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# Cardiac $\alpha_1$ -Adrenergic Receptors: Novel Aspects of Expression, Signaling Mechanisms, Physiologic Function, and Clinical Importance

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**Abstract**—Adrenergic receptors (AR) are G-protein-coupled receptors (GPCRs) that have a crucial role in cardiac physiology in health and disease. Alpha<sub>1</sub>-ARs signal through  $G_{\alpha_q}$ , and signaling through  $G_q$ , for example, by endothelin and angiotensin receptors, is thought to be detrimental to the heart. In contrast, cardiac alpha<sub>1</sub>-ARs mediate important protective and adaptive functions in the heart, although alpha<sub>1</sub>-ARs are only a minor fraction of total cardiac ARs. Cardiac alpha<sub>1</sub>-ARs activate pleiotropic downstream signaling to prevent pathologic remodeling in heart failure. Mechanisms defined in animal and cell models include activation of adaptive hypertrophy, prevention of cardiac myocyte death, augmentation of contractility, and induction of ischemic preconditioning. Surprisingly,

at the molecular level, alpha<sub>1</sub>-ARs localize to and signal at the nucleus in cardiac myocytes, and, unlike most GPCRs, activate “inside-out” signaling to cause cardioprotection. Contrary to past opinion, human cardiac alpha<sub>1</sub>-AR expression is similar to that in the mouse, where alpha<sub>1</sub>-AR effects are seen most convincingly in knockout models. Human clinical studies show that alpha<sub>1</sub>-blockade worsens heart failure in hypertension and does not improve outcomes in heart failure, implying a cardioprotective role for human alpha<sub>1</sub>-ARs. In summary, these findings identify novel functional and mechanistic aspects of cardiac alpha<sub>1</sub>-AR function and suggest that activation of cardiac alpha<sub>1</sub>-AR might be a viable therapeutic strategy in heart failure.

**ABBREVIATIONS:**  $\alpha_1$ -AR,  $\alpha_1$ -adrenergic receptor;  $\beta$ -AR,  $\beta$ -adrenergic receptor;  $\alpha_1A$ -subtype,  $\alpha_1A$ -adrenergic receptor;  $\alpha_1B$ -subtype,  $\alpha_1B$ -adrenergic receptor;  $\alpha_1$ -blocker,  $\alpha_1$ -adrenergic receptor antagonist;  $\alpha_1AKO$ ,  $\alpha_1A$ -adrenergic receptor knockout;  $\alpha_1BKO$ ,  $\alpha_1B$ -adrenergic receptor knockout;  $\alpha_1ABKO$ ,  $\alpha_1AB$ -adrenergic receptor double knockout;  $\alpha$ SkAct,  $\alpha$ -skeletal actin; AR, adrenergic receptor; ATR, angiotensin receptor; ALLHAT, Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial; BPH, benign prostatic hyperplasia; BEST,  $\beta$ -Blocker Evaluation of Survival Trial; cav-3, caveolin-3; CAM, constitutively active mutant; DAG, diacylglycerol; ETR, endothelin receptor; EMT, extraneuronal monoamine transporter; ERK, extracellular signal-regulated kinase; GFP, green fluorescent protein; GPCR, G-protein-coupled receptor; HEK, human embryonic kidney; HW, heart weight; IP, inositol phosphate; IP<sub>3</sub>, inositol 1,4,5-trisphosphate; KO, knockout; MEK, mitogen-activated protein kinase kinase; MOXCON, Moxonidine Congestive Heart Failure Trial; MOXSE, Moxonidine Safety and Efficacy Trial; MyHC, myosin heavy chain; NE, norepinephrine; NFAT, nuclear factor of activated T cells; NRVM, neonatal rat ventricular myocyte; NYHA, New York Heart Association; OCT3, organic cation transporter 3; PLC $\beta_1$ , phospholipase C $\beta_1$ ; PKC, protein kinase C; PKD, protein kinase D; V-HeFT, Vasodilator-Heart Failure Trial; WT, wild type.

## I. Introduction

Adrenergic receptors (ARs) bind to and are activated by the endogenous catecholamine hormones epinephrine and norepinephrine (NE). Epinephrine is primarily produced in and released to the circulation from the adrenal gland, whereas NE is synthesized in and released by sympathetic nerve terminals in the peripheral nervous system and brain. In the heart, the two main ARs are the  $\beta$ -ARs, which comprise roughly 90% of the total cardiac ARs, and  $\alpha_1$ -ARs, which account for approximately 10% (see section II).

In general, acute activation of cardiac  $\beta_1$ -ARs, the predominant  $\beta$ -AR subtype (80% or more of total  $\beta$ -ARs in heart), induces positive inotropic and chronotropic responses, although in heart failure, where sympathetic activation and catecholamine levels are increased, long-term activation of  $\beta_1$ -ARs exacerbates pathologic remodeling (Bristow, 2000; Naga Prasad et al., 2001; Lohse et al., 2003).

Less is known about cardiac  $\alpha_1$ -ARs, but studies from the last thirty years indicate that long-term activation of cardiac  $\alpha_1$ -ARs activates beneficial trophic signaling in the developing heart and that these  $\alpha_1$ -AR-mediated trophic effects in the adult, in many ways, counteract the negative effects of overstimulation of  $\beta_1$ -ARs in heart failure. This review will focus on these trophic effects of cardiac  $\alpha_1$ -ARs and how activation of  $\alpha_1$ -ARs might be beneficial in heart failure.

There are three  $\alpha_1$ -AR subtypes, the  $\alpha_1A$ ,  $\alpha_1B$ , and  $\alpha_1D$ , and all three are expressed in the heart in a cell-type specific manner (section II). All three  $\alpha_1$ -ARs are G-protein-coupled receptors (GPCR), and classic  $\alpha_1$ -AR signaling mechanisms involve coupling to the  $G_q/11$  ( $G_{\alpha_q}$ ) family of G-proteins and activation of phospholipase  $C\beta_1$  ( $PLC\beta_1$ ) at the plasma membrane. Activation of  $PLC\beta_1$  cleaves phosphatidylinositol (PI), increasing inositol triphosphate ( $IP_3$ ) and diacylglycerol (DAG).  $IP_3$  binds to the  $IP_3$ -receptor to release calcium from intracellular stores, and DAG activates protein kinase C (PKC).

Other  $G_q$ -coupled GPCRs that signal through  $G_{\alpha_q}$ , such as endothelin receptors (ETRs) and angiotensin receptors (ATRs), are believed to play an important role in the pathogenesis of heart failure. Hallmarks of cardiomyopathy with heart failure include contractile dysfunction (both systolic and diastolic), myocyte hypertrophy, fibrosis, and increased cardiac cell death (Anand and Florea, 2003), which can all be worsened by  $G_q$ -coupled receptors (Salazar et al., 2007).

However, it also needs to be recalled that the view that  $G_q$ -coupled receptor signaling is toxic is based in large part on a transgenic mouse model with  $G_q$  overexpression that markedly exceeds the 2-fold increase found in human heart failure (Adams et al., 1998; Ponick et al., 1998; Sakata et al., 1998) and thus cannot be considered to simulate human pathophysiology. In human heart failure, the maximal increase in

$G_q$  abundance is 2-fold (Ponick et al., 1998), and transgenic mice with 2-fold cardiomyocyte-specific  $G_q$  overexpression have no discernible cardiac phenotype (Adams et al., 1998; Sakata et al., 1998).

Furthermore,  $\alpha_1$ -ARs differ from other  $G_q$ -coupled receptors in several important ways, including expression limited to myocytes within the heart (section II) and localization and signaling at the nucleus, as discussed in section III.

Thus, unlike what can be seen with some  $G_q$ -coupled receptors,  $\alpha_1$ -ARs protect the heart by activating an adaptive or physiologic hypertrophy, preventing cardiac myocyte death, augmenting contractile function in heart failure and inducing preconditioning (section IV). Finally, clinical trials indicate that blockade of  $\alpha_1$ -ARs exacerbates heart failure (section V), which could be explained by the cardioprotective functions of  $\alpha_1$ -ARs identified in cell and animal models. This review summarizes these data, which span decades, and emphasizes recent findings from our laboratories.

## II. $\alpha_1$ -Adrenergic Receptor Expression in the Heart

### A. $\alpha_1$ -Adrenergic Receptor Expression in the Heart in Animal Models

In mice and rats, all three  $\alpha_1$ -AR subtype mRNAs,  $\alpha_1A$ ,  $\alpha_1B$ , and  $\alpha_1D$ , are detected in the heart (Rokosh et al., 1994; Stewart et al., 1994; Cavalli et al., 1997; O'Connell et al., 2003). Interestingly, among most species, including mouse, guinea pig, rabbit, pig, and cow, heart  $\alpha_1$ -AR levels determined by ligand binding are relatively constant (mouse: mean of six studies,  $\sim 12$  fmol/mg protein) (Steinfath et al., 1992a; Cavalli et al., 1997; Yang et al., 1998; Lin et al., 2001; O'Connell et al., 2003; Rokosh and Simpson, 2002), with the exception of rat heart, in which  $\alpha_1$ -AR levels are approximately 10-fold higher (rat: mean of four studies,  $\sim 114$  fmol/mg) (Steinfath et al., 1992a; Michel et al., 1994; Noguchi et al., 1995; Stewart et al., 1994).

Determination of cell-type specific expression of  $\alpha_1$ -ARs in the heart, or any tissue, is hampered by the lack of validated, subtype-specific  $\alpha_1$ -AR antibodies (Jensen et al., 2009c), which is a general problem with antibodies for GPCRs, as reviewed (Michel et al., 2009). However, studies in  $\alpha_1$ -AR knockout mice demonstrate that cardiac myocytes express only the  $\alpha_1A$ - and  $\alpha_1B$ -subtypes, based on lack of [ $^3H$ ]prazosin binding or functional responses in hearts from  $\alpha_1AB$ -double knockout mice ( $\alpha_1ABKO$ ) (McCloskey et al., 2003; O'Connell et al., 2003; Turnbull et al., 2003) as well as lack of binding to a fluorescent  $\alpha_1$ -AR antagonist or signaling in cardiac myocytes isolated from  $\alpha_1ABKO$  hearts (O'Connell et al., 2003; Wright et al., 2008).

Ligand binding studies further indicate that the  $\alpha_1B$  is predominant, with the  $\alpha_1A$ - and  $\alpha_1B$ -subtypes

expressed in a 1:2–4 ratio in cardiac myocytes (Rokosh and Simpson, 2002; O'Connell et al., 2003). Despite the presence of  $\alpha_1$ D-subtype mRNA, rodent cardiac myocytes do not appear to express the  $\alpha_1$ D-subtype protein by binding (O'Connell et al., 2003). However, the  $\alpha_1$ D might be expressed in the coronary vasculature, based on studies demonstrating  $\alpha_1$ -AR mediated reductions in coronary flow in isolated  $\alpha_1$ -AR knockout hearts (Chalothorn et al., 2003; Turnbull et al., 2003). This idea is supported by human studies (below). Conversely, rodent cardiac fibroblasts do not express  $\alpha_1$ -ARs (Stewart et al., 1994; O'Connell et al., 2001), and  $\alpha_1$ -agonist infusion induces hypertrophy without fibrosis (Marino et al., 1991), suggesting that  $\alpha_1$ -AR activation does not exacerbate fibrosis associated with heart failure. In contrast with  $\alpha_1$ -ARs, most ATRs and ET<sub>B</sub>Rs are in fibroblasts, not cardiac myocytes (Kim et al., 1995; Gray et al., 1998; Modesti et al., 1999).

Long-term activation of  $\alpha_1$ -ARs and other hypertrophic agonists increases the  $\alpha_1$ A-subtype, without desensitizing  $\alpha_1$ -mediated inositol phosphate (IP) turnover or growth, while decreasing  $\alpha_1$ B-subtype mRNA and protein levels in cultured neonatal rat cardiac myocytes (NRVM) and in rats subjected to aortic banding (Rokosh et al., 1996). Moreover, total  $\alpha_1$ -AR levels are not altered in vivo by hypertrophy or heart failure in rats (Rokosh et al., 1996; Sjaastad et al., 2003), and  $\alpha_1$ -AR inotropic effects are maintained or increased (Wang et al., 2010), in contrast to  $\beta$ -ARs that are desensitized and downregulated in heart failure (Bristow et al., 1982; Bristow et al., 1988).

Partial explanation for the differences in desensitization of  $\alpha_1$ -AR and  $\beta$ -ARs might reside in expression and regulation of G-protein receptor kinases (GRKs). GRK3 is found exclusively in myocytes, regulates  $\alpha_1$ -ARs, and is not upregulated in heart failure (Vinge et al., 2001, 2007; Agüero et al., 2012). In contrast, GRK2 and GRK5 that desensitize  $\beta$ -ARs but not  $\alpha_1$ -ARs are expressed in many myocardial cell types and are upregulated in heart failure (Rockman et al., 1996; Eckhart et al., 2000; Vinge et al., 2001, 2007; Agüero et al., 2012).

### *B. Unique Aspects of $\alpha_1$ -Adrenergic Receptor Expression Profiles in Cardiac Myocytes*

Recent studies provide unique information on the expression and distribution of  $\alpha_1$ -ARs in cardiac myocytes. First, both the  $\alpha_1$ A- and  $\alpha_1$ B-subtypes localize to and signal at the nuclear membrane, but not the plasma membrane, in adult mouse cardiac myocytes (Huang et al., 2007; Wright et al., 2008; Wright et al., 2012), as reviewed in section III. Second,  $\alpha_1$ A-subtype expression and function are graded in adult cardiac myocytes, from high levels to none, whereas the  $\alpha_1$ B-subtype is expressed in all cardiac myocytes (unpublished data).

### *C. $\alpha_1$ -Adrenergic Receptor Expression in Human Heart*

In human heart, all three  $\alpha_1$ -AR subtype mRNAs are detected (Jensen et al., 2009a). Furthermore,  $\alpha_1$ -AR expression levels in human heart determined by ligand binding are similar to mouse and most other species (human: mean of 6 studies,  $\sim$ 12 fmol/mg protein) (Bohm et al., 1988; Bristow et al., 1988; Vago et al., 1989; Steinfath et al., 1992b; Hwang et al., 1996; Jensen et al., 2009a). Human myocardium has the  $\alpha_1$ A- and  $\alpha_1$ B-subtypes, with the  $\alpha_1$ B predominant, similar to other species (Jensen et al., 2009a,b), and the  $\alpha_1$ A is functional in signaling (R. C. Thomas and P. C. Simpson, unpublished data). These data suggest that the mouse is a more appropriate model to approximate cardiac  $\alpha_1$ -AR function than the rat, which as mentioned above, has roughly 10-fold more  $\alpha_1$ -ARs.

