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## Alpha-1-Adrenergic Receptors in Heart Failure: The Adaptive Arm of the Cardiac Response to Chronic Catecholamine Stimulation

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### Abstract

Alpha-1-adrenergic receptors are G-protein coupled receptors (GPCRs) activated by catecholamines. The alpha-1A and alpha-1B subtypes are expressed in mouse and human myocardium, whereas the alpha-1D protein is found only in coronary arteries. There are far fewer alpha-1-ARs than beta-ARs in the non-failing heart, but their abundance is maintained or increased in the setting of heart failure, which is characterized by pronounced chronic elevation of catecholamines and beta-AR dysfunction. Decades of evidence from gain- and loss-of-function studies in isolated cardiac myocytes and numerous animal models demonstrate important adaptive functions for cardiac alpha-1-ARs, to include physiological hypertrophy, positive inotropy, ischemic preconditioning, and protection from cell death. Clinical trial data indicate that blocking alpha-1-ARs is associated with incident heart failure in patients with hypertension. Collectively, these findings suggest that alpha-1-AR activation might mitigate the well-recognized toxic effects of beta-ARs in the hyperadrenergic setting of chronic heart failure. Thus, exogenous cardioselective activation of alpha-1-ARs might represent a novel and viable approach to the treatment of heart failure.

### I. Introduction

Marked chronic elevation in the endogenous catecholamines, epinephrine and norepinephrine (NE) is one of the central neurohormonal abnormalities of heart failure. Catecholamines activate two classes of adrenergic receptors (ARs) in the myocardium: beta-ARs ( $\beta$ -ARs) and alpha-1-ARs ( $\alpha$ 1-ARs).  $\beta$ -ARs are the predominant cardiac AR, and the toxic effects of chronic activation of  $\beta$ 1-ARs on cardiac myocytes and  $\beta$ 2-ARs on cardiac fibroblasts are central to the pathobiology of heart failure.<sup>1,2</sup>

$\alpha$ 1-ARs are coupled to the  $G_{q/11}$  ( $G_{\alpha q}$ ) family of G-proteins and activate phospholipase C $\beta$ 1 (PLC $\beta$ 1), leading to intracellular calcium release through an increase in inositol trisphosphate (IP<sub>3</sub>). Conventional wisdom views activation of myocardial Gq-coupled receptors as inherently pathological. However, this view is based in large part on a transgenic mouse model with Gq overexpression that markedly exceeds the two-fold increase found in human heart failure,<sup>3-5</sup> and thus cannot be considered to simulate human pathophysiology. In

human heart failure, the maximal increase in Gq abundance is two-fold,<sup>5</sup> and transgenic mice with two-fold cardiomyocyte-specific Gq overexpression have no discernible cardiac phenotype.<sup>3,4</sup>  $\alpha$ 1-ARs also differ from other Gq-coupled receptors with respect to both cellular distribution in the heart, and intracellular localization within myocytes, as described later.

Signaling cascades downstream of  $\alpha$ 1-ARs are diverse and well-characterized, with over 70 known signaling partners.<sup>6</sup> The three subtypes of  $\alpha$ 1-ARs,  $\alpha$ 1A,  $\alpha$ 1B, and  $\alpha$ 1D, have differential expression in many tissues. The heart contains all three subtypes and substantial evidence demonstrates that myocardial  $\alpha$ 1-AR activation confers benefits through multiple pathways and processes.<sup>6</sup>

In the rodent and human heart,  $\alpha$ 1A and  $\alpha$ 1B-ARs are expressed predominantly in cardiac myocytes,<sup>7,8</sup> whereas the  $\alpha$ 1D is found in coronary smooth muscle cells.<sup>9</sup> Myocardial  $\alpha$ 1-ARs are essential for normal post-natal growth of the heart, and exert adaptive and protective effects in the setting of chronic stress, such as heart failure. Indeed,  $\alpha$ 1-AR abundance and function are maintained or increased in the setting of heart failure, in contrast to  $\beta$ -ARs, which are down-regulated and dysfunctional.<sup>7,10</sup>

