiac dimensions or cardiac function in α_{1D}-AR knockout mice (data not shown).

Effects on Coronary Perfusion Pressure

In contrast to having little effect on cardiac contractile responses, knockout of the α_{1D}-AR has prominent effects on coronary vascular responses. The basal coronary flow rate required to maintain the coronary perfusion pressure was found to be significantly greater in α_{1D}-AR knockout animals when compared to non-transgenic controls (Figure 6). In hearts from control mice, 100 uM phenylephrine infusion caused a significant

increase in coronary perfusion pressure (Figure 7). Phenylephrine-induced increases in perfusion pressure were significantly reduced in hearts from α_{1D}-AR knockout mice. Prominent effects on vascular function were also noted by Tanoue *et al.,* (2002) in α_{1D}-AR knockout animals. These workers noted that the response of the aorta to phenylephrine was significantly impaired in knockout animals (Tanoue *et al.,* 2002).

Discussion

While it is clear that the α_1-AR family plays a prominent role in the regulation of cardiac and vascular function, the specific function of each subtype has been difficult to discern. Despite the fact that many tissues express multiple α_1-AR, we do not believe that there is redundancy in the regulatory activities of these receptors. Rather, we hypothesize that each subtype is coupled to distinct regulatory processes. We propose that the α_{1B}-AR plays a role in the modulation of cardiac function while the α_{1D}-AR is a specific regulator of vascular contractile function

These hypotheses were tested using two newly developed lines of transgenic mice. While transgenic models offer a unique and powerful approach to receptor research, they are not without shortcomings. The assumption is that the observed biochemical or physiologic alterations are a direct result of transgenic receptor expression or deletion. However, we must concede that any effects we observe could also be non-specific and occur as result of interference in the expression of vital signaling molecules unrelated to the α_1-ARs whose expression were altered.

To examine the regulatory activity of the α_{1B}-AR we chose a transgenic line of mice over-expressing a constitutively active mutant of this α_{1B}-AR. An α_{1B}-AR knockout line of mice is also available (Cavalli *et al.,* 1997). Studying these knockout animals would essentially be a loss of function protocol. However, by studying constitutively active receptors we are able to use the gain of function as a read out of receptor activity. The use of constitutively active receptors offers another advantage in studying

receptor systems like the α_{1B}-AR for which there are no selective agonists. Without such selective ligands, wild-type receptor activation can only be achieved by administering non-selective agonists such as phenylephrine that would activate all α_1-AR subtypes. Because constitutively active receptors engage signaling pathways in the absence of agonists, we can observe the results of α_{1B}-AR activation without the need to administer agonist compounds.

In previous work we showed that in the absence of agonist, the α_{1B}-AR_{C128F} can couple to inositol phosphate formation (Zuscik *et al.,* 2001). In this work we show that there is an increase in the activity of MAPKs (see Figure 1) in α_{1B}-AR_{C128F} animals. This would imply that this receptor is indeed constitutively coupled to signaling pathways. Coupling of the α_{1B}-AR to MAPKs. would be in agreement with a great deal of data from non-transgenic sources (see reviews of García-Sáinz *et al.,* 1999; Varma and Deng, 2000; Piascik and Perez, 2001). However, considering the uncertainties of experiments with transgenic animals we cannot be completely sanguine that the observed increases in kinase activity are a direct result of receptor expression as opposed to being non-specific and secondary to other pathophysiologic alterations in cardiac function.

Echocardiographic analysis of mice over-expressing the α_{1B}-AR_{C128F} revealed a statistically significant reduction in fractional shortening when compared to non-transgenic controls (Table 1). A decrease in fractional shortening is evidence for contractile dysfunction in these animals.

Further evidence that over-expression of the α_{1B}-AR_{C128F} interferes with

myocardial contractility was obtained in the isolated-perfused heart where we observed that the ability of isoproterenol to increase contractile force was significantly reduced in hearts from transgenic animals (see Figures 2B and 2C). We also noted an impaired ability of isoproterenol to promote increases in cAMP levels (see Figure 3) in homogenates from transgenic hearts. This indicates the possibility that tonic unregulated activation of the α_{1B}-AR impairs β_1- AR signaling and could be the underlying reason for the decrease in contractile function.