Competition binding experiments do not detect the  $\alpha_1$ D-subtype in explanted human heart (Jensen et al., 2009a,b). However, the  $\alpha_1$ D-subtype is expressed and functional in coronary artery smooth muscle cells and might cause vasoconstriction (Jensen et al., 2009b). The  $\alpha_1$ B-subtype is expressed in coronary artery endothelial cells and might induce vasodilation and angiogenesis (Jensen et al., 2010).

### *D. $\alpha_1$ -Adrenergic Receptor Levels Increase Proportionately in Human Heart Failure*

In heart failure,  $\beta_1$ -ARs are desensitized and downregulated. In contrast, radioligand-binding studies indicate that myocardial  $\alpha_1$ -AR levels are slightly increased in human heart failure (mean of six studies, increased from  $\sim$ 12 to  $\sim$ 19 fmol/mg protein) (Bohm et al., 1988; Bristow et al., 1988; Vago et al., 1989; Steinfath et al., 1992b; Hwang et al., 1996; Jensen et al., 2009a). This means that  $\alpha_1$ -AR levels in the heart, which normally represent approximately 11% of the total AR population at baseline (range 2–23%, mean of six studies), are proportionately increased to approximately 25% of the total AR population in heart failure (range 9–41%) (Bohm et al., 1988; Bristow et al., 1988; Vago et al., 1989; Steinfath et al., 1992b; Hwang et al., 1996; Jensen et al., 2009a). Given that sympathetic drive and catecholamine levels are increased in heart failure (Cohn et al., 1984), this could imply that  $\alpha_1$ -ARs sustain adrenergic function when  $\beta_1$ -ARs are downregulated. In fact,  $\alpha_1$ -AR-induced positive inotropy, which at baseline is minimal, can be equal to  $\beta$ -AR-mediated inotropy in ventricular muscle strips isolated from human heart failure patients (Skomedal et al., 1997), as reviewed in more detail below.

### *E. Conclusions on $\alpha_1$ -Adrenergic Receptor Heart Expression*

In summary,  $\alpha_1$ -ARs constitute a minority of the total cardiac AR population in humans at baseline, and

this seems to hold across species, with the exception of rats where  $\alpha_1$ -ARs levels are  $\sim 10$ -fold higher than any other species. This should be considered when interpreting results from studies of  $\alpha_1$ -ARs in rats, particularly in cultured NRVMs, the most common cardiac myocyte culture model.

Cardiac myocytes of all species have all three  $\alpha_1$ -AR subtype mRNAs, but only the  $\alpha_1A$ - and  $\alpha_1B$ -subtype receptor proteins are detected. In humans, the  $\alpha_1D$ -subtype is present in coronary smooth muscle and might regulate coronary vasoconstriction, whereas the  $\alpha_1B$ -subtype is in coronary endothelial cells and might regulate vasodilation and angiogenesis. In contrast,  $\alpha_1$ -ARs are not expressed by cardiac fibroblasts.

In heart failure,  $\alpha_1$ -ARs are not downregulated as are  $\beta_1$ -ARs and thus become a greater share (25%) of ARs in the heart. This increase in  $\alpha_1$ -ARs could suggest that  $\alpha_1$ -ARs have a compensatory or adaptive role in heart failure, as suggested by studies showing that  $\alpha_1$ -mediated inotropy can be similar to  $\beta$ -AR-mediated inotropy in heart failure (Skomedal et al., 1997). The idea that  $\alpha_1$ -ARs might have an adaptive and protective role in the heart is a central theme of this review and is discussed in following sections.

### III. $\alpha_1$ -AR Signaling in Cardiac Myocytes

The following sections review the conventional models of  $\alpha_1$ -AR localization and signaling at the plasma membrane, or “outside-in” signaling, and evidence for novel models, suggesting that  $\alpha_1$ -ARs and other GPCRs signal from the cardiac myocyte nucleus, or “inside-out” signaling.

#### A. Conventional Models of $\alpha_1$ -Adrenergic Receptor Signaling

Conventional models of GPCR signaling describe receptor activation at the plasma membrane leading to initiation of downstream signaling within the cell, commonly referred to as “outside-in” signaling. Furthermore, classic models of GPCR function suggest that GPCRs are expressed on the cell membrane and are only internalized after receptor phosphorylation and subsequent desensitization (Drake et al., 2006).  $\alpha_1$ -ARs signal through the  $G_q/11$  class of G-proteins, leading to activation of  $PLC\beta_1$  and increases in  $IP_3$ /calcium signaling and activation of PKC (Graham et al., 1996; Piascik and Perez, 2001). The  $\alpha_1B$ -subtype might also signal through  $G_i$  (Hu and Nattel, 1995; Steinberg et al., 1985; Akhter et al., 1997; Melien et al., 2000; Snabaitis et al., 2005). In NRVM, historically the primary cell model used to study cardiac  $\alpha_1$ -AR signaling,  $\alpha_1$ -AR-induced increases in  $IP_3$  are readily observed, but in adult cardiac myocytes this is controversial. The general consensus is that  $\alpha_1$ -ARs signal through the  $G_q/PLC\beta_1$ - $IP_3$ /PKC pathway, but downstream signaling pathways are diverse, as reviewed

elsewhere (Hein and Michel, 2007; Cotecchia, 2010; Jensen et al., 2011). To date, over 70 downstream signaling molecules have been implicated in cardiac  $\alpha_1$ -AR signaling, using the NRVM model of  $\alpha_1$ -AR-stimulated cardiac myocyte hypertrophy (Jensen et al., 2011). Some data suggest interactions with  $\beta$ -arrestin (Pediani et al., 2005; Stanasila et al., 2008; Hennenberg et al., 2011) and  $G\beta\gamma$  (Vettel et al., 2012).

#### B. New Model for General G-Protein-Coupled Receptor Signaling: G-Protein-Coupled Receptors at the Nucleus

It is now clear that several GPCRs localize to and signal at the nucleus, or “inside-out” signaling. Nuclear signaling is seen in several cell types, including neurons, hepatocytes, and cardiac myocytes, as reviewed previously (Gobeil et al., 2006; Boivin et al., 2008; Bkaily et al., 2009; Tadevosyan et al., 2012). The GPCRs include receptors for prostaglandin  $E_2$  in the brain (Gobeil et al., 2002), angiotensin II (AT1R) in the brain and in HEK and Chinese hamster ovary cells (Lu et al., 1998; Chen et al., 2000; Lee et al., 2004), platelet activating factor in the liver and brain (Marrache et al., 2002), apelin in the brain (Lee et al., 2004), bradykinin in HEK cells (Lee et al., 2004), and glutamate in neurons (O'Malley et al., 2003).

Several recent studies show that GPCRs localize to nuclei in binucleate adult cardiac myocytes, as reviewed previously (Tadevosyan et al., 2012). Specifically, ETRs are detected on nuclei isolated from adult cardiac myocytes, and endothelin stimulates nuclear calcium transients (Boivin et al., 2003). ATRs and  $\beta$ -ARs are also detected on nuclei isolated from adult cardiac myocytes and mediate increased RNA synthesis (Boivin et al., 2006; Tadevosyan et al., 2010; Vaniotis et al., 2011). These findings indicate that GPCR localization to the nucleus could regulate important physiologic functions in adult cardiac myocytes. However, the majority of these other receptors can localize also to the myocyte plasma membrane; for example, 95% of ETRs are on the sarcolemma (Boivin et al., 2003; Wright et al., 2012), so that the relative functional significance of nuclear versus surface localization is uncertain.

Despite the data reviewed above, the prevalent view is that GPCRs, including  $\alpha_1$ -ARs, are localized primarily to the plasma membrane in heart and myocytes. This impression is based predominantly on radioligand binding to membrane fractions, binding assays in whole cells (Filipeanu et al., 2006) and studies with  $\alpha_1$ -AR antibodies. Difficulties with these approaches are discussed in the next section.

#### C. $\alpha_1$ -Adrenergic Receptors in the Nuclei in Cardiac Myocytes

Cellular localization of signaling molecules determines function, emphasizing the importance of  $\alpha_1$ -AR subcellular localization in cardiac myocytes. The following

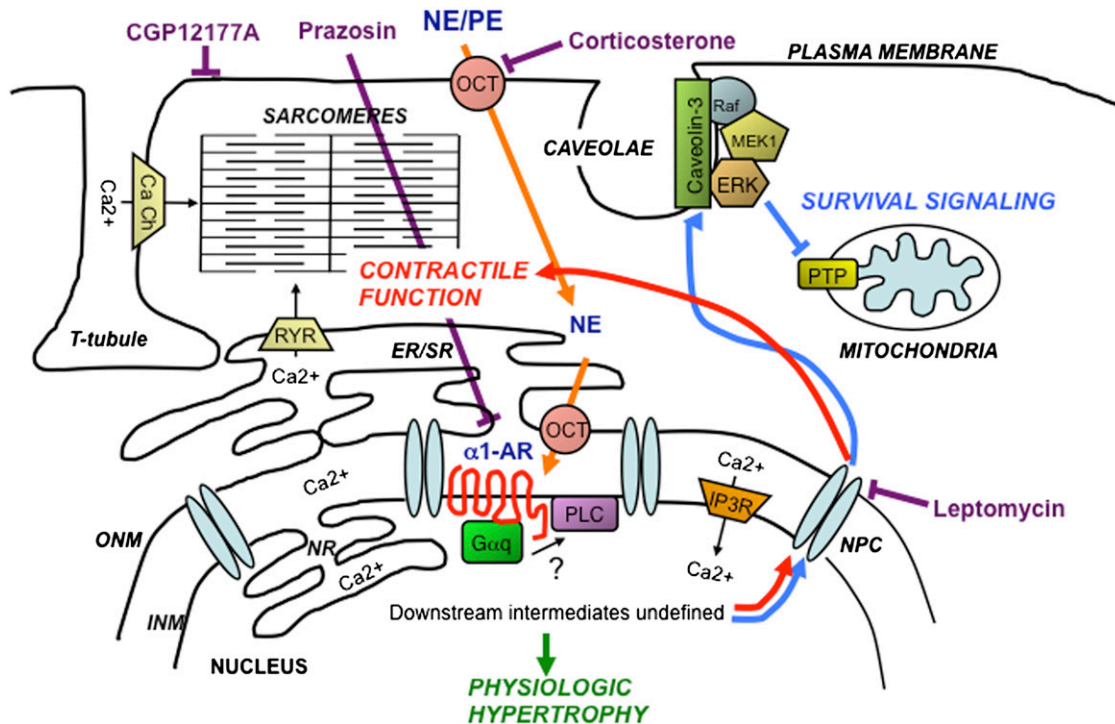
sections review the limitations and advantages of different approaches to detect  $\alpha_1$ -AR subcellular localization and the evidence that  $\alpha_1$ -ARs are in the cardiac myocyte nucleus, derived from studies of localization, agonist uptake, and signaling. Physiologic implications of nuclear  $\alpha_1$ -ARs are also suggested. This novel nuclear  $\alpha_1$ -AR signaling paradigm in cardiac myocytes is illustrated in Fig. 1.

**1. Nuclear Localization of  $\alpha_1$ -Adrenergic Receptors in Cardiac Myocytes.** Limitations with the techniques used to detect  $\alpha_1$ -ARs, radioligand binding and  $\alpha_1$ -AR antibodies, might explain the conventional view that  $\alpha_1$ -ARs localize mainly to the plasma membrane. Ligand binding assays typically involve homogenization of heart tissue or cultured cells followed by a high-speed ultracentrifugation to isolate total membrane fractions. This high-speed ultracentrifugation pulls down all membranes, and if subcellular markers are not used, this technique does not distinguish between plasma, sarcoplasmic, and nuclear membranes (Lin et al., 2001; Rokosh and Simpson, 2002; O'Connell et al., 2003). Furthermore, most purified membrane preparations exclude over 65 to 85% of total heart  $\alpha_1$ -ARs that are found in "debris" and low-speed pellets

discarded normally (Simpson, 2006). Whole-cell binding assays are limited by the lack of radioligands that do not enter the cell (Filipeanu et al., 2006).

Immunochemical detection, either by immunoblot or cell/tissue staining, is another commonly used technique to detect  $\alpha_1$ -ARs. However, none of 10 commercial  $\alpha_1$ -AR antibodies are specific for  $\alpha_1$ -ARs in general or for any subtype, as documented by the fact that no antibody detects a band in wild-type (WT) tissue that is absent in tissue from  $\alpha_1$ -AR knockout (KO) mice (Jensen et al., 2009c). This nonspecificity of anti-PCR antibodies is a general problem, reviewed recently, emphasizing that  $\alpha_1$ -AR antibodies need to be validated using KO tissue (Michel et al., 2009). Nonspecificity of  $\alpha_1$ -AR antibodies calls into question previous reports with these reagents, for example, work suggesting  $\alpha_1$ -AR localization to the plasma membrane and t-tubules in adult rat cardiac myocytes (O-Uchi et al., 2008) or a study using immunoprecipitation of  $\alpha_1$ -ARs with potential signaling partners (Fujita et al., 2001).

An antibody to the 1D4 epitope tag at the C terminus of the  $\alpha_1A$  detects surface membrane expression in heart sections of a transgenic mouse (Lin et al., 2001).



**Fig. 1.** Model for  $\alpha_1$ -AR signaling at the nuclear membrane. In adult cardiac myocytes, catecholamine  $\alpha_1$ -AR agonists (NE/PE) are actively transported into the myocyte via organic cation transporter 3 (OCT), which can be inhibited by corticosterone. The membrane-permeable  $\alpha_1$ -AR antagonist prazosin (and similar derivatives) can cross the plasma membrane to inhibit signaling, whereas the membrane impermeable  $\alpha_1$ -AR antagonist CGP12177A fails to inhibit signaling. The model suggests that active  $\alpha_1$ -ARs localize to the inner nuclear membrane with the ligand-binding domain facing the space between the outer and inner nuclear membranes (ONM and INM, respectively). On the basis of this orientation, binding of agonist to  $\alpha_1$ -ARs induces signaling inside the nucleus, possibly through  $G_{\alpha_q}$ , although downstream intranuclear signaling pathways remain to be defined. We propose that activation of nuclear  $\alpha_1$ -ARs can induce intranuclear hypertrophic signaling as well as extranuclear signaling, including activation of ERK in caveolae and survival signaling or phosphorylation of cardiac troponin I at the sarcomere and contractile function. HDAC, histone deacetylase; Ca Ch, calcium channel; RYR, ryanodine receptor; PTP, mitochondrial permeability transition pore; ER/SR, endoplasmic/ sarcoplasmic reticulum; NR, nucleoplasmic reticulum; NPC, nuclear pore complex.