In vitro and animal studies have established that  $\alpha$ 1-ARs mediate cardioprotection through numerous adaptive processes, including inhibition of cardiac myocyte death, enhanced protein synthesis, increased glucose metabolism, and positive inotropy. Human clinical trials suggest that the use of medications that antagonize the effects of  $\alpha$ 1-ARs is associated with an increased incidence of heart failure. Here we review briefly the data from cell, animal, and human studies that collectively indicate benefit from activation of  $\alpha$ 1-ARs in the heart, with a particular focus on the adaptive role of myocardial  $\alpha$ 1-ARs in the setting of heart failure, as contrasted to the maladaptive effects of  $\beta$ 1-ARs (Figure 1).

## II. There are 3 $\alpha$ 1-AR subtypes, A, B, and D, with distinct patterns of expression in the heart

### IIa. Myocardial $\alpha$ 1-ARs: animal models

Cardiac  $\alpha$ 1-ARs were identified initially in the rat by Williams and Lefkowitz<sup>11</sup> and confirmed shortly thereafter in guinea pig by Karliner and colleagues.<sup>12</sup>  $\alpha$ 1-ARs were studied subsequently in the hearts of mice, rabbits, pigs, dogs, and cows. There is species-to-species variation in cardiac  $\alpha$ 1-AR abundance,<sup>13,14</sup> though the basal level of expression is uniformly lower than  $\beta$ -ARs.  $\alpha$ 1-AR levels are 5- to 8-fold higher in rats than in other species.<sup>14</sup>

The rodent heart contains mRNAs for all three  $\alpha$ 1-AR subtypes, though only the  $\alpha$ 1A and  $\alpha$ 1B are expressed as proteins (Figure 1).<sup>8,14-17</sup> The  $\alpha$ 1A is the most abundant transcript in the rodent heart, but competition radioligand binding identifies higher levels of  $\alpha$ 1B.<sup>8,18</sup> Importantly,  $\alpha$ 1-AR proteins can only be detected reliably using radioligand binding, as there are no commercially available antibodies with  $\alpha$ 1-AR specificity.<sup>19</sup>

Analysis of the major constituent myocardial cell types in rodents reveals that both neonatal and adult cardiac myocytes contain  $\alpha$ 1A and  $\alpha$ 1B-ARs, and cardiac fibroblasts contain no  $\alpha$ 1-ARs whatsoever (Figure 2).<sup>17,20</sup> Interestingly, recent data demonstrate also that the  $\alpha$ 1A subtype is expressed in only a subpopulation of myocytes.<sup>21</sup>

Thus, signaling events within cardiac myocytes primarily drive the cardiac effects of  $\alpha$ 1-AR activation, and  $\alpha$ 1-ARs cannot participate directly in the fibroblast proliferation and fibrosis that characterizes heart failure. Experimental chronic infusion of an  $\alpha$ 1-AR agonist causes

physiological cardiac hypertrophy without fibrosis,<sup>22</sup> whereas  $\beta$ 2-AR stimulation drives proliferation of both rodent and human cardiac fibroblasts.<sup>1,23-26</sup>

It is axiomatic that receptor localization must determine function, and  $\alpha$ 1-,  $\alpha$ 2-, and  $\beta$ -ARs all have distinct distributions among cardiac cells (Figures 1 and 2). These locations need to be kept in mind, when considering the roles of cardiac ARs.