Activation of members of the α_1-AR subtype family has been associated with increases in myocardial contraction (see Varma and Deng, 2000 and references therein). This present work and that of others (Akhter *et al*., 1997; Lemire *et al*., 2001) shows that the α_{1B}-AR is not the subtype coupled to this positive inotropic effect. In other work with the α_{1B}-AR_{C128F} over-expressing mice, we have shown that it is the α_{1A}-AR that mediates the positive inotropic actions of phenylephrine (Ross *et al.,* 2003 in revision). We have further shown that constitutive activation of the α_{1B}-AR decreases the ability of the α_{1A}-AR to activate myocardial contraction (Ross *et al.,* 2003 in revision) as well as decreasing α_{1A}-AR mRNA levels. Taking into consideration the caveats raised above regarding the use of transgenic models, our data can also be used to argue that tonic unregulated activation of the α_{1B}-AR diminishes cardiac contractile activity by decreasing the positive inotropic signaling emanating not only from the β_1-AR but the α_{1A}-AR as well.

In addition to contractile dysfunction, echocardiographic analysis also revealed

increases in the left ventricular internal dimensions of the α_{1B}-AR_{C128F} heart. This is evidence of an increase in chamber size. This phenotype of contractile dysfunction and increased chamber dimensions has also been seen in a distinctly different mouse model that uses cardiac targeting to over-express the wild-type α_{1B}-AR (Grupp *et al*., 1998; Lemire *et al*., 2001). In contrast to these results, other reports with a cardiac-targeted constitutively active α_{1B}-AR (Milano *et al*., 1994) or our systemic over-expression model provide evidence of contractile dysfunction and cardiac hypertrophy. It is not clear as to why studies in the same mouse models reveal differences in cardiac phenotype. What is clear is that tonic unregulated activation of the α_{1B}-AR has significant and negative effects on cardiac function that can progress into hypertrophy or dilated cardiomyopathy. Factors that determine how biosignals emanating from the α_{1B}-AR lead to these pathophysiologies are being investigated.

Consistent with published works (see Piascik and Perez, 2001; García-Sáinz *et al*., 1999 and references therein) we propose that the α_{1B}-AR has minimal activity as a regulator of vascular function. Previously, we showed that over-expression of the α_{1B}-AR_{C128F} does not increase resting systemic arterial blood pressure (Zuscik *et al.,* 2001). Knockout of the α_{1B}-AR also had no effect on resting blood pressure (Cavalli *et al.,* 1997). Herein we show that over-expression of the α_{1B}-AR_{C128F} does not alter the response characteristics in the isolated aorta. Therefore in the same mouse line where over-expression of a constitutively active α_{1B}-AR has demonstrable effects on cardiac function, we are unable to detect any increases in systemic arterial blood pressure or

contractility in the aorta. If over-expression of the constitutively active α_{1B}-AR produced non-specific effects on cardiovascular function then it would be reasonable to suppose that vascular function would also be impaired. These data support our hypothesis that there is specificity in coupling amongst the α_1-AR subtype family and that the α_{1B}-AR is coupled to regulatory events in the heart without participating in the contraction of vascular smooth muscle.

The α_{1D}-AR is an enigmatic and the least well-studied member of the α_1-AR subtype family. In previous work, it has been shown that this receptor is expressed mainly in intracellular compartments (McCune *et al.,* 2000; Chalothorn *et al.,* 2002). We do not yet know the reason for this atypical localization pattern or if the regulatory activities of the α_{1D}-AR are accomplished by these intracellular receptors. Recently, it has been shown that the α_{1D}-AR is constitutively active (García-Sáinz and Torres-Padilla, 1999; Gisbert *et al*., 2000; McCune *et al.,* 2000). D'Ocon's group has shown that the constitutively active α_{1D}-ARs are capable of mediating vascular smooth muscle contraction. This constitutive activation could account for the intracellular expression. Other studies have demonstrated that the α_{1D}-AR is expressed throughout the cardiovascular system (Rudner *et al*., 1999; Hrometz *et al*., 1999). This includes being expressed on vascular beds such as the renal artery where the α_{1D}-AR has not been shown to have a function (see Piascik and Perez, 2001). We do not yet understand why members of the α_1-AR family are expressed on tissues in the cardiovascular system and do not participate in regulatory events. However, in keeping with this

conundrum, we observed little effect of α_{1D}-AR gene detection on dimensions or contractility as assessed echocardiographically or in the isolated-perfused heart (see also Tanoue *et al.,* 2002).

We hypothesize that the major regulatory activity of the α_{1D}-AR is the regulation of vascular smooth muscle contraction in specific blood vessels (Piascik and Perez, 2001). Evidence supporting this postulate also comes from work with the α_{1D}-AR knockout line of mice (Tanoue *et al.,* 2002). Tanoue *et al.* (2002) showed that knockout of the α_{1D}-AR significantly decreased systemic arterial blood pressure as well as the pressor responses to norepinephrine and responses in the isolated aorta. In this present work we show that knockout of the α_{1D}-AR significantly impaired the ability of phenylephrine to promote increases in coronary perfusion pressure. Therefore, in the same mouse line, where we can demonstrate prominent effects on vascular function, we do not see measurable effects on the examined cardiac parameters. This adds support to our hypothesis that the α_{1D}-AR serves predominantly in vascular function.