However, it is problematic whether receptor localization with 170-fold overexpression simulates that of endogenous  $\alpha_1$ -ARs (Lin et al., 2001).

A few studies use membrane fractionation combined with the caveolar marker caveolin-3 (*cav-3*) to detect  $\alpha_1$ -AR binding in caveolae in NRVMs (Fujita et al., 2001; Lanzafame et al., 2006). In NRVMs, a caveolar fraction defined in this way contains most or all  $\alpha_1$ -mediated IP turnover (Morris et al., 2006) and 27% of total  $\alpha_1$ -AR binding, both  $\alpha_1A$  and  $\alpha_1B$  (Lanzafame et al., 2006). The value of 27%  $\alpha_1$ -AR binding in caveolae in NRVMs agrees well with a more recent study finding 20% of total  $\alpha_1$ -ARs in adult myocyte membranes defined by high levels of *cav-3* (Wright et al., 2008).

The contrary notion that  $\alpha_1$ -ARs localize primarily to the nucleus arises from three main lines of evidence. First, 80% of total  $\alpha_1$ -AR binding in adult mouse cardiac myocytes is found in nuclear membranes defined by the marker LAP2 (Wright et al., 2008, 2012). In NRVMs, nuclear  $\alpha_1$ -AR binding is also observed (Buu et al., 1993), and 73% of total  $\alpha_1$ -ARs are in noncaveolar membranes that might be nuclear (Lanzafame et al., 2006), in good agreement with the results in adult myocytes.

Second, BODIPY-prazosin is a fluorescent analog of the  $\alpha_1$ -AR antagonist prazosin that binds all three  $\alpha_1$ -AR subtypes with equal affinity and fluoresces only when bound to receptor (Daly et al., 1998; Mackenzie et al., 2000; Pediani et al., 2005). BODIPY-prazosin staining of living adult cardiac myocytes identifies endogenous  $\alpha_1$ -ARs on the nuclear membrane but does not detect receptors at the plasma membrane (Wright et al., 2008). Nuclei isolated from adult cardiac myocytes confirm positive BODIPY-prazosin staining of endogenous nuclear  $\alpha_1$ -ARs (Wright et al., 2012).

Third, a reconstitution system, in which  $\alpha_1$ -AR-GFP fluorescent fusion proteins are expressed in cultured adult  $\alpha_1$ ABKO cardiac myocytes, recapitulates the nuclear localization of the endogenous  $\alpha_1$ -ARs (Huang et al., 2007; Wright et al., 2008, 2012).

Studies in other cells provide some support for the results in cardiac myocytes. In recombinant cells expressing  $\alpha_1$ -ARs, for example, HEK293 cells, all  $\alpha_1$ -AR subtypes show some intracellular localization (Daly et al., 1998; Mackenzie et al., 2000; Chalothorn et al., 2002). In primary cultures of smooth muscle cells, endogenous  $\alpha_1$ -ARs are also found on both the plasma membrane and intracellular, using a fluorescent ligand, BODIPY-FL prazosin (Mackenzie et al., 2000).

**2. Mechanism of  $\alpha_1$ -Adrenergic Receptor Nuclear Localization.** The mechanism for nuclear targeting involves nuclear localization sequences embedded in the protein. These nuclear localization sequences typically consist of mono- or bi-partite basic residues, usually lysines and arginines or glycine-arginine repeats (Dono et al., 1998; Hock et al., 1998; Lu et al., 1998). Nuclear

localization sequences are recognized by a class of proteins known as importins that bind these sequences and facilitate transport of the target protein to the nucleus. This importin-mediated nuclear localization not only occurs for proteins that target to the nucleoplasm but for proteins that target the inner nuclear membrane as well (King et al., 2006; Cook et al., 2007; Lusk et al., 2007). Importin-mediated nuclear localization was previously described for the type 1 parathyroid hormone receptor (Pickard et al., 2006, 2007) and more recently for the gonadotropin-releasing hormone type 1 receptor (Re et al., 2010). Recent experiments identify nuclear localization sequences in the  $\alpha_1A$ - and  $\alpha_1B$ -subtypes, and mutation of these sequences results in loss of nuclear localization for each subtype in adult mouse cardiac myocytes (Wright et al., 2012).

**3. Receptor Orientation in the Inner Nuclear Membrane.** As described above, nuclear membrane proteins are targeted to the inner nuclear membrane through nuclear localization sequences, similar to proteins in the nucleoplasm. Also important is the orientation of inner nuclear membrane proteins, which could affect how they signal. For GPCRs, such as  $\alpha_1$ -ARs, if the ligand-binding domain faces the inside of the nucleus, the ligand would have to enter the nucleus and signaling would be initiated on the cytoplasmic side in the space between the inner and outer nuclear membranes. Conversely, if the ligand-binding domain faces the space between the outer and inner nuclear membranes, then signaling would be activated inside the nucleus.

Recent studies with nuclear GPCRs detect signaling in isolated nuclei, implying that nuclear receptors are likely oriented with the ligand-binding domain facing outward and the C terminus facing the nucleoplasm. ETRs induce calcium transients in isolated nuclei (Boivin et al., 2003), and  $\beta$ -ARs and ATRs induce transcriptional responses in isolated nuclei (Tadevosyan et al., 2010; Vaniotis et al., 2011). These studies suggest that nuclear GPCR signaling is activated inside the nucleus, thereby indicating an orientation in the inner nuclear membrane similar to GPCRs at the plasma membrane where the C terminus faces the cytoplasm.

**4. Catecholamine Uptake in Cardiac Myocytes.** A prerequisite for nuclear  $\alpha_1$ -AR signaling is that NE and other  $\alpha_1$ -AR ligands must traverse the plasma membrane, transit to the nucleus, and bind to and activate receptors in a time course consistent with signaling. In nonneuronal cells, this process is known as NE "uptake-2" (Obst et al., 1996) and is facilitated by extraneuronal monoamine transporter/organic cation transporter 3 (EMT/OCT3) (Zwart et al., 2001; Schomig et al., 2006). EMT/OCT3 is expressed most abundantly in heart (Zwart et al., 2001), where it is present on both the plasma and nuclear membranes in

adult cardiac myocytes (Wright et al., 2008). In neonatal myocytes, uptake of [ $^3$ H]NE is observed, but the time scale of nearly an hour before NE is detected in the nucleus is not sufficiently rapid to account for  $\alpha_1$ -AR signaling (Buu et al., 1993).

However, a more sensitive fluorescent-based catecholamine uptake assay shows that catecholamines are taken up very rapidly in cultured adult mouse cardiac myocytes. In this system, catecholamine uptake begins within seconds, is clearly increased by 5 minutes, peaks at 30 minutes, and is antagonized by addition of unlabeled NE 15 minutes prior to catecholamine uptake measurement, indicating specificity (Wright et al., 2008).

Further consistent with rapid uptake, the intrinsic uptake kinetics of OCT3, which is the rate at which one transporter moves a cation, show that OCT3-mediated cation transport is in the time frame of seconds. Thus, the uptake kinetics of catecholamines by recombinant OCT3 expressed in HEK293 cells is a  $V_{\max} \sim 30,000$  pmol/mg protein/min and a  $K_m \sim 900$   $\mu$ M for NE, and a  $V_{\max} \sim 13,000$  pmol/mg protein/min and a  $K_m \sim 500$   $\mu$ M for epinephrine (Duan and Wang, 2010). Catecholamine uptake is observed in seconds, with half-maximum response seen in  $\sim 2$  minute (Duan and Wang, 2010). It is likely that the kinetic properties of the transporter are relatively consistent from cell to cell and that expression level will dictate the absolute amount of uptake and OCT3 expression is highest in heart (Zwart et al., 2001).

In mice, OCT3-mediated heart uptake of the neurotoxin cation methyl-4-phenylpyridinium acetate is observed within minutes of infusion ( $\sim 4000$  ng/g tissue 5 minutes after infusion), and this uptake is inhibited by 75% in OCT3KO mice (Zwart et al., 2001), indicating a rapid and robust uptake system. The phenotype of OCT3KO mice is further interesting. Thus, OCT3KO mice have a trend toward reduced heart size in males [WT heart weight (HW) 160 mg, OCT3KO HW 145 mg,  $n = 7$ ,  $P = 0.138$ , a 10% reduction] (Zwart et al., 2001), reminiscent of the small heart phenotype seen in male  $\alpha_1$ ABKO mice (WT HW 147 mg,  $\alpha_1$ ABKO 122 mg,  $n = 33-27$ ,  $P < 0.05$ , a 17% reduction) (O'Connell et al., 2003), but the number of OCT3KO mice analyzed was small ( $n = 7$ ) (Zwart et al., 2001).

Thus, the kinetics of catecholamine uptake in myocytes (Wright et al., 2008) and the biochemistry and biology of OCT3 (Zwart et al., 2001; Duan and Wang, 2010) are consistent with  $\alpha_1$ -AR responses initiated by agonist activation of nuclear receptors.

In agreement with the idea that  $\alpha_1$ -AR agonist must be transported into the myocyte for signaling, there is a long latency of  $\alpha_1$ -AR responses after agonist addition, in contrast to the rapid onset for  $\beta$ -AR agonism. Specifically, the latency for contractile or calcium responses to  $\alpha_1$ -agonism in isolated myocytes is 2 to 5 minutes in nine studies (Tohse et al., 1990;

Terzic et al., 1992; Gambassi et al., 1998; Zhang et al., 1998; Woo and Lee, 1999; Ross et al., 2003; O-Uchi et al., 2005; Luo et al., 2007; Ichishima et al., 2010), rather than seconds as would be expected for a receptor at the sarcolemma.

Finally, there is functional evidence that agonist uptake is required for  $\alpha_1$ -AR signaling. Inhibition of EMT/OCT3-mediated catecholamine uptake with corticosterone, an EMT/OCT3 antagonist, prevents  $\alpha_1$ -AR activation of ERK in cultured adult mouse cardiac myocytes (Wright et al., 2008).

In summary, the kinetics of agonist uptake in myocytes, EMT/OCT3 biochemistry and biology, including kinetics, heart expression, and inhibition by corticosterone, and the latency of  $\alpha_1$ -AR physiologic responses are all consistent with agonist uptake and activation of nuclear  $\alpha_1$ -ARs.

*5. Functional Evidence for Nuclear  $\alpha_1$ -Adrenergic Receptor Signaling.*  $\alpha_1$ -ARs have numerous signaling effects in the cytosol, as reviewed later. Thus, if  $\alpha_1$ -ARs signal in the nucleus, then that signal must be transduced out of the nucleus to reach these cytosolic targets, defining an inside-out (nuclear-to-cytoplasmic) signaling mechanism. Three sets of data support the idea that  $\alpha_1$ -signaling is initiated in the nucleus. First, CGP-12177A [4-[3-[(1,1-dimethylethyl)amino]2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one hydrochloride], an  $\alpha_1$ -antagonist that does not cross membranes (Staehelin et al., 1983; Levin et al., 2002; Brahmadevara et al., 2003, 2004), does not block  $\alpha_1$ -AR-ERK signaling in cultured adult mouse cardiac myocytes, whereas the prototypical  $\alpha_1$ -AR antagonist prazosin, which freely crosses the plasma membrane, does block  $\alpha_1$ -AR-ERK signaling (Wright et al., 2008). Second, mislocalization mutants of both the  $\alpha_1A$ - and  $\alpha_1B$ -subtype, in which the nuclear localization sequences are mutated, do not activate ERK in cultured adult mouse cardiac myocytes (Wright et al., 2012). These mislocalization mutants are not redirected to the plasma membrane, which would provide a more crucial test of the requirement for nuclear localization, but the mutants do show that nuclear localization is required for  $\alpha_1$ -AR signaling in adult cardiac myocytes. Finally, activation of nuclear  $\alpha_1$ -ARs leads to the activation of ERK in caveolae at the plasma membrane (Wright et al., 2008), and the nuclear export inhibitor leptomycin B blocks  $\alpha_1$ -AR-mediated activation of ERK, suggesting that  $\alpha_1$ -AR signaling to ERK at caveolae must originate in the nucleus (Wright et al., 2012). How signals are transported from the nucleus to cytosolic targets is uncertain. However,  $\alpha_1$ -ARs activate PKC, a molecule known to translocate upon activation, suggesting a possible mechanism to transmit a signal out of the nucleus. Taken together, these studies provide functional evidence for  $\alpha_1$ -AR signaling initiated in the nucleus.



6. *Localization of Signaling Partners with  $\alpha_1$ -Adrenergic Receptors in Cardiac Myocyte Nuclei.* To effect nuclear  $\alpha_1$ -AR signaling in cardiac myocytes,  $\alpha_1$ -ARs must colocalize with downstream signaling partners in the nuclear membrane. However, their identities so far remain unclear.

A fraction of  $G_{\alpha_q}$  colocalizes with  $\alpha_1$ -ARs at the nucleus, based on immunocytochemistry in  $\alpha_1$ ABKO cardiac myocytes expressing  $\alpha_1$ -AR-GFP fluorescent fusion proteins, and on subcellular fractionation of WT adult cardiac myocytes (Wright et al., 2008). In NRVMs, approximately 56% of  $G_{\alpha_q}$  is in caveolar membranes defined by cav-3, and the remainder is in noncaveolar membranes, some of which might be nuclear (Morris et al., 2006).

A role in nuclear signaling is uncertain for  $PLC\beta_1$ , the classic  $\alpha_1$ -coupled PLC.  $PLC\beta_1$  is detected in the nuclei of adult cardiac myocytes using the G12  $PLC\beta_1$  antibody from Santa Cruz Biotechnology (Santa Cruz, CA) (Wright et al., 2008), but this and other commercial  $PLC\beta_1$  antibodies are not proven specific for  $PLC\beta_1$  using KO tissues. In NRVMs, 91% of  $PLC\beta_1$  is detected in a caveolar fraction using a Santa Cruz antibody (Lanzafame et al., 2006), and forced expression of  $PLC\beta_1b$  with an N-terminal enhanced GFP tag detects localization on the sarcolemma but not the nucleus (Grubb et al., 2008). Also in NRVMs, expression of a C-terminal peptide from  $PLC\beta_1b$  blocks  $\alpha_1$ - and  $G_q$ -mediated IP turnover and aspects of hypertrophy (Grubb et al., 2008; Filtz et al., 2009). Finally, the substrate for  $PLC\beta_1$ , phosphatidylinositol 4,5-bisphosphate, is not detected in nuclear membranes (Zhang et al., 2013). Taken together, these data suggest that  $PLC\beta_1$  might not be involved in  $\alpha_1$ -AR nuclear signaling.

An interesting alternate mediator of nuclear  $\alpha_1$ -AR signaling is  $PLC\epsilon$ , which might be regulated by small GTPases (Rho, Ras, Rap) and  $G\beta\gamma$  subunits, but not by  $G_{\alpha_q}$  (Lopez et al., 2001).  $PLC\epsilon$  in NRVMs and heart is scaffolded to muscle-specific A kinase-anchoring protein at the nuclear envelope with PKD, one key hypertrophic signaling molecule (Zhang et al., 2011, 2013). Knockdown of  $PLC\epsilon$  inhibits  $\alpha_1$ - and  $G_{\alpha_q}$ -stimulated hypertrophy in NRVMs, but has no effect on IP turnover, and expression of  $PLC\epsilon$  causes hypertrophy, an effect that requires  $PLC\epsilon$  catalytic activity (Zhang et al., 2011, 2013). Myocyte-specific  $PLC\epsilon$  KO also inhibits hypertrophy with pressure overload in vivo (Zhang et al., 2013). Extensive evidence suggests that  $PLC\epsilon$  mediates hypertrophy by hydrolysis of phosphatidylinositol 4-phosphate (PI4P) at the nuclear envelope, with generation of DAG and activation of PKD, but upstream mechanisms are uncertain (Zhang et al., 2013). Clearly, definition of nuclear  $\alpha_1$ -AR signaling partners is a promising area for the future.

7. *Nuclear  $\alpha_1$ -Adrenergic Receptor Localization in Vivo.* It is possible that localization observed in isolated or cultured cardiac myocytes does not reflect the true

localization of  $\alpha_1$ -ARs in vivo. As mentioned, overexpressed  $\alpha_1A$ -ARs in transgenic mice localize to the plasma membrane based on immunohistochemical staining for an epitope tag (Lin et al., 2001). However, very high receptor levels, about 170-fold over basal, might cause artifactual localization, and the lack of validated  $\alpha_1$ -AR antibodies (Jensen et al., 2009c) make conventional immunohistochemical approaches problematic.

Conversely, in a different  $\alpha_1A$ -subtype transgenic model, in which an  $\alpha_1A$ -subtype GFP fusion protein is expressed at a much lower level, approximately 5-fold over basal,  $\alpha_1$ -ARs are detected at the nuclei in ventricular tissue sections with a GFP antibody (Wright et al., 2008). This result with the  $\alpha_1A$ -GFP transgenic mice suggests that  $\alpha_1$ -AR nuclear localization observed in cultured cardiac myocytes can represent  $\alpha_1$ -AR localization in vivo.

8. *Pathophysiologic Implications of Nuclear  $\alpha_1$ -Adrenergic Receptor Signaling.* ETRs, ATRs, and  $\beta$ -ARs signal in isolated nuclei from adult cardiac myocytes (Boivin et al., 2003; Tadevosyan et al., 2010; Vaniotis et al., 2011). However, it is difficult to assign a functional significance to nuclear signaling by these GPCRs in cardiac myocytes, because the majority of ETRs, ATRs, and  $\beta$ -ARs localize to the plasma membrane (although quantitative ligand binding in subcellular fractions for ATRs is not possible due to low level of expression). Conversely, approximately 80% of  $\alpha_1$ -ARs localize to the nuclei in adult mouse cardiac myocytes. Interestingly, in pathologic settings,  $\alpha_1$ -AR signaling is clearly protective (sections IV and V), whereas ETR and ATR signaling can exacerbate pathologic remodeling (Harada et al., 1999; Yang et al., 2004). This raises the possibility that differences in receptor localization might lead to differences between physiologic and pathologic signaling. In other words, nuclear receptors, like  $\alpha_1$ -ARs, might be protective, whereas ETRs and ATRs at the plasma membrane might induce pathologic signaling (Wright et al., 2012). Although these ideas remain to be tested, differential localization of  $G_q$ -coupled receptors could have significant implications for their physiologic functions and for therapeutic targeting of  $G_q$ -coupled receptors in heart disease.

9. *Summary of  $\alpha_1$ -Adrenergic Receptor Nuclear Localization.* Overall, the majority of current data supports the idea that  $\alpha_1$ -ARs localize to and signal from the nuclei in adult cardiac myocytes in vitro and in vivo. Identification of functional nuclear localization sequences in each  $\alpha_1$ -subtype provides a mechanistic basis to support nuclear  $\alpha_1$ -AR localization, oriented with the C-terminal tail in the nucleoplasm. Ligand uptake into the cell via EMT/OCT3 provides a mechanism for receptor activation. The signaling mechanisms of nuclear  $\alpha_1$ -ARs remain unclear, as do the physiologic implications of nuclear versus

sarcolemmal signaling by  $\alpha_1$ -ARs and other  $G_q$ -coupled receptors.

#### IV. $\alpha_1$ -Adrenergic Receptor Physiologic Function in the Heart

In the heart, AR physiology is largely focused on acute  $\beta$ -AR mediated regulation of contractile function, whereas chronic  $\beta$ -AR signaling is maladaptive and  $\beta$ -AR antagonists are now standard therapy in heart failure. Short-term  $\alpha_1$ -AR signaling can increase contractility, as reviewed below, but this has not been studied in detail in vivo. On the other hand, many studies now indicate that chronic  $\alpha_1$ -AR signaling is adaptive, protecting the heart from pathologic stress through activation of physiologic hypertrophy, survival signaling, augmentation of contractility, and ischemic preconditioning. These data are described below.

##### A. $\alpha_1$ -Adrenergic Receptors Activate Physiologic or Adaptive Hypertrophy

Cardiac myocyte hypertrophy is the most common cellular response in the heart to pathologic stress, but hypertrophy is not always maladaptive (Frey and Olson, 2003). Cardiac hypertrophy occurs during normal physiologic development and in response to exercise and also as an adaptive response to pathologic stress. Physiologic or adaptive hypertrophy is characterized by an increase in heart and cardiac myocyte size without fibrosis and an overall improvement in function.

In contrast, pathologic or maladaptive hypertrophy is characterized by an increase in heart and cardiac myocyte size accompanied by combinations of cardiac cell death, fibrosis, vessel loss, reduced innervation, and, most importantly, declining function. Clinically, cardiac hypertrophy in Framingham adults is correlated with a significantly increased risk of heart failure and sudden death (Levy et al., 1990). Thirty years of research from cell culture to genetically modified mice, summarized below, demonstrates clearly that  $\alpha_1$ -ARs can mediate a physiologic or adaptive form of cardiac hypertrophy that offsets pathologic remodeling in heart failure.

1.  *$\alpha_1$ -Adrenergic Receptor-Mediated Hypertrophy in Cell Culture Models.* Primary cultures of NRVMs are the most common cell culture model used to examine signaling in cardiac myocytes. The original and now classic experiments (Glembotski, 2013) demonstrated that catecholamines acting through  $\alpha_1$ -ARs produce a direct trophic response in NRVMs (Simpson et al., 1982; Simpson, 1983, 1985). At the time, there was debate as to whether catecholamines induced hypertrophy through increasing blood pressure or through a direct action on cardiac myocytes, that is, whether myocyte hypertrophy was regulated in some way only by "load" or whether growth factors and their receptors

were involved, as in other types of cells. These papers were the first demonstration that catecholamines induce cardiac myocyte hypertrophy directly. This finding was later confirmed in cultured adult rat and cat cardiac myocytes (Simpson, 1988; Fuller et al., 1990; Ikeda et al., 1991; Volz et al., 1991; Clark et al., 1993).

Cardiac hypertrophy is clearly linked to induction of gene transcription, and in NRVMs,  $\alpha_1$ -ARs induce a pattern of hypertrophic gene transcription characterized by re-expression of genes normally expressed only in the fetal heart (Simpson et al., 1989). Early studies identified a group of these "fetal genes" induced by  $\alpha_1$ -ARs, including c-myc (Starksen et al., 1986), atrial natriuretic factor (Knowlton et al., 1991, 1993),  $\alpha$ -skeletal actin ( $\alpha$ SkAct) (Bishopric et al., 1987; Long et al., 1989; Karns et al., 1995), and  $\beta$ -myosin heavy chain ( $\beta$ MyHC) (Waspe et al., 1990; Kariya et al., 1993, 1994).

A crucial study of endogenous transcription in intact NRVMs proved that  $\alpha_1$ -ARs stimulate transcription not only of a fetal gene ( $\alpha$ SkAct), but also all RNA species, including the mRNA for an adult gene (cardiac actin), and total RNA (ribosomal and transfer) (Long et al., 1989). Several subsequent studies focusing on  $\alpha_1$ -AR-mediated transcriptional regulation delineated a host of transcriptional factors and modifiers activated by  $\alpha_1$ -ARs in cardiac myocytes, including TEF-1 (Kariya et al., 1993, 1994; Karns et al., 1995; McLean et al., 2003), GATA-4 (Morimoto et al., 2000; Liang et al., 2001a,b), Egr-1 (Jin et al., 2000), Elk 1 (McWhinney et al., 2000), Vgl-4 (Chen et al., 2004), Rlf (Post et al., 2002), CREB (Markou et al., 2004), Zfp260 (Debrus et al., 2005), and class 2 histone deacetylase (Vega et al., 2004; Liu et al., 2009).

Mechanistically, the  $\alpha_1A$ -subtype is implicated in  $\alpha_1$ -AR mediated hypertrophy in NRVMs through the use of  $\alpha_1$ -AR subtype-specific pharmacologic agents (Autelitano and Woodcock, 1998). Several mechanisms for  $\alpha_1$ -AR-mediated hypertrophic signaling are proposed, and a multitude of signal transducers are implicated (Jensen et al., 2011). Certain molecules, based on frequency in the literature, might be considered essential or "core" molecules required for  $\alpha_1$ -AR-mediated hypertrophic signaling, including PLC (Filtz et al., 2009; Zhang et al., 2013), PKC ( $\alpha$ ,  $\delta$ , and  $\epsilon$ , three main isotypes activated by  $\alpha_1$ -ARs) (Henrich and Simpson, 1988; Kariya et al., 1991, 1993, 1994; Karns et al., 1995; Haworth et al., 2000; Rohde et al., 2000; Braz et al., 2002, 2004; Vega et al., 2004; Carnegie et al., 2008), PKD (Haworth et al., 2000; Vega et al., 2004; Harrison et al., 2006; Avkiran et al., 2008; Bossuyt et al., 2008, 2011; Carnegie et al., 2008; Liu et al., 2009), ERK (Bueno et al., 2000; Xiao et al., 2001; Barron et al., 2003; O'Connell et al., 2003), and class 2 histone deacetylase (Vega et al., 2004; Backs et al., 2006, 2008; Harrison et al., 2006; Liu et al., 2009).

Evidence also exists that transactivation of the EGFR is involved in  $\alpha_1$ -mediated hypertrophy (Morris et al., 2004; Guo et al., 2009; Li et al., 2011; Papay et al., 2013).

In total,  $\alpha_1$ -mediated hypertrophy in NRVMs is characterized by  $\alpha_1$ A-subtype-mediated activation of the "fetal gene program" along with general increases in transcription of all RNA species and protein synthesis (Simpson, 1985; Long et al., 1989). Because the fetal gene program is often associated with pathologic hypertrophy, it was believed originally that  $\alpha_1$ -ARs induce a pathologic hypertrophy, similar to high levels of  $G_q$  overexpression (Dorn and Brown, 1999). This idea proved to be incorrect.

**2.  $\alpha_1$ -Adrenergic Receptor-Mediated Hypertrophy in Animal Models.** Early studies in mice, cats, and dogs showed that long-term catecholamine infusion in various species and at doses that do not increase blood pressure produces cardiac hypertrophy in vivo, which is "physiological," in that cardiac function is normal or improved and there is no fibrosis (Laks et al., 1973; King et al., 1987; Marino et al., 1991; Patel et al., 1991; Stewart et al., 1992; Vecchione et al., 2002). Whereas these studies suggest clearly that activation of ARs induces cardiac hypertrophy directly, which was debated at the time, the lack of subtype-specific AR pharmacologic agents limits mechanistic insight. However, in preliminary experiments, infusion of a subpressor dose of an  $\alpha_1$ A agonist in mice can increase fetal gene expression (unpublished data).

The advent of transgenic mouse technology provided a platform to address AR subtype-specific function in vivo, and transgenic gain-of function and gene deletion loss-of-function models have mostly confirmed cell culture studies indicating that  $\alpha_1$ -ARs regulate hypertrophy, with some prominent exceptions.

Cardiac myocyte-specific transgenic overexpression of the WT  $\alpha_1$ A-subtype, even at very high levels (148- to 170-fold), does not alter heart size, although  $\alpha_1$ A transgenic mice eventually develop dilated cardiomyopathy and die prematurely (Lin et al., 2001; Chaulet et al., 2006). On the other hand, transgenic overexpression of constitutively active mutant (CAM)  $\alpha_1$ A with the endogenous  $\alpha_1$ A-promoter induces cardiac hypertrophy without an effect on systemic blood pressure (Papay et al., 2013).