Acknowledgements

We thank Joseph Tinney (University of Kentucky) for his assistance in the preparation of the animals for the echocardiographic study, and Robert Papay (Cleveland Clinic Foundation) for his help in establishing the α_{1B}-AR_{C128F} transgenic line at the University of Kentucky.

References

Akhter, S.A., Milano, C.A., Shotwell, K.F., Cho, M.C., Rockman, Lefkowitz, R.J., and Koch, W.J. (1997) Transgenic mice with cardiac overexpression of the alpha1B-adrenergic receptors. In vivo alpha1-adrenergic receptor-mediated regulation of beta-adrenergic signaling. *J. Biol. Chem.* **272**: 21253-21259.

Besse, J.C. and Furchgott, R.F. (1976) Dissociation constants and relative efficacies of agonists acting on alpha adrenergic receptors in rabbit aorta. *J. Pharmacol. Exp. Ther.* **197**: 66-78.

Cavalli, A., Lattion, A.L., Hummler, E., Nenniger, M., Pedrazzini, T., Aubert, J.F., Michel, M.C., Yang, M., Lembo, G., Vecchione, C., Mostardini, M., Schmidt, A., Beermann, F., and Cotecchia, S. (1997) Decreased blood pressure response in mice deficient of the α_{1b}-adrenergic receptor. *Proc. Natl. Acad. Sci. USA* **94**: 11589-11594.

Chalothorn, D., McCune, D.F., Edelmann, S.E., García-Cazarín, M.L., Tsujimoto, G., and Piascik, M.T. (2002) Differences in the Cellular Localization and Agonist-Mediated Internalization Properties of the α_1-Adrenoceptor Subtypes. *Mol. Pharmacol.* **61**: 1008-1016.

García-Sáinz, J.A. and Torres-Padilla, M.E. (1999) Modulation of basal intracellular calcium by inverse agonists and phorbol myristate acetate in rat-1 fibroblasts stably

expressing alpha1d-adrenoceptors. *FEBS Lett*. **443**: 277-281.

García-Sáinz, J.A., Vázquez-Prado, J., and Villalobos-Molina, R. (1999) Alpha 1-adrenoceptors: subtypes, signaling, and roles in health and disease (Review). *Arch. Med. Res.* **30**: 449-58.

Gardin, J.M., Siri, F.M., Kitsis, R.N., Edwards, J.G., and Leinwand, L.A. (1995) Echocardiographic Assessment of Left Ventricular Mass and Systolic Function in Mice. *Circ. Res.* **76**: 907-914.

Gisbert, R., Noguera, M.A., Ivorra, M.D., and D'Ocon, P. (2000) Functional evidence of a constitutively active population of alpha(1D)-adrenoceptors in rat aorta. *J. Pharmacol. Exp. Ther.* **295**: 810-817.

Grupp, I.L., Lorenz, J.N., Walsh, R.A., Boivin, G.P., and Rindt, H. (1998) Overexpression of α_{1B}-adrenergic receptor induces left ventricular dysfunction in the absence of hypertrophy. *Am. J. Physiol.* **275** (*Heart Circ. Physiol*. **44**): H1338-H1350.

Hrometz, S.L., Edelmann, S.E., McCune, D.F., Olges, J.R., Hadley, R.W., Perez, D.M., and Piascik, M.T. (1999) Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: correlation with the regulation of contraction. *J. Pharmacol. Exp. Ther*. **290**: 4524-63.

Lazou, A., Bogoyevitch, M.A., Clerk, A., Fuller, S.J., Marshall, C. J., and Sugden, P.H. (1994) Regulation of mitogen-activated protein kinase cascade in adult rat heart preparations in vitro. *Circ. Res.* **75**: 932-941.

Lemire, I., Ducharme, A., Tardif, J.C., Poulin, F., Jones, L.R., Allen, B.G., Hébert, T.E., and Rindt, H. (2001) Cardiac-directed overexpression of wild-type α_{1B}-adrenergic receptor induces dilated cardiomyopathy. *Am. J. Heart Circ. Physiol.* **281**: H931-H938.