Similarly, the  $\alpha_1$ B-subtype shows a variable ability to induce hypertrophy when overexpressed, depending on the model. Overexpression of a CAM of the  $\alpha_1$ B-subtype with the  $\alpha$ -myosin heavy chain ( $\alpha$ MyHC) promoter at low levels (2- to 3-fold) induces hypertrophy (Milano et al., 1994) and exacerbates pathologic remodeling after aortic constriction (Wang et al., 2000). Likewise, systemic overexpression of a CAM  $\alpha_1$ B with the endogenous  $\alpha_1$ B-promoter also induces cardiac hypertrophy, along with *hypotension*, and a decreased pressor response, clearly dissociating hypertrophy from

blood pressure (Zuscik et al., 2001). In the same study, overexpression of a wild-type (WT)  $\alpha_1$ B with the endogenous  $\alpha_1$ B-promoter shows a lesser degree of hypertrophy (Zuscik et al., 2001). More recently, a different result was found in that overexpression of the CAM  $\alpha_1$ B caused hypertrophy, but only in older mice, and hypertrophy was associated with fibrosis (Papay et al., 2013). Furthermore, hypertrophy seen in both the CAM  $\alpha_1$ A and  $\alpha_1$ B mice was not observed when the mice were interbred to derive a systemic CAM  $\alpha_1$ AB transgenic mouse (Papay et al., 2013). In contrast with these results, relatively high-level overexpression of the WT  $\alpha_1$ B with the  $\alpha$ MyHC promoter (>40-fold) does not induce hypertrophy but results in dilated cardiomyopathy and death (Akhter et al., 1997; Grupp et al., 1998; Iaccarino et al., 2001; Lemire et al., 2001).

$\alpha_1$ -AR gene-deletion models are reviewed in detail (Simpson, 2006). In brief, heart size is not different in the  $\alpha_1$ A-knockout on a mixed FVB/129SvJ background ( $\alpha_1$ AKO) (Rokosh and Simpson, 2002) or in the  $\alpha_1$ B-knockout on a mixed C57Bl/6/129SvJ background ( $\alpha_1$ BKO) (Cavalli et al., 1997). Knockout of the  $\alpha_1$ D-subtype, which is not expressed in cardiac myocytes, also has no effect on heart size (Tanoue et al., 2002; Chalothorn et al., 2003; Hosoda et al., 2005).

However, double knockout of both the  $\alpha_1$ A- and  $\alpha_1$ B-subtypes, which eliminates  $\alpha_1$ -AR binding in the heart, on a congenic C57Bl/6J background ( $\alpha_1$ ABKO) causes a 15% reduction in heart and cardiac myocyte size during normal postnatal development (O'Connell et al., 2003). Mechanistically, ERK activity is reduced 30% in  $\alpha_1$ ABKO hearts, and  $\alpha_1$ -mediated activation of ERK is absent in cultured  $\alpha_1$ ABKO cardiac myocytes, suggesting that  $\alpha_1$ -AR-ERK signaling might regulate hypertrophic growth during postnatal development (O'Connell et al., 2003). Importantly,  $\alpha_1$ ABKO mice have normal basal blood pressure, normal body and organ weights, normal home cage locomotor activity, and normal overall health (O'Connell et al., 2003, 2006). These data show that any effects of the  $\alpha_1$ A- and  $\alpha_1$ B-subtypes on maintenance of basal blood pressure can be compensated by other receptors, but that heart growth requires the  $\alpha_1$ A and/or  $\alpha_1$ B. Maximal contractile responses to phenylephrine in  $\alpha_1$ ABKO isolated arteries are reduced by 35% in carotid and by 77% in mesenteric, with little or no changes in  $pEC_{50}$ , indicating compensation by  $\alpha_1$ D (Methven et al., 2009). We have not tested  $\alpha_1$ -mediated pressor response in the intact  $\alpha_1$ ABKO, but they are reduced in both the  $\alpha_1$ AKO and  $\alpha_1$ BKO (Cavalli et al., 1997; Rokosh and Simpson, 2002).

Interestingly, a preliminary re-examination of the  $\alpha_1$ -AR single knockouts on a congenic C57Bl/6J background reveals that  $\alpha_1$ BKO mice have small hearts, similar to the  $\alpha_1$ ABKO, suggesting that the  $\alpha_1$ B-subtype alone is required for *physiologic* postnatal growth of the heart (unpublished data). In support of

this, subpressor catecholamine infusion does not cause hypertrophy in an  $\alpha_1$ BKO on a mixed genetic background (Vecchione et al., 2002).

Aortic constriction in the  $\alpha_1$ ABKO mice results in a worse dilated cardiomyopathy, with fibrosis, apoptosis, decreased contractility, and increased mortality contrasted to WT mice (O'Connell et al., 2006). Interestingly, the final degree of hypertrophy in  $\alpha_1$ ABKO hearts after aortic constriction is similar to that in WT hearts, but induction of the fetal gene program is lost (O'Connell et al., 2006). This shows that the absence of  $\alpha_1$ -ARs exacerbates *pathologic* hypertrophic responses and that induction of the fetal-gene program can be uncoupled from pathologic hypertrophy.

**3. Summary of  $\alpha_1$ -Adrenergic Receptor in Hypertrophy.** Early studies in NRVMs demonstrated that the  $\alpha_1$ A-subtype induces hypertrophy with activation of the fetal-gene program and overall RNA and protein synthesis. In vivo, infusions of subpressor doses of catecholamines cause a physiologic hypertrophy but generally do not pinpoint which  $\alpha_1$ -subtype might be responsible. Although studies from  $\alpha_1$ -AR transgenic mice provide inconsistent results, studies from  $\alpha_1$ -AR knockout mice suggest that the  $\alpha_1$ B-subtype is required for hypertrophic growth during postnatal cardiac development, a period of physiologic heart growth, and that  $\alpha_1$ -ARs are not required for *pathologic* hypertrophy after aortic constriction. In fact, pathologic hypertrophy is worse in the absence of  $\alpha_1$ -ARs.

Therefore, in vivo studies, either with catecholamine infusion or  $\alpha_1$ -AR knockout mice, collectively suggest that  $\alpha_1$ -ARs stimulate an adaptive or *physiologic* hypertrophy, with no decrease in contractile function. Interestingly, findings from NRVMs suggesting that  $\alpha_1$ -ARs induce pathologic hypertrophy based on activation of the fetal gene program are not supported by findings in  $\alpha_1$ ABKO mice. In  $\alpha_1$ ABKO mice, activation of the fetal gene program is absent despite significant hypertrophy and worse cardiomyopathy after aortic constriction.

A new consideration with regard to the fetal gene program is that a classic fetal gene,  $\beta$ -MyHC, is re-expressed only in a minor subpopulation of cardiac myocytes in the mouse heart after aortic constriction, and the myocytes with  $\beta$ -MyHC are smaller than the cells with  $\alpha$ -MyHC, not larger (Lopez et al., 2011). These data question whether fetal genes are even markers of hypertrophy or pathology (Lopez et al., 2011).

### *B. $\alpha_1$ -Adrenergic Receptors Prevent Cardiac Myocyte Death*

Cell death, either apoptotic, necrotic, or autophagic, plays a significant role in the development of heart failure (Guerra et al., 1999; Kostin et al., 2003; Wencker et al., 2003; Baines et al., 2005; Foo et al., 2005; Nakayama et al., 2007; Nishida and Otsu, 2008;

Baines, 2010; Whelan et al., 2010; Nemchenko et al., 2011). Whereas substantial evidence indicates that  $\beta_1$ -ARs induce cell death, a growing body of research summarized below indicates that  $\alpha_1$ -ARs prevent cardiac myocyte cell death in direct opposition to  $\beta_1$ -ARs.

**1.  $\alpha_1$ -Adrenergic Receptor-Mediated Myocyte Survival Signaling in Cell Culture Models.** In cultured cardiac myocytes, NE stimulates apoptotic cell death through activation of  $\beta_1$ -ARs, whereas  $\beta_2$ -ARs are believed to be cytoprotective (Mann et al., 1992; Xiao et al., 2004). Interestingly, several studies indicate that  $\alpha_1$ -ARs are also cytoprotective and act antithetically to  $\beta_1$ -ARs. In NRVM, the  $\alpha_1$ -AR agonist phenylephrine inhibits apoptosis induced by the  $\beta$ -agonist isoproterenol (Iwai-Kanai et al., 1999; Zhu et al., 2000), nonhydrolyzable cAMP analogs (Iwai-Kanai et al., 1999; Zhu et al., 2000), hypoxia (Zhu et al., 2000), serum starvation (Zhu et al., 2000), 2-deoxyglucose (Valks et al., 2002), and doxorubicin (Aries et al., 2004). Similar results are observed in cultured adult rat cardiac myocytes, where NE-induced apoptosis is abolished by the  $\beta$ -AR antagonist propranolol, but not the  $\alpha_1$ -AR antagonist prazosin (Communal et al., 1998; O'Connell et al., 2006).

Mechanistically, a variety of pathways are implicated in  $\alpha_1$ -AR-mediated survival signaling in cardiac myocytes.  $\alpha_1$ -AR survival signaling requires activation of ERK and subsequent regulation of Bcl-2 family members to stabilize the mitochondrial membrane (Iwai-Kanai et al., 1999; Zhu et al., 2000; Valks et al., 2002; Communal et al., 2003; Huang et al., 2007; Wright et al., 2008, 2012). In NRVM, phenylephrine inhibits apoptosis induced by serum starvation and hypoxia by preventing downregulation of Bcl-2 and Bcl-X mRNA and protein levels (Zhu et al., 2000). In addition, phenylephrine induces the phosphorylation of Bcl-2 family member Bad at Ser112 and Ser155, preventing 2-deoxyglucose-induced apoptosis (Valks et al., 2002). Interestingly, cAMP-dependent protein kinase (PKA), known to be downstream of  $\beta_1$ -ARs, also stimulates phosphorylation of Bad at Ser136 (Valks et al., 2002). This finding might suggest that both  $\beta_1$ -AR and  $\alpha_1$ -AR signaling converge on a single molecule to modulate cell survival, implying that the balance between  $\beta_1$ - and  $\alpha_1$ -AR signaling could control cell fate. However, how the interplay between  $\alpha_1$ -AR and  $\beta$ -AR phosphorylation of Bad impacts cardiac myocyte survival is unclear.

Other studies propose a role for ERK as a regulator of  $\alpha_1$ -AR survival signaling. ERK mediates cytoprotective signaling in cardiac myocytes (Lips et al., 2004), and  $\alpha_1$ -AR mediated activation of ERK is a well-characterized signaling pathway involved in hypertrophy (Bueno et al., 2000; Xiao et al., 2001; Barron et al., 2003; O'Connell et al., 2003). In NRVM, the MEK-1 inhibitor PD098059 [2-(2-amino-3-methoxyphenyl)-4H-

1-benzopyran-4-one] negates phenylephrine-mediated survival signaling (Iwai-Kanai et al., 1999). Furthermore, in cultured adult rat cardiac myocytes, NE-mediated activation of ERK upregulates  $\beta_1$ -integrin and protects cells against  $\beta_1$ -AR-mediated apoptosis (Communal et al., 2003).

The most convincing evidence for ERK in  $\alpha_1$ -mediated survival signaling comes from  $\alpha_1$ ABKO myocytes. Cultured  $\alpha_1$ ABKO mouse myocytes have markedly increased necrosis and apoptosis with toxic stimuli, including hydrogen peroxide, doxorubicin, and  $\beta$ -AR stimulation (O'Connell et al., 2006; Huang et al., 2007). This sensitivity to death stimuli in  $\alpha_1$ ABKO myocytes is rescued by expression of the  $\alpha_1A$ -subtype but not by the  $\alpha_1B$ , indicating that the  $\alpha_1A$  is necessary and sufficient for myocyte survival (Huang et al., 2007). Furthermore, rescue is mimicked by expression of constitutively activated MEK, which increases ERK activity, and rescue by the  $\alpha_1A$  is prevented by dominant negative MEK, which inhibits ERK (Huang et al., 2007). Together, these experiments define an  $\alpha_1A$ -ERK pathway for myocyte survival (Huang et al., 2007).

Potential mediators downstream of ERK include p90Rsk, a potential kinase for  $\alpha_1$ -AR-mediated phosphorylation of Ser112 in Bad (Valks et al., 2002), and the transcription factors GATA4 and nuclear factor of activated T cells (NFAT) (Pu et al., 2003; Aries et al., 2004).

Overall, the data from cultured cardiac myocytes indicate that  $\alpha_1$ -ARs mediate survival signaling, potentially through  $\alpha_1A$  activation of ERK, leading to regulation of Bcl-2 family members and stabilization of the mitochondrial membrane, and/or by induction of GATA4 and NFAT.

*2.  $\alpha_1$ -Adrenergic Receptor-Mediated Myocyte Survival Signaling in Animal Models.* In mice, cats, and dogs, long-term infusion of subpressor doses of the mixed  $\alpha_1/\beta$ -AR agonist NE produces an adaptive hypertrophy without increased cell death or fibrosis (Laks et al., 1973; King et al., 1987; Marino et al., 1991; Patel et al., 1991; Stewart et al., 1992; Vecchione et al., 2002). Furthermore,  $\alpha_1$ -AR stimulation in isolated, perfused hearts prevents ischemia-reperfusion-induced cell apoptosis and necrosis in mice (Tejero-Taldo et al., 2002), rats (Banerjee et al., 1993; Mitchell et al., 1995; Tosaki et al., 1995; Meng et al., 1996a,b, 1999; Meldrum et al., 1997; Imani et al., 2008), rabbits (Bankwala et al., 1994; Tsuchida et al., 1994; Cope et al., 1997; Baghelai et al., 1999a,b), and dogs (Kitakaze et al., 1987, 1991, 1994; Node et al., 1997).

However, gain-of-function  $\alpha_1$ -AR transgenic models are inconsistent in demonstrating that  $\alpha_1$ -ARs protect against cardiac cell death. Cardiac myocyte-specific transgenic overexpression of the  $\alpha_1A$ -subtype at high levels (66-fold) protects against pathologic stress from pressure overload induced by aortic constriction and

ischemic injury induced by coronary artery ligation, although the mechanism is linked to a basal hypercontractile phenotype rather than prevention of cardiac myocyte death (Du et al., 2004, 2006). Moreover, by 1 year, ventricles from these  $\alpha_1A$ -transgenic mice show increased fibrosis and apoptotic labeling, and the mice die prematurely, indicating that long-term, very high-level  $\alpha_1A$ -subtype overexpression can be associated with cell death rather than survival signaling (Chaulet et al., 2006).

Although transgenic overexpression of the  $\alpha_1B$ -subtype shows a variable ability to induce hypertrophy, prolonged overexpression of the  $\alpha_1B$ -subtype in some models, although not directly linked to increased cell death, induces a pathologic remodeling (Grupp et al., 1998; Iaccarino et al., 2001; Lemire et al., 2001; Wang et al., 2000). The failure of these gain-of-function models to recapitulate the findings in NRVM or in other animal models might be linked to the high levels of overexpression in most of these models or failure of overexpressed receptors to recapitulate signaling by ligand-activated endogenous receptors.