McCune, D.F., Edelmann, S.E., Olges, J.R., Post, G.R., Waldrop, B.A., Waugh, D.J.J., Perez, D.M., and Piascik, M.T. (2000) Regulation of the cellular localization and signaling properties of the alpha(1B)- and alpha(1D)-adrenoceptors by agonists and inverse agonists. *Mol. Pharmacol*. **57**: 659-666.

Milano, C.A., Dolber, P.C., Rockman, H.A., Bond, R.A., Venable, M.E., Allen, L.F., and Lefkowitz, R.J. (1994) Myocardial expression of a constitutively active α_{1B}-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA.* **91**: 10109-10113.

Minneman, K.P. and Esbenshade, T.A. (1994) Alpha 1-adrenergic receptor subtypes.

Review. *Annu. Rev. Pharmacol. Toxicol.* **34**: 117-133.

Perez, D.M., DeYoung, M.B., and Graham, R.M. (1993) Coupling of expressed alpha 1B- and alpha 1D-adrenergic receptor to multiple signaling pathways is both G protein and cell type specific. *Mol. Pharmacol*. **44**: 784-795.

Piascik, M.T., Guarino, R.D., Smith, M.S., Soltis, E.E., Saussy, D.L. Jr., and Perez, D.M. (1995) The specific contribution of the novel alpha-1D adrenoceptor to the contraction of vascular smooth muscle. *J. Pharmacol. Exp. Ther.* **275**: 1583-1589.

Piascik, M.T., Hrometz, S.L., Edelmann, S.E., Guarino, R.D., Hadley, R.W., and Brown, R.D. (1997) Immunocytochemical localization of the alpha-1B adrenergic receptor and the contribution of this and the other subtypes to vascular smooth muscle contraction: analysis with selective ligands and antisense oligonucleotides. *J. Pharmacol. Exp. Ther.* **283**: 854-868.

Piascik, M.T. and Perez, D.M. (2001) α_1-Adrenergic Receptors: New Insights and Directions. *J. Pharmacol. Expt. Ther.* **298**: 403-410.

Piascik, M.T, Smith, M.S., Soltis, E.E., and Perez, D.M. (1994) Identification of the mRNA for the novel alpha 1D-adrenoceptor and two other alpha 1-adrenoceptors in

vascular smooth muscle. *Mol. Pharmacol.* **46**: 30-40.

Pombo, J.F., Troy, B.L., and Russell, R.O. (1971) Left ventricular volumes and ejection fraction by echocardiography. *Circulation*. **43**: 480-490.

Rudner, X.L., Berkowitz, D.E., Booth, J.V., Funk, B.L., Cozart, K.L., D'Amico, E.B., El-Moalem, H., Page, S.O., Richardson, C.D., Winters, B., Marucci, L., and Schwinn, D.A. (1999) Subtype specific regulation of human vascular alpha(1)-adrenergic receptors by vessel bed and age. *Circulation*. **100**: 2336-2343.

Sayet, I., Neuilly, G., Rakotoarisoa, L., Mironneau, C., and Mironneau, J. (1993) Relation between alpha 1-adrenoceptor subtypes and noradrenaline-induced contraction in rat portal vein smooth muscle. *Br. J. Pharmacol.* **110**: 207-212.

Schwinn, D.A., Page, S.O., Middleton, J.P., Lorenz, W., Liggett, S.B., Yamamoto, K., Lapetina, E.G., Caron, M.G., Lefkowitz, R.J., and Cotecchia, S. (1991) The alpha 1C-adrenergic receptor: characterization of signal transduction pathways and mammalian tissue heterogeneity. *Mol. Pharmacol.* **40**: 619-626.

Tanoue, A., Nasa, Y., Koshimizu, T., Shinoura, H., Oshikawa, S., Kawai, T., Sunada, S., Takeo, S., and Tsujimoto, G. (2002) The α 1D-adrenergic receptor directly regulates

arterial blood pressure via vasoconstriction. *J. Clin. Invest.* **109**: 765-775.

Varma, D.R. and Deng, X.F. (2000) Cardiovascular alpha1-adrenoceptor subtypes: functions and signaling. Review. *Can. J. Physiol. Pharmacol.* **78**: 267-292.

Wu, D., Katz, A., Lee, C.H., and Simon, M.I. (1992) Activation of phospholipase C by alpha 1-adrenergic receptors is mediated by the alpha subunits of Gq family. *J. Biol. Chem.* **267**: 25798-25802.

Zuscik, M.J., Sands, S., Ross, S.A., Waugh, D.J., Gaivin, R.J., Morilak, D., and Perez, D.M. (2000) Overexpression of the alpha1B-adrenergic receptor causes apoptotic neurodegeneration: multiple system atrophy. *Nat.Med.* **6**: 1388-1394.

Zuscik, M.J., Chalothorn, D., Hellard, D., Deighan, C., McGee, A., Daly, C.J., Waugh, D.J., Ross, S.A., Gaivin, R.J., Morehead, A.J., Thomas, J.D., Plow, E.F., McGrath, J.C., Piascik, M.T., and Perez, D.M. (2001) Hypotension, autonomic failure, and cardiac hypertrophy in transgenic mice overexpressing the alpha 1B-adrenergic receptor. *J. Biol. Chem.* **276**: 13738-13743.

Footnotes

This work was supported by National Institutes of Health Grant (HL38120) (M.T.P.), a Predoctoral Fellowship from the Pharmaceutical Research and Manufacturers of America Foundation (D.F.M.), and a Predoctoral Fellowship from the American Heart Association (D.C.).

* indicates equal contributions

Figure Legends

Figure 1: MAPK activity in transgenic mouse hearts. A) ERK activity and B) JNK activity measured by in-gel kinase assays, where each bar represents the mean and the S.E. of 7 independent determinations. The asterisk (*) indicates significantly different values from non-transgenic control values.

Figure 2: Functional responses of mouse hearts to 3, 10, 30, and 100 nM of isoproterenol. A) Heart rate (HR), B) left ventricular developed pressure (LVDP), C) positive change in the developed pressure as a function of time (+dP/dt), and D) negative change in the developed pressure as a function of time (-dP/dt). Each bar or point on the curve represents the mean and the S.E. of 7 and 10 independent experiments for the non-transgenic control and α_{1B}-AR_{C128F} hearts, respectively. The asterisk (*) indicates statistical differences from the non-transgenic control value at the respective isoproterenol concentration.

Figure 3: The ability of isoproterenol to increase cAMP levels in ventricular slices from non-transgenic control and α_{1B}-AR_{C128F} animals. cAMP levels are presented as pmol of cAMP/20 mg of protein. Data are the mean and the S.E. of 5 and 8 heart samples from experiments performed in duplicate from the non-transgenic control and the α_{1B}-AR_{C128F} hearts, respectively. The asterisk (*) indicates significantly different cAMP levels from non-transgenic control cAMP levels.

Figure 4: Log-dose response curves of the phenylephrine-induced contraction in mouse thoracic aortae in the absence and the presence of 30 nM BMY 7378. A) Non-

transgenic control, where the curves in the absence and the presence of BMY 7378 are composed of the average and the S.E. of 52 and 23 independent experiments, respectively and B) α_{1B}-AR_{C128F}, where the curves in the absence and the presence of BMY 7378 are composed of the average and the S.E. of 39 and 10 independent experiments, respectively.

Figure 5: Functional responses of α_{1D}-AR KO mouse hearts to 3, 10, 30, and 100 nM of isoproterenol. A) Heart rate (HR), B) left ventricular developed pressure (LVDP), C) positive change in the developed pressure as a function of time (+dP/dt), and D) negative change in the developed pressure as a function of time (-dP/dt). Each bar or point on the curve represents the mean and the S.E. of 12 and 11 independent experiments for the control and the α_{1D}-AR KO hearts, respectively.

Figure 6: Basal coronary flow rate required to maintain a constant perfusion pressure. Each bar represents the average and the S.E. of 7 independent experiments. The asterisk (*) indicates statistical significance from the control group.

Figure 7: Effect of 100 μM of phenylephrine on relative changes in the coronary perfusion pressure (CPP) of hearts lacking the α_{1D}-AR. The initial CPPs were 83.3 ± 2.3 and 81.9 ± 2.2 mm Hg for the control and the α_{1D}-AR KO hearts, respectively. The recordings were performed over a 7 min period. Each curve is composed of the average and the S.E. for 7 different experiments where the asterisk (*) indicates statistical significance between the α_{1D}-AR KO and the control group at the respective time point.