Conversely, loss-of-function models clearly indicate that  $\alpha_1$ -ARs prevent cardiac myocyte cell death. In  $\alpha_1$ ABKO mice, which lack the two  $\alpha_1$ -AR subtypes expressed in cardiac myocytes, aortic constriction induces a worse dilated cardiomyopathy, accompanied by a significant increase in cardiac cell apoptosis and fibrosis, leading to decreased function and increased mortality compared with WT. Cultured  $\alpha_1$ ABKO cardiac myocytes have increased susceptibility to several pro-death agonists, as reviewed in the preceding section (O'Connell et al., 2006; Huang et al., 2007, 2008), and this is rescued by adenoviral mediated reconstitution of the  $\alpha_1A$ -subtype, but not the  $\alpha_1B$ -subtype, in a pathway that requires ERK (Huang et al., 2007). The absence of this  $\alpha_1A$ -subtype ERK survival signaling might explain, at least partially, the negative outcome in  $\alpha_1$ ABKO mice subjected to aortic constriction (O'Connell et al., 2006).

In support of the finding that the  $\alpha_1A$ -subtype is both sufficient and necessary to prevent cardiac myocyte death, long-term infusion of a subpressor concentration of the  $\alpha_1A$ -subtype-specific agonist A61603 [*N*-[5-(4,5-dihydro-1*H*-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulphonamide hydrobromide] prevents cell death and pathologic remodeling associated with doxorubicin-induced cardiotoxicity (Chan et al., 2008; Dash et al., 2011).

*3. Summary of  $\alpha_1$ -Adrenergic Receptor-Mediated Myocyte Survival Signaling.* Studies in cultured neonatal and adult cardiac myocytes show that  $\alpha_1$ -ARs mediate survival signaling, most likely through activation of ERK and subsequent regulation of Bcl-2 family members to preserve mitochondrial membrane stability, as well as induction of protective transcription factors, such as GATA4 and NFAT, with their own

downstream effectors. These findings generally support early studies in which catecholamine infusion in several animal models stimulated an adaptive hypertrophy without cell death or fibrosis. However, gain-of-function studies in  $\alpha_1$ A- and  $\alpha_1$ B-transgenic mouse models are inconsistent with regard to survival signaling, possibly due to massive levels of overexpression and/or aberrant signaling by constitutively activated receptors. More importantly, loss-of-function models, particularly  $\alpha_1$ ABKO mice, support the notion that  $\alpha_1$ -ARs mediate survival signaling. Furthermore, studies in cultured  $\alpha_1$ ABKO cardiac myocytes with reconstitution of the  $\alpha_1$ A-subtype define an  $\alpha_1$ A-subtype ERK survival signaling pathway, the absence of which could at least partially explain the maladaptive responses to pathologic stress in  $\alpha_1$ ABKO mice.

### C. $\alpha_1$ -Adrenergic Receptors Augment Contractile Function

Contractile dysfunction is a major component of and a causative factor in heart failure progression. Furthermore, as  $\beta$ -AR-mediated inotropy declines in heart failure due to receptor desensitization and down-regulation,  $\alpha_1$ -AR mediated inotropy, which contributes little to basal contractile function, might function in a compensatory role to preserve contractile function in the failing heart.

1.  *$\alpha_1$ -Adrenergic Receptor Activation of Contraction in In Vitro Models.* In many species,  $\alpha_1$ -ARs induce a positive inotropic response in left ventricular myocytes, trabeculae, and the perfused heart (Endoh and Blinks, 1988; Terzic et al., 1992; Turnbull et al., 2003). However, in mice, the  $\alpha_1$ -AR inotropic response can be negative in a few left ventricular preparations, including isolated papillary muscles and a minority of isolated cardiac myocytes (Hirano et al., 2006; Chu et al., 2013). Interestingly, populations of cardiac myocytes from the right and left ventricle have a fraction of myocytes that have a positive inotropic response to phenylephrine and an increased  $\text{Ca}^{2+}$  transient, prominent in the left ventricle, and a fraction of myocytes that have a negative inotropic response to phenylephrine and a decreased  $\text{Ca}^{2+}$  transient, mainly in the right ventricle (Chu et al., 2013).

Another unexpected finding in mouse heart is that  $\alpha_1$ -ARs mediate negative inotropy in myocardium from the normal right ventricle but positive inotropy in left ventricular myocardium (Wang et al., 2010). Surprisingly, in heart failure caused by myocardial infarction,  $\alpha_1$ -AR inotropy in right ventricular myocardium switches from negative to positive, and  $\alpha_1$ -AR positive inotropy in left ventricular myocardium is preserved (Litwin et al., 1995; Wang et al., 2010). These two aspects of  $\alpha_1$ -AR-mediated positive inotropy in heart failure after myocardial infarction can be interpreted as adaptive, that is, undiminished inotropy in the left ventricle (Litwin et al., 1995; Wang et al., 2010) and

the appearance of positive inotropy in the right ventricle (Wang et al., 2010). Notably, right ventricular failure predicts worse outcomes in patients with left ventricular failure (Bleasdale and Frenneaux, 2002).

Interestingly, some find that the  $\alpha_1$ A- and  $\alpha_1$ B-subtypes differentially regulate contraction, with the  $\alpha_1$ A-subtype mediating a positive response and the  $\alpha_1$ B-subtype a negative response (Gambassi et al., 1998; Lin et al., 2001; Ross et al., 2003; O-Uchi et al., 2008).

A multitude of mechanisms are proposed to explain  $\alpha_1$ -AR-mediated positive or negative inotropy. Proposed mechanisms include inhibition of outward  $\text{K}^+$  currents and increased action potential duration (Apkon and Nerbonne, 1988; Fedida et al., 1989, 1990, 1991; Ravens et al., 1989; Tohse et al., 1990, 1992; Wang et al., 1991, 2001; Braun et al., 1992; Sato and Koumi, 1995; Gaughan et al., 1998); inhibition of L-type  $\text{Ca}^{2+}$  channel current (Chen et al., 1996; Gaughan et al., 1998; Belevych et al., 2001) or activation (Zhang et al., 1998; Mohl et al., 2011; Chu et al., 2013); activation of the  $\text{Na}^+/\text{H}^+$  exchanger and intracellular acidification (Gambassi et al., 1992; Terzic et al., 1992); and regulation of myofilament  $\text{Ca}^{2+}$  sensitivity through phosphorylation of myosin light chain and/or cardiac troponin I (Hartmann et al., 1995; Andersen et al., 2002; McCloskey et al., 2003; MacGowan et al., 2005; Wang et al., 2006, 2010).

2.  *$\alpha_1$ -Adrenergic Receptor-Mediated Contraction in Transgenic and Gene-Deletion Mouse Models.* Cardiac myocyte-specific transgenic overexpression of the  $\alpha_1$ A-subtype at high levels (148- to 170-fold) increases basal contractile function (Lin et al., 2001) and limits pathologic remodeling from pressure overload and ischemic injury (Du et al., 2004, 2006). These results suggest that the  $\alpha_1$ A-subtype mediates positive inotropic responses, in agreement with recent in vitro studies (Mohl et al., 2011; Chu et al., 2013). Conversely, transgenic overexpression of the  $\alpha_1$ B-subtype can be associated with depressed contractile function and pathologic remodeling in the heart (Grupp et al., 1998; Wang et al., 2000; Iaccarino et al., 2001; Lemire et al., 2001).

Loss-of-function models suggest that  $\alpha_1$ -AR-mediated inotropic responses are not required for basal contractile function, but might prevent contractile decline in response to pathologic stress. Basal contractile function by echocardiography is normal in both  $\alpha_1$ AKO and  $\alpha_1$ BKO mice (Rokosh and Simpson, 2002; Vecchione et al., 2002). In a similar fashion, basal contractile function assessed by echocardiography in  $\alpha_1$ ABKO mice is similar to WT, although cardiac output is decreased, due to a slight bradycardia and reduced left-ventricular volume, and exercise performance is impaired, presumably for the same reasons (O'Connell et al., 2003). Interestingly, calcium sensitivity is increased and maximal force is decreased in isolated

muscle strips from  $\alpha_1$ ABKO mice, suggesting subtle abnormalities in basal contractile function with long-term absence of  $\alpha_1$ -ARs (McCloskey et al., 2003). More strikingly, contractile function is impaired significantly by aortic constriction in  $\alpha_1$ ABKO mice (O'Connell et al., 2006). This contractile dysfunction might be caused by both the loss of  $\alpha_1$ -AR-mediated positive inotropy and the absence of  $\alpha_1$ -AR-mediated survival signaling and adaptive hypertrophic effects, such as increased myosin synthesis. The exaggerated contractile dysfunction in the  $\alpha_1$ ABKO confirms that  $\alpha_1$ -AR-mediated inotropy could play an important protective role in response to pathologic stress in the heart.

**3.  $\alpha_1$ -Adrenergic Receptor Activation of Contraction in Humans.** In healthy young women, systemic infusion of the  $\alpha_1$ -AR agonist methoxamine increases contractility determined noninvasively (Curiel et al., 1989). In both healthy patients and patients with New York Heart Association (NYHA) II–IV heart failure, infusion of the  $\alpha_1$ -AR agonist phenylephrine into the left main coronary artery increases contractility measured as dp/dt, demonstrating  $\alpha_1$ -AR inotropy in both healthy and heart failure patients (Landzberg et al., 1991). Interestingly, in the same patients, the  $\alpha_1$ -AR antagonist phentolamine shows no effect on baseline contractile function, suggesting that there is little contribution of  $\alpha_1$ -AR inotropy to basal contractile function in humans (Landzberg et al., 1991).

Surprisingly,  $\alpha_1$ -AR-mediated inotropy can equal  $\beta$ -AR-mediated inotropy in trabeculae isolated from failing human hearts (Skomedal et al., 1997). This suggests that in human heart failure, as  $\beta$ -ARs are desensitized and downregulated,  $\alpha_1$ -AR mediated contractility might act in a compensatory role to maintain contractile function. Similarly, inhalation of the  $\alpha_1$ -AR agonist methoxamine improves exercise performance in patients with significant left ventricular contractile dysfunction (Cabanès et al., 1992). Furthermore, in a small cohort of patients with hypotension due to end stage heart failure, the use of the  $\alpha_1$ -AR agonist midodrine is associated with increased contractile function (Zakir et al., 2009). The authors attributed the benefit to a modest increase in blood pressure that resulted from activation of vascular  $\alpha_1$ -ARs, permitting up-titration of recommended heart failure medications (Zakir et al., 2009). However, it is possible that direct activation of cardiac  $\alpha_1$ -ARs contributed to this beneficial effect.

**4. Summary of  $\alpha_1$ -Adrenergic Receptor Activation of Contraction.** Studies with isolated myocytes, muscle strips, and perfused hearts show that  $\alpha_1$ -ARs can induce either positive or negative inotropic responses by altering  $K^+$  and  $Ca^{2+}$  currents, intracellular pH, and myofilament  $Ca^{2+}$  sensitivity. Mouse models confirm that the  $\alpha_1A$ -subtype can induce positive inotropic responses in vitro and in vivo and that  $\alpha_1A$ -subtype-mediated inotropy might protect the heart from pathologic

stress. Importantly,  $\alpha_1$ -AR-mediated positive inotropy is documented in intact humans and can be equal to  $\beta$ -AR inotropy in isolated trabeculae from human heart failure patients, identifying a clinically relevant adaptive function for  $\alpha_1$ -ARs.

#### *D. $\alpha_1$ -Adrenergic Receptors Induce Ischemic Preconditioning*

Ischemic preconditioning is an intrinsic protective mechanism in the heart whereby transient periods of ischemia protect the myocardium from damage due to longer bouts of ischemia, and protection can be observed both early (minutes to hours after ischemia) and late (hours to days). Pharmacologic agents can also induce preconditioning, and  $\alpha_1$ -ARs are among the most effective (Jensen et al., 2011).

**1.  $\alpha_1$ -Adrenergic Receptor-Mediated Preconditioning in Animal Models.** In several animal models, including dog (Kitakaze et al., 1987, 1991, 1994; Node et al., 1997), rabbit (Bankwala et al., 1994; Tsuchida et al., 1994; Cope et al., 1997; Baghelai et al., 1999a,b), rats (Banerjee et al., 1993; Mitchell et al., 1995; Tosaki et al., 1995; Meng et al., 1996a,b, 1999; Meldrum et al., 1997; Imani et al., 2008) and mice (Tejero-Taldo et al., 2002),  $\alpha_1$ -ARs induce both early and late preconditioning, through a variety of mechanisms including adenosine release (Kitakaze et al., 1991), activation of 5'-nucleotidase activity (Kitakaze et al., 1994; Node et al., 1997), activation of PKC (Tsuchida et al., 1994; Mitchell et al., 1995; Node et al., 1997; Meng et al., 1999), regulation of Bcl2 family members (Baghelai et al., 1999a), induction of heat-shock proteins and protein synthesis (Meng et al., 1996a,b), activation of mitochondrial K-ATP channels (Imani et al., 2008), and induction of iNOS (Tejero-Taldo et al., 2002; Zhao et al., 2012). Once again though, poor specificity of antagonists for the  $\alpha_1$ -subtypes has made it difficult to define the  $\alpha_1$ -AR subtype(s) responsible for ischemic preconditioning.

However, transgenic overexpression of constitutively active mutants of the  $\alpha_1A$ - and  $\alpha_1B$ -subtypes reveals that the  $\alpha_1A$ -subtype, but not the  $\alpha_1B$ , mediates ischemic preconditioning that might not involve PKC (Rorabaugh et al., 2005). Preconditioning by the  $\alpha_1A$  in a rat transgenic model could be mediated by MEK/ERK phosphorylation and iNOS (Zhao et al., 2012). Similarly, cardiac myocyte-specific transgenic overexpression of the  $\alpha_1A$ -subtype suppresses ischemia-reperfusion-induced IP<sub>3</sub> generation in isolated, perfused hearts (Amirahmadi et al., 2008). Conversely, cardiac myocyte-specific transgenic overexpression of the  $\alpha_1B$ -subtype does not prevent ischemic preconditioning or prevent ischemic-reperfusion injury (Gao et al., 2000).

**2.  $\alpha_1$ -Adrenergic Receptor-Mediated Preconditioning in Humans.** In human atrial and ventricular muscle strips,  $\alpha_1$ -ARs mediate ischemic preconditioning through activation of PKC, p38 MAPK, and opening of

mitochondrial K-ATP channels (Cleveland et al., 1996, 1997; Loubani and Galinanes, 2001, 2002).

3. *Summary of  $\alpha_1$ -Adrenergic Receptor-Mediated Preconditioning.* In short, the data indicate that  $\alpha_1$ -ARs, most likely the  $\alpha_1$ A-subtype based on studies in mouse and rat, induce ischemic preconditioning and prevent cardiac myocyte death from ischemic injury.

### *E. Conclusions: $\alpha_1$ -Adrenergic Receptors Are Cardioprotective*

Three fundamental conclusions emerge based on 30 years of studies examining the physiologic function of  $\alpha_1$ -ARs in the heart that tend to contradict the conventional wisdom regarding cardiac  $\alpha_1$ -ARs.

1.  *$\alpha_1$ -Adrenergic Receptors are Cardioprotective and Prevent Pathologic Remodeling in Heart Failure Unlike Other  $G_q$ -Coupled Receptors.* Specifically, the  $\alpha_1$ A-subtype induces cardioprotection and positive inotropy, whereas the  $\alpha_1$ B-subtype might be required for an adaptive, physiologic hypertrophy. Cardiac remodeling mediated by  $G_q$ -coupled receptors, such as  $\alpha_1$ -ARs, ETRs, and ATRs, is arguably the most significant physiologic function of these receptors in the heart. Currently, it is widely believed that all  $G_q$ -coupled receptors mediate a pathologic hypertrophic response. The idea that  $G_q$ -signaling is pathologic is based primarily on three lines of evidence, as reviewed previously (Dorn and Brown, 1999; Adams and Brown, 2001). First,  $G_q$ -agonists, such as phenylephrine (an  $\alpha_1$ -AR agonist), endothelin, and angiotensin, induce hypertrophy with expression of the “fetal genes” in cultured NRVM, and re-expression of the “fetal genes” is classically associated with pathologic ventricular remodeling, as reviewed previously (Dorn and Brown, 1999). Second, mouse models targeting overexpression of ETRs or ATRs suggest that these receptors generally induce pathologic remodeling (Ainscough et al., 2009; Paradis et al., 2000; Yang et al., 2004). Clinically, ATR blockers are used to treat heart failure (Chrysant, 2008), although current ETR antagonists have no proven efficacy for heart failure (Mylona and Cleland, 1999; McMurray et al., 2007). Third, cardiac-specific transgenic overexpression of  $G\alpha_q$  in mice induces pathologic remodeling with increased cardiac myocyte apoptosis, as reviewed (Dorn and Brown, 1999; Dorn, 2005). However, high-level  $G\alpha_q$ -overexpression is required for pathology, as reviewed previously (Jensen et al., 2011). Furthermore, the data summarized in this section clearly challenge the dogma that all  $G_q$ -coupled receptor signaling is pathologic. Instead, the data indicate  $\alpha_1$ -AR signaling can be cardioprotective.

2.  *$\alpha_1$ -Adrenergic Receptor-Mediated Cardioprotective Signaling Can Explain the Worsening of Heart Failure with  $\alpha_1$ -Blockers Observed in Clinical Trials.* As detailed in the next section (section V),  $\alpha_1$ -blockers exacerbate heart failure and are associated with worse outcomes in patients with hypertension (ALLHAT),

heart failure (V-HeFT), and benign prostatic hyperplasia (Cohn, 1993; Cohn et al., 1986; ALLHAT, 2000, 2003; Dhaliwal et al., 2009). Hallmarks of ventricular remodeling in heart failure include contractile dysfunction (both systolic and diastolic), pathologic hypertrophy, and increased cardiac cell death and fibrosis (Anand and Florea, 2003). Importantly,  $\alpha_1$ ABKO mice by virtue of their lack of cardiac myocyte  $\alpha_1$ -ARs approximate the use of  $\alpha_1$ -blockers (O’Connell et al., 2003). In the  $\alpha_1$ ABKO mouse model, pathologic stress from aortic constriction causes hypertrophy with failed gene transcription, increased cardiac cell death, increased fibrosis, and worsened contractile function, leading to dilated cardiomyopathy, heart failure, and ultimately 50% mortality (O’Connell et al., 2003, 2006). Follow-up studies in cultured  $\alpha_1$ ABKO cardiac myocytes define a cardioprotective  $\alpha_1$ A-subtype signaling pathway, identifying a direct requirement for protective  $\alpha_1$ -AR signaling in cardiac myocytes, the absence of which could at least partially explain the negative outcomes in  $\alpha_1$ ABKO mice (Huang et al., 2007, 2008). Support for the assertion that  $\alpha_1$ -ARs are cardioprotective can also be drawn from studies in gain-of-function models, where  $\alpha_1$ A-subtype overexpression protects against pathologic stress (Du et al., 2004, 2006; Lin et al., 2001). In combination, these studies indicate that  $\alpha_1$ -ARs are both sufficient to induce and required for cardioprotective signaling. In summary, these data provide a mechanistic basis to explain the negative outcomes in clinical trials with  $\alpha_1$ -blockers.

3.  *$\alpha_1$ -Agonist Therapies Might Improve Heart Failure Outcomes.* On the basis of the accumulated evidence,  $\alpha_1$ -AR activation of adaptive hypertrophy, prevention of cardiac myocyte death, augmentation of contractility, and induction of ischemic preconditioning could prevent worsened outcomes from systolic heart failure. This provides the foundation of the argument for  $\alpha_1$ -AR agonist therapy in heart failure. However, several counterarguments exist (Jensen et al., 2011). First, in transgenic models, the  $\alpha_1$ B-subtype can worsen function and induce dilated cardiomyopathy (Grupp et al., 1998; Wang et al., 2000; Iaccarino et al., 2001; Lemire et al., 2001). However, pharmacology and knockouts argue against the results seen in certain cardiac transgenics. Specifically, studies with  $\alpha_1$ -agonists in mouse (Chan et al., 2008; Dash et al., 2011) and human (Cleveland et al., 1996, 1997; Loubani and Galinanes, 2001, 2002), as well as in loss-of-function mouse models (O’Connell et al., 2003, 2006; Huang et al., 2007) as reviewed above, support  $\alpha_1$ -mediated cardioprotective effects.

Second,  $\alpha_1$ -ARs induce vasoconstriction, which is contraindicated in heart failure. However, in mice, suppressor doses of an  $\alpha_1$ A-subtype-specific ligand prevent doxorubicin cardiotoxicity (Chan et al., 2008; Dash et al., 2011); in humans, some small trials



indicate  $\alpha_1$ -ARs agonists might improve function in heart failure (Cabanès et al., 1992; Zakir et al., 2009); and numerous studies identify adaptive hypertrophy with suppressor  $\alpha_1$ -agonist infusion, as reviewed in the section on hypertrophy. These data provide a preliminary proof-of-principle demonstration of the efficacy of  $\alpha_1$ -agonists in heart failure, and justify further study.

A third argument against  $\alpha_1$ -agonist therapy is that the mixed  $\alpha_1/\beta$ -blocker carvedilol has proven efficacy in heart failure. However, as discussed in the next section (section V), current evidence suggests that the  $\alpha_1$ -blocking properties of carvedilol are not sustained in long-term dosing (Kubo et al., 2001; Hryniewicz et al., 2003), and conversely carvedilol might potentiate  $\alpha_1$ -AR signaling (Van Tassell et al., 2008).

Fourth,  $\alpha_1$ -ARs are  $G_q$ -coupled receptors, and the conventional wisdom is that  $G_q$ -signaling exacerbates pathologic remodeling (Jensen et al., 2011). However, as mentioned already,  $\alpha_1$ -ARs clearly do not fit this paradigm. In summary, despite the caveats listed,  $\alpha_1$ -AR agonist therapy might present a novel effective treatment of heart failure.

## V. $\alpha_1$ -Adrenergic Receptors in Human Heart Disease

The classic physiologic function of  $\alpha_1$ -ARs is to increase vascular smooth muscle contractility and hence blood pressure.  $\alpha_1$ -ARs are found in vascular beds throughout the body, including smooth muscle cells in arteries of the heart, brain, kidneys, and gut, as well as in smooth muscle in the prostate and bladder (Michelotti et al., 2000). By virtue of their ability to block  $\alpha_1$ -AR-mediated smooth muscle contractions,  $\alpha_1$ -AR antagonists ( $\alpha_1$ -blockers) are used to treat hypertension (Lund-Johansen and Omvik, 1991; Frishman and Kotob, 1999; Sica, 2005) and benign prostatic hyperplasia (Caine et al., 1976, 1978; Schwinn and Roehrborn, 2008; Michel, 2010).

More recently, it has become clear that  $\alpha_1$ -ARs might play a significant role in preventing the clinical progression of heart failure, as reviewed above. Heart failure is a clinical syndrome of varied etiology in which the heart cannot pump enough blood to meet the body's needs. Heart failure is characterized by neuro-hormonal augmentation, leading to increased catecholamine levels, which are thought to play a causative role in pathologic ventricular remodeling through increased activation of ARs. Indeed, increased blood NE levels are a primary finding in heart failure patients and predict disease severity and mortality (Cohn et al., 1984). Whereas this does not establish a causal relationship between increased NE levels and induction of heart failure, it was part of the rationale to block NE activation of ARs for therapy in heart failure.

In fact, clinical trials with  $\beta$ -AR antagonists or  $\beta$ -blockers, such as Metoprolol CR/XL Randomized

Intervention Trial in Congestive Heart Failure (metoprolol) (MERIT, 1999), Cardiac Insufficiency Bisoprolol Study II (bucindolol) (CIBIS-II, 1999) and Carvedilol Prospective Randomized Cumulative Survival Trial (carvedilol) (Packer et al., 2001), show significant reductions in mortality in patients with heart failure (Foody et al., 2002; Teerlink and Massie, 1999; Chatterjee et al., 2013). On the basis of the success of  $\beta$ -blockers in improving outcomes in heart failure,  $\beta$ -blockers are standard of care in heart failure therapy (Hunt et al., 2005). This success has led to the notion that blocking all AR signaling in heart failure would be beneficial, and tests of this idea are reviewed below. Currently, 5.7 million Americans have heart failure, 1 million are admitted to the hospital each year, and the 5-year survival rate is only 50% (Roger et al., 2011). Thus, new drugs to treat heart failure are needed (Simpson, 2011). Here, we review the recent clinical trials suggesting that some AR signaling, particularly  $\alpha_1$ -AR signaling, might be beneficial in human heart failure. Table 1 summarizes the trials.

*A. Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial: An  $\alpha_1$ -Adrenergic Receptor Antagonist in Hypertension Increases the Risk of Heart Failure.* The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) was a large, randomized, double-blind, active controlled trial initiated in 1994 and funded by the National Heart, Lung, Blood Institute (ALLHAT, 2000, 2003). ALLHAT was designed to compare new treatments for hypertension versus older, standard treatments. Primary outcomes were fatal coronary heart disease and nonfatal myocardial infarction, and secondary outcomes included all-cause mortality, stroke, and combined cardiovascular disease. In one arm, 24,335 patients with hypertension and at least one other risk factor for coronary heart disease were randomized to the diuretic chlorthalidone (15,268) or the nonselective  $\alpha_1$ -AR antagonist ( $\alpha_1$ -blocker) doxazosin (9067) and were to be followed for 4–8 years.

In 2000, the ALLHAT data safety and monitoring board stopped this arm of the trial early, citing that patients on doxazosin had 25% more cardiovascular events and a significant doubling in the risk of heart failure versus patients on chlorthalidone (SoRelle, 2000). Although systolic blood pressure was approximately 3 mm Hg higher in the doxazosin group, the ALLHAT investigators concluded that this difference was unlikely to account for the doubling in the risk of heart failure (Davis et al., 2002; ALLHAT, 2003), and heart failure events were validated (Piller et al., 2002). Furthermore, approximately 60% of patients in both groups were on additional treatments to reduce blood pressure, but a follow up analysis revealed that the risk of heart failure was not reduced by this additional antihypertensive treatment, thereby confirming the initial findings (Davis et al., 2002). Subsequently, the

TABLE 1  
Summary of clinical trials involving  $\alpha_1$ -blockers in cardiovascular disease