Table 1: Echocardiographic assessment of the murine left ventricular dimensions and function in mice over-expressing the α_{1B}-AR_{C128F}. The values are the mean S.E. and * indicates statistical difference from the non-transgenic value ($P<0.05$).

	n	Body Weight (g)	Left Ventricular Internal Dimension (mm/g)		Posterior Wall Thickness (mm/g)		Left Ventricular Mass (g)	Left Ventricular Mass/Body Weight (10^{-3})
			Diastole	Systole	Diastole	Systole		
Control	10	30.03 ± 1.43	0.1364 ± 0.0046	0.0770 ± 0.0055	0.0197 ± 0.0022	0.0358 ± 0.0041	0.0782 ± 0.0074	2.659 ± 0.302
α_{1B}-AR_{C128F}	12	27.99 ± 1.16	0.1523 ± 0.0065*	0.1038 ± 0.0066*	0.0171 ± 0.0009	0.0313 ± 0.0032	0.0683 ± 0.0051	2.441 ± 0.149

	n	Ejection Time (msec)	Mean Velocity for Circumferential Fiber Shortening corrected for Heart Rate ($1/\sqrt{sec}$)	Heart Rate (beats/min)	Stroke Volume (mm^3)	Cardiac Output (mm^3/min)	% Fractional Shortening
Control	10	57.23 ± 3.83	0.6935 ± 0.1195	454.1 ± 22.7	0.0273 ± 0.0018	12.31 ± 0.83	43.10 ± 4.11
α_{1B}-AR_{C128F}	12	51.76 ± 2.00	0.5550 ± 0.0984	484.6 ± 20.9	0.0274 ± 0.0023	13.21 ± 1.22	32.05 ± 2.44*

Table 2: Characteristics of the phenylephrine response in control and α_{1B}-AR_{C128F} over-expressing aortae.

Calculated pA_2 values and their 95% confidence intervals (C.I.) are listed along with the dissociation constant (K_i).

	Phenylephrine			**Characterization of BMY 7378 Antagonism**			
	n	**Log EC_{50}**	**S.E.**	**pA_2**	**95% C.I.**	**K_i (nM)**	**95% C.I. (nM)**
Control	52	-6.869	0.0662	9.531	0.219	0.294	0.149
α_{1B}-AR_{C128F}	39	-6.795	0.1036	9.414	0.452	0.385	0.401

Figure 1

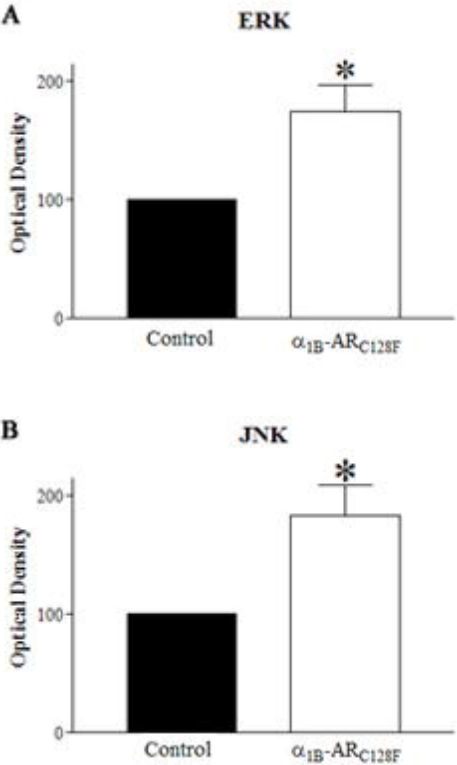

A
ERK
Optical Density
200
100
0
*
Control
α1B-AR C128F
B
JNK
Optical Density
200
100
0
*
Control
α1B-AR C128F