Trial	Treatment Groups and Patient Numbers		Inclusion Criteria	Result	Interpretation
	Control Groups	$\alpha_1$ -Blocker Groups			
ALLHAT (2000) (ALLHAT, 2000, 2003; SoRelle, 2000; Davis et al., 2002; Piller et al., 2002)	Chlorthalidone (diuretic) [12.5–25 mg/day ( $N = 15,268$ )]	Doxazosin ( $\alpha_1$ -blocker) [2–8 mg/day ( $N = 9067$ )]	Men and women age 55 or older with hypertension (systolic 140 mm Hg and/or diastolic 90 mm Hg, or on medication for hypertension) and at least 1 other risk factor for coronary heart disease	Trial stopped early. Doxazosin increased cardiovascular events by 25% and doubled the risk of heart failure.	$\alpha_1$ -Blockade worsens outcomes in hypertension with cardiac risk factors.
V-HeFT I and II (Cohn et al., 1986; Cohn, 1993)	V-HeFT I Placebo ( $N = 273$ ); Hydralazine/ isosorbide dinitrate (direct vasodilators) [300/160 mg/day ( $N = 186$ )] V-HeFT II Hydralazine/ isosorbide dinitrate Enalapril (ACE inhibitor) ( $N = 403$ )	Prazosin ( $\alpha_1$ -blocker) [20 mg/day ( $N = 183$ )]	Male veterans mean age 58 with symptomatic heart failure due to dilated cardiomyopathy (ischemic or nonischemic), on digoxin and diuretics	Prazosin did not change left ventricular ejection fraction or mortality versus placebo; both were improved with hydralazine/isosorbide. Trend toward increased mortality at 5 years in prazosin group versus placebo, survival improved by other vasodilators.	$\alpha_1$ -Blockade shows no benefit in heart failure and might worsen mortality.
$\alpha_1$ -Blockers in BPH effect on HF (Dhaliwal et al., 2009)		Tamsulosin (58%), terazosin (40%), or doxazosin (2%) ( $N = 98$ )	Male veterans with dilated cardiomyopathy admitted with heart failure ( $N = 388$ overall)	Among the 25% of patients taking $\alpha_1$ -blockers, most for BPH, subsequent hospitalizations for heart failure were increased in patients receiving $\alpha_1$ -blockers without $\beta$ -blockers.	$\alpha_1$ -Blockade worsens outcomes in BPH in the absence of $\beta$ -blockade.
COMET (Carvedilol or Metoprolol European Trial) (Poole-Wilson et al., 2002, 2003)	Metoprolol tartrate ( $\beta_1$ -selective antagonist) (50 mg bid; $N = 1518$ )	Carvedilol ( $\beta_{1/2}$ - $\alpha_1$ -AR antagonist) [25 mg bid ( $N = 1511$ )]	Men (80%) and women mean age 62 with NYHA class II–IV heart failure due to dilated cardiomyopathy (ischemic or nonischemic) on stable therapy	Mortality reduced in carvedilol group versus metoprolol. Subsequent analyses indicates that benefit might not be due to $\alpha_1$ -blockade (Kubo et al., 2001; Hryniewicz et al., 2003; Van Tassel et al., 2008)	Carvedilol reduces mortality, possibly related in part to potentiation of $\alpha_1$ -AR signaling.
MOXSE (Swedberg et al., 2002)	Placebo ( $N = 38$ )	Moxonidine (sympatholytic) [0.3–1.5 mg bid ( $N = 227$ total)]	Patients NYHA class II–IV heart failure due to dilated cardiomyopathy (ischemic or nonischemic)	Moxonidine reduced plasma NE and heart rate but increased adverse events	Some degree of AR signaling is cardioprotective.
MOXCON (Coats, 1999; Cohn et al., 2003; Pocock et al., 2004)	Placebo ( $N = 944$ )	Moxonidine (1.5 mg bid [ $N = 990$ ])	As in MOXSE	Trial stopped early Moxonidine reduced NE and increased morbidity and mortality	Some degree of AR signaling is cardioprotective.
BEST (Bristow et al., 2004)	Placebo [3 month ( $N = 845$ ); 12 month ( $N = 654$ )]; Total for BEST ( $N = 2708$ )	Bucindolol ( $\beta_{1/2}$ -AR antagonist, sympatholytic) [3 month ( $N = 841$ ); 12 month ( $N = 642$ )]	Patients NYHA class III–IV heart failure due to dilated cardiomyopathy (ischemic or nonischemic) on stable therapy, subgroup with NE measured at 3 and 12 months	Bucindolol reduced NE and increased mortality.	Some degree of AR signaling is cardioprotective.

ALLHAT results led to the recommendation against  $\alpha_1$ -blockers as a primary treatment of high blood pressure (Messerli, 2001); compliance with this recommendation has been modest (Stafford et al., 2004).

When it was initiated, ALLHAT was the largest trial yet to compare the efficacy of different methods for treating hypertension on cardiovascular outcomes. At the time, it was assumed that lowering blood pressure, regardless of mechanism, would by itself reduce morbidity and mortality (Messerli, 2000). However, ALLHAT shows that clinical trials involving new antihypertensive treatments must examine multiple cardiovascular outcomes.

More importantly, ALLHAT demonstrates that blocking  $\alpha_1$ -ARs has a negative impact on the heart, further challenging the established dogma that elevated AR signaling in heart disease is always pathologic. Although ALLHAT does not prove that the negative effect of  $\alpha_1$ -blockade is a direct effect on the heart, a direct effect is supported by studies on the  $\alpha_1$ ABKO mice and other animal and human data discussed above.

*B. Vasodilator-Heart Failure Trial: An  $\alpha_1$ -Adrenergic Receptor Antagonist Does Not Improve Survival in Heart Failure.* The Vasodilator-Heart Failure Trial (V-HeFT), phase I, was a small trial initiated in 1980 involving 642 men diagnosed with chronic congestive heart failure. V-HeFT was designed to evaluate the effects of vasodilators on mortality as the primary outcome (Cohn et al., 1986). Prior to V-HeFT, studies indicated that reducing systemic vascular resistance improved hemodynamics in patients with heart failure (Cohn and Franciosa, 1977a,b), and V-HeFT was designed to test whether this principal would translate into clinical benefit. Patients with heart failure and taking digoxin and a diuretic, a common therapeutic regimen for heart failure at the time, were randomized to receive placebo, the  $\alpha_1$ -blocker prazosin, or the combination of hydralazine and isosorbide dinitrate. At a mean follow-up of 2.3 years, the combination of hydralazine/isosorbide increased cardiac function (ejection fraction) and reduced mortality, but function and mortality were the same as placebo for prazosin (Cohn et al., 1986).

In phase II, the angiotensin converting enzyme inhibitor enalapril was included, and mortality was tracked in all groups from phase I and II for 5 years. As in phase I, prazosin showed no benefit in phase II, and at 5 years, a trend toward increased mortality was observed (Cohn, 1993). Interestingly, prazosin was the only vasodilator that failed to show any positive outcome. Although not as clear-cut as the results from ALLHAT, the negative results with prazosin in V-HeFT again suggest that  $\alpha_1$ -AR blockade is harmful to the heart and that  $\alpha_1$ -AR activation is indeed beneficial.

A caveat to consider with both prazosin (V-HeFT) and doxazosin (ALLHAT) is that both antagonists can cause myocyte apoptosis, independent of their  $\alpha_1$ -blocking activity (Gonzalez-Juanatey et al., 2003). However, the

dose required to cause apoptosis in culture (10  $\mu$ M) (Gonzalez-Juanatey et al., 2003) is far higher than the peak plasma levels attained in clinical use, e.g.,  $\sim$ 200 nM for doxazosin (Fawzy et al., 1999). Furthermore, the concern that the  $\alpha_1$ -blockers prazosin and doxazosin might be maladaptive for the human heart via an off-target effect is largely obviated by the observation of maladaptive cardiac effects in the  $\alpha_1$ ABKO, as reviewed above.

*C.  $\alpha_1$ -Adrenergic Receptor Antagonist Therapy in Benign Prostatic Hyperplasia Might Exacerbate Heart Failure.* In the prostate,  $\alpha_1$ -ARs mediate smooth muscle contraction, which is the basis for  $\alpha_1$ -blocker use in benign prostatic hyperplasia (BPH). Currently, estimates indicate that 9.5 million men over 65 are diagnosed with benign prostatic hyperplasia (Vaughan, 2003), with common comorbidities for hypertension (87%) and a previous admission for heart failure (40%) (Dhaliwal et al., 2009). The U.S. Food and Drug Administration has approved five  $\alpha_1$ -blockers for BPH: silodosin (Rapaflo), an  $\alpha_1$ A-subtype-selective antagonist, and the nonselective  $\alpha_1$ -antagonists terazosin (Hytrin), doxazosin (Cardura), tamsulosin (Flomax), and alfuzosin (Uroxatral).

A recent meta-analysis of 388 men with heart failure revealed that cotreatment with an  $\alpha_1$ -blocker increased the risk of heart failure hospitalizations, unless patients were concurrently treated with a  $\beta$ -blocker for heart failure (Dhaliwal et al., 2009). Given that only two-thirds of patients in this trial were being treated with a  $\beta$ -blocker (Dhaliwal et al., 2009) and had the common comorbidities of BPH, hypertension, and heart failure, there is reason for concern regarding the safety of  $\alpha_1$ -blockers in BPH.

*D. Carvedilol: A Nonselective  $\beta_{1/2}$ -Adrenergic Receptor/ $\alpha_1$ -Adrenergic Receptor Antagonist for Heart Failure.* Carvedilol is a nonselective  $\beta_{1/2}$ -AR/ $\alpha_1$ -AR antagonist indicated for the treatment of heart failure based on its efficacy as established in several trials, for example, Carvedilol Prospective Randomized Cumulative Survival Trial (Packer et al., 2001, 2002) and US Carvedilol (Packer et al., 1996), as reviewed previously (Teerlink and Massie, 1999; Foody et al., 2002; Wollert and Drexler, 2002; Chatterjee et al., 2013). Interestingly, the Carvedilol or Metoprolol European Trial suggested that carvedilol extended survival relative to metoprolol, a  $\beta_1$ -AR selective antagonist, in patients with NYHA Class II–IV heart failure (Poole-Wilson et al., 2002, 2003). Among other explanations for the benefit provided by carvedilol (Bristow et al., 2003), one hypothesis is that by blocking both  $\beta_2$ - and  $\alpha_1$ -ARs, as well as  $\beta_1$ -ARs, carvedilol confers additional benefit over selective  $\beta_1$ -AR antagonism alone (Poole-Wilson et al., 2002).

As a mixed acting  $\beta_1/\beta_2/\alpha_1$ -blocker, carvedilol is proposed to block  $\alpha_1$ -mediated vasoconstriction to account for the added benefit. Indeed, in HEK cells expressing the human  $\alpha_1$ -subtypes, carvedilol has higher

binding affinity for the  $\alpha_1B$  and  $\alpha_1D$  than for  $\beta$ -ARs or the  $\alpha_1A$  and selectively inhibits  $\alpha_1B$ - and  $\alpha_1D$ -subtype-specific calcium transients (Koshimizu et al., 2004). However, the idea that benefit with carvedilol relies on its  $\alpha_1$ -blocking properties might seem counterintuitive, given the failure of the  $\alpha_1$ -blocker prazosin to improve mortality in V-HeFT (Cohn et al., 1986; Cohn, 1993). Indeed,  $\alpha_1$ -mediated vasopressor responses are not reduced during chronic treatment with carvedilol (Kubo et al., 2001; Hryniewicz et al., 2003). These studies show that chronic carvedilol treatment does not inhibit  $\alpha_1$ -AR-mediated vascular contraction and further suggest that the long-term benefits of carvedilol are not likely due to  $\alpha_1$ -AR antagonism.

In another smaller study of patients with heart failure, the blood pressure responses to  $\alpha_1$ -AR agonist infusion (phenylephrine) were actually increased in patients receiving carvedilol (Van Tassell et al., 2008). These data were interpreted to mean that chronic carvedilol treatment actually *potentiates*  $\alpha_1$ -mediated vasoconstriction (Van Tassell et al., 2008). Although this was a small study, an effect on the heart is also possible, and the implications are that carvedilol might provide an added benefit in heart failure through augmented  $\alpha_1$ -AR signaling.

*E. Sympatholytics: Reducing Norepinephrine Levels Does Not Improve Heart Failure.* If the central tenet of heart failure therapy for the last several decades is correct, namely that increased catecholamine signaling exacerbates heart failure, then by extension, reducing catecholamine levels should improve heart failure outcomes. Following that logic, both the Moxonidine Safety and Efficacy Trial (MOXSE) (Swedberg et al., 2002) and the Moxonidine Congestive Heart Failure Trial (MOXCON) (Cohn et al., 2003) examined the effects of the sympatholytic imidazoline receptor agonist moxonidine on mortality in heart failure. MOXSE examined 268 patients with New York Heart Association class II–IV systolic heart failure and found that moxonidine reduced heart rate and modestly improved ejection fraction but increased adverse events.

MOXCON, the larger of the two trials, also targeted patients with New York Heart Association class II–IV systolic heart failure. After enrolling roughly 1900 patients, the trial was stopped early due to increased mortality in the moxonidine group (Coats, 1999; Cohn et al., 2003; Pocock et al., 2004).

Another clinical trial showed that the  $\beta_1/\beta_2$ -blocker/sympatholytic agent bucindolol increased mortality associated with pronounced NE reduction in the  $\beta$ -Blocker Evaluation of Survival Trial (BEST) (Bristow et al., 2004). In total, the failure of these trials suggests that some catecholamine signaling is beneficial in heart failure. Although the mechanism whereby sympatholysis leads to increased mortality is uncertain, it is possible that decreasing catecholamine levels abrogates the cardioprotective effects of  $\alpha_1$ -AR signaling.

*F. Conclusions and Implications: Are Myocardial  $\alpha_1$ -Adrenergic Receptors Cardioprotective in Humans?* In summary, one large clinical trial and several smaller studies show that  $\alpha_1$ -blockers or a reduction in NE levels worsens outcomes in patients with hypertension or heart failure, as summarized in Table 1. One implication of these results is to force a reconsideration of the notion that all AR signaling in heart failure is pathologic. Although  $\beta$ -AR blockade is clearly beneficial in heart failure, the negative results of trials involving sympatholytics indicate that reducing NE levels excessively can be harmful. Perhaps more importantly, another implication of these results would be to suggest that myocardial  $\alpha_1$ -ARs are protective, based on the negative results in trials with  $\alpha_1$ -blockers. None of the trials involving  $\alpha_1$ -blockers were designed to address mechanisms whereby  $\alpha_1$ -AR inhibition worsens heart failure. However, as discussed in section IV,  $\alpha_1$ -ARs are clearly cardioprotective in cell and animal models.

## VI. Final Summary

The functional significance of cardiac  $\alpha_1$ -ARs has dramatically advanced over the last 30 years since  $\alpha_1$ -ARs were first demonstrated to have a direct trophic effect on cardiac myocytes (Simpson, 1983). Clinical data now show that  $\alpha_1$ -blockers cause heart failure in hypertensive patients and possibly in patients taking  $\alpha_1$ -blockers for BPH. These clinical data imply a protective function for cardiac  $\alpha_1$ -ARs, and data indicate that unlike  $\beta_1$ -ARs,  $\alpha_1$ -ARs are not downregulated in human heart failure but are proportionally increased, available to mediate protective signaling in heart failure. In fact, studies in cultured myocytes and animal models show that  $\alpha_1$ -ARs are cardioprotective and prevent pathologic remodeling in heart failure. The mechanisms are multifactorial, including activation of adaptive or physiologic hypertrophy, prevention of cardiac myocyte death, augmentation of positive inotropic responses, and induction of ischemic preconditioning. Importantly, these studies provide a mechanistic basis to explain the failure of  $\alpha_1$ -blockers in humans. Perhaps most surprising is the finding that  $\alpha_1$ -ARs localize to and signal at the nuclear membrane in adult cardiac myocytes and engage “inside-out” signaling to regulate  $\alpha_1$ -AR cardioprotective signaling. Overall, these findings raise several new questions regarding the functional role of cardiac  $\alpha_1$ -ARs. Perhaps the most important are whether activation of cardiac  $\alpha_1$ -ARs, especially the  $\alpha_1A$  subtype, might be a viable therapeutic strategy in heart failure and how nuclear  $\alpha_1$ -AR signaling might differ functionally from  $\alpha_1$ -AR signaling at the plasma membrane.

### Authorship Contributions

Wrote or contributed to the writing of the manuscript: O'Connell, Jensen, Baker, Simpson.

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