Figure 2

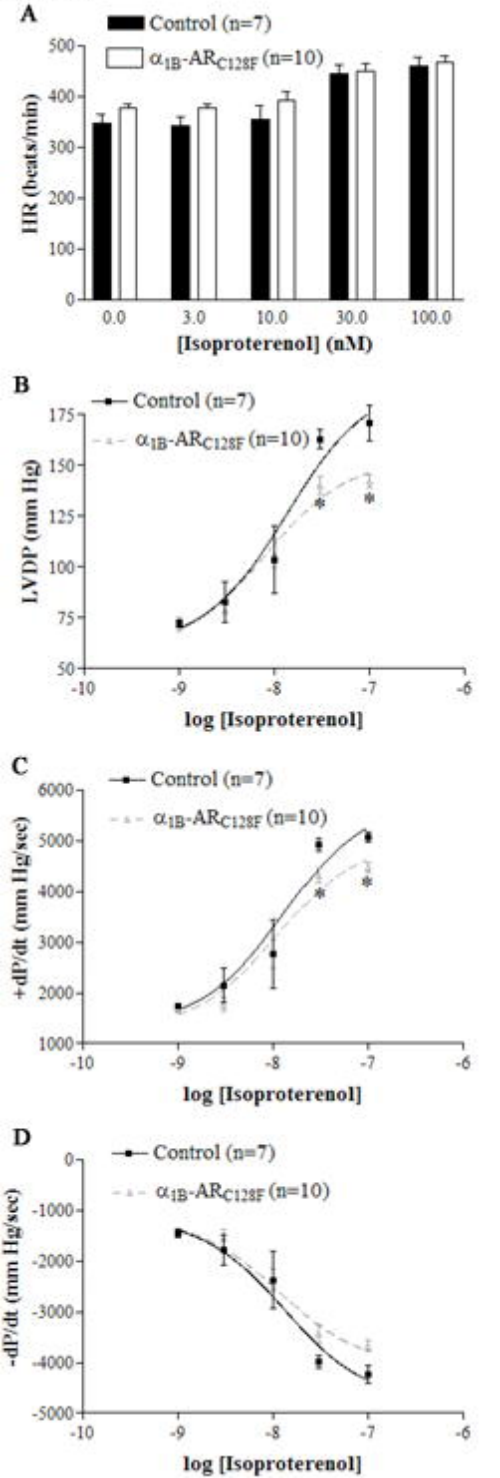

A
Control (n=7)
α1B-ARC128F (n=10)
HR (beats/min)
500
400
300
200
100
0
0.0
3.0
10.0
30.0
100.0
[Isoproterenol] (nM)
B
Control (n=7)
α1B-ARC128F (n=10)
LVDP (mm Hg)
175
150
125
100
75
50
-10
-9
-8
-7
-6
log [Isoproterenol]
C
Control (n=7)
α1B-ARC128F (n=10)
+dP/dt (mm Hg/sec)
6000
5000
4000
3000
2000
1000
-10
-9
-8
-7
-6
log [Isoproterenol]
D
Control (n=7)
α1B-ARC128F (n=10)
-dP/dt (mm Hg/sec)
0
-1000
-2000
-3000
-4000
-5000
-10
-9
-8
-7
-6
log [Isoproterenol]

Figure 3

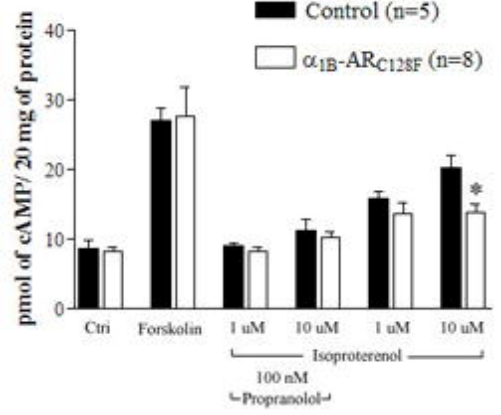

Control (n=5)
α1B-AR C128F (n=8)
pmol of cAMP/ 20 mg of protein
40
30
20
10
0
*
Ctri
Forskolin
1 uM
10 uM
1 uM
10 uM
Isoproterenol
100 nM
Propranolol

Figure 4

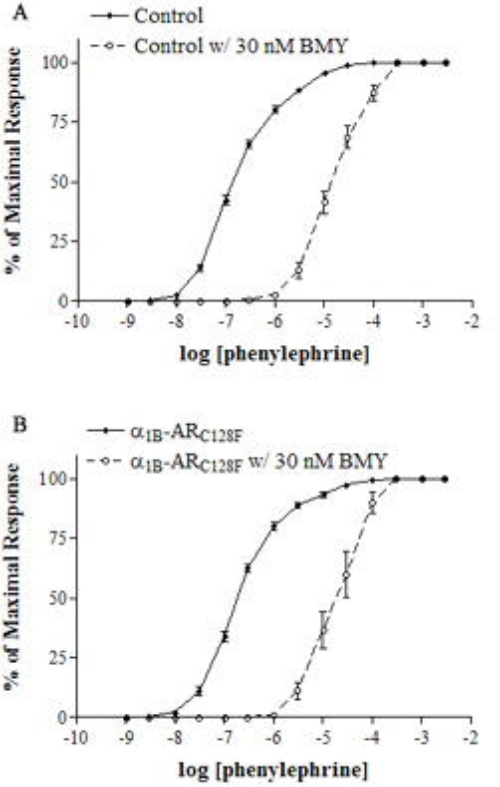

A
Control
Control w/ 30 nM BMY
% of Maximal Response
100
75
50
25
0
-10
-9
-8
-7
-6
-5
-4
-3
-2
log [phenylephrine]
B
α1B-AR C128F
α1B-AR C128F w/ 30 nM BMY
% of Maximal Response
100
75
50
25
0
-10
-9
-8
-7
-6
-5
-4
-3
-2
log [phenylephrine]

Figure 5

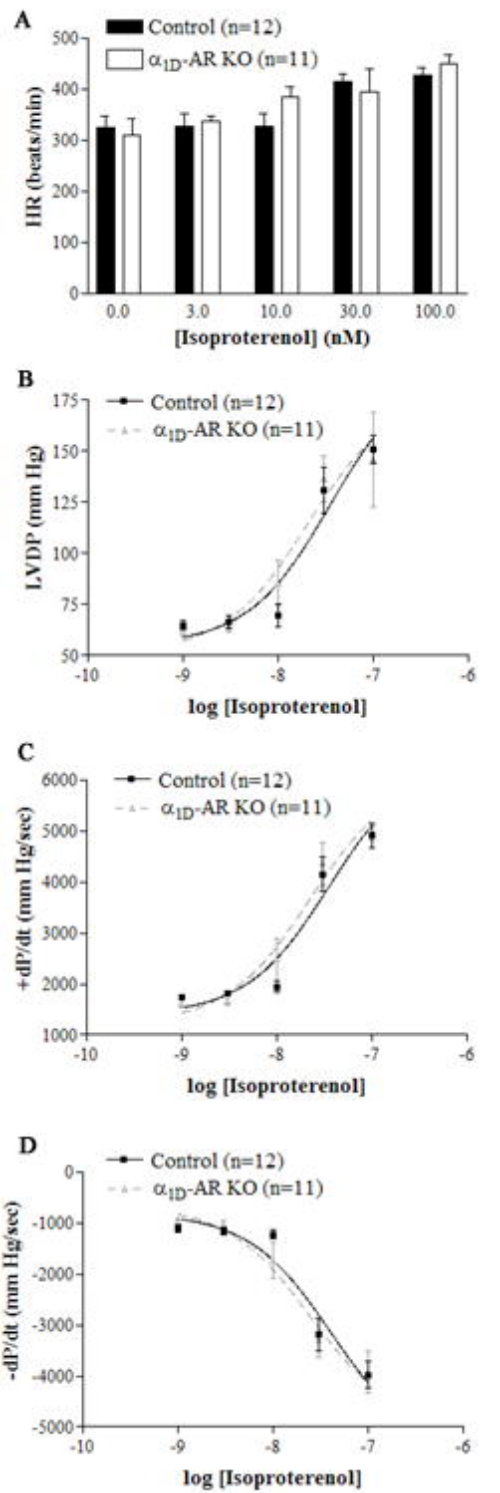

A
Control (n=12)
α1D-AR KO (n=11)
HR (beats/min)
500
400
300
200
100
0
0.0
3.0
10.0
30.0
100.0
[Isoproterenol] (nM)
B
Control (n=12)
α1D-AR KO (n=11)
LVDP (mm Hg)
175
150
125
100
75
50
-10
-9
-8
-7
-6
log [Isoproterenol]
C
Control (n=12)
α1D-AR KO (n=11)
+dP/dt (mm Hg/sec)
6000
5000
4000
3000
2000
1000
-10
-9
-8
-7
-6
log [Isoproterenol]
D
Control (n=12)
α1D-AR KO (n=11)
-dP/dt (mm Hg/sec)
0
-1000
-2000
-3000
-4000
-5000
-10
-9
-8
-7
-6
log [Isoproterenol]

Figure 6

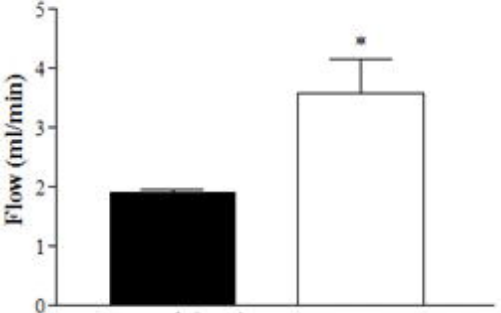
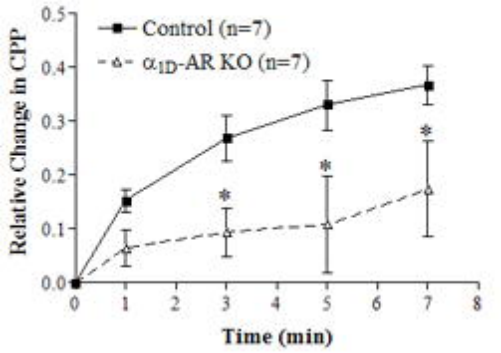


Figure 7


Control (n=7)
α1D-AR KO (n=7)
Relative Change in CPP
Time (min)
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
7
8
*
*
*