Cellular distribution also distinguishes  $\alpha$ 1-ARs from the other major myocardial Gq-coupled receptors. The majority of cardiac angiotensin II receptors are found in fibroblasts, with minimal expression in cardiomyocytes,<sup>27,28</sup> which may account for pathological effects when angiotensin II Gq-coupled receptors are activated. ET(B) receptors are found on cardiac fibroblasts, with minimal expression on cardiomyocytes,<sup>29</sup> and participate in pathological fibrosis in heart failure. The effects of ET(A) stimulation are complex and not uniformly pathological,<sup>30</sup> with evidence to support cardioprotective effects such as positive inotropy,<sup>31</sup> antiapoptosis,<sup>32</sup> and ischemic preconditioning.<sup>33</sup> Thus, many of the detrimental effects of Gq-coupled receptor activation can be attributable to effects in cardiac fibroblasts, not cardiomyocytes. As a corollary, cardiac-specific,  $\alpha$ -MHC-driven transgenic models of some other Gq-coupled receptors should be viewed with caution, given the extremely low abundance of the wild type receptors in cardiomyocytes relative to non-myocytes; transgenic over-expression in cardiac myocytes might have minimal relevance to actual pathophysiology.

$\alpha$ 1-ARs, as most GPCRs, historically were thought to localize to the plasma membrane. However, early studies suggested that  $\alpha$ 1-ARs were present on the nucleus of neonatal rat ventricular myocytes (NRVMs),<sup>34</sup> and recent compelling evidence confirms nuclear localization of a substantial proportion of  $\alpha$ 1-ARs in adult mouse cardiac myocytes. Radioligand binding of fractionated adult mouse cardiac myocytes detects 80% of  $\alpha$ 1-ARs in the nuclear fraction, and this finding is substantiated using BODIPY-prazosin, a fluorescently labeled  $\alpha$ 1-AR antagonist.<sup>35</sup> Subsequent studies extended this finding, demonstrating that nuclear localization was required for normal  $\alpha$ 1-AR signaling and function in adult cardiac myocytes.<sup>36</sup> These discoveries, coupled with the identification in adult cardiac myocyte nuclei of angiotensin receptors<sup>37</sup> and  $\beta$ 1-ARs<sup>38</sup>, challenge the longstanding paradigm of 'outside-in' GPCR signaling in the heart. Endothelin-1 receptors also have been identified at the nucleus,<sup>39</sup> although endothelin receptors can function at the plasma membrane.<sup>40</sup> Thus nuclear compartmentalization of functional  $\alpha$ 1-ARs may also distinguish them from other Gq-coupled receptors in the heart.

### **IIb. Myocardial $\alpha$ 1A- and $\alpha$ 1B-ARs: human studies**

Human and mouse cardiac  $\alpha$ 1-AR abundance and subtype distribution appear to be very similar. All three  $\alpha$ 1-AR subtype mRNAs are found in both ventricles of explanted human hearts; the  $\alpha$ 1A is the most abundant, followed by the  $\alpha$ 1B and  $\alpha$ 1D. As in the mouse, only the  $\alpha$ 1A and  $\alpha$ 1B proteins are detectable by radioligand binding, and the  $\alpha$ 1B is more abundant.<sup>7</sup> Collectively, these findings suggest that the mouse likely represents an accurate model for human cardiac  $\alpha$ 1-AR biology.

### **IIc. Coronary vascular $\alpha$ 1D-ARs in rodents and humans**

$\alpha$ 1-ARs are well known for their effects in vascular tissue, where they typically mediate vasoconstriction. The stimulation of vascular  $\alpha$ 1-ARs by catecholamines produces little change in the diameter of normal human coronaries.<sup>41,42</sup> However, in coronary arteries with disrupted endothelium,  $\alpha$ 1-AR stimulation produces pronounced vasoconstriction and can lead to myocardial ischemia.<sup>43-45</sup> The  $\alpha$ 1D is the predominant  $\alpha$ 1-AR subtype in human epicardial coronary arteries and is pharmacologically active in primary human coronary

smooth muscle cells and ex vivo coronary rings.<sup>9</sup> Functional  $\alpha 1B$ -ARs are expressed in primary cultures of human epicardial coronary endothelial cells.<sup>46</sup>  $\alpha 1A$  mRNA represents 4% of total  $\alpha 1$ -AR mRNA in human epicardial coronary arteries and has no discernible function in either smooth muscle or endothelial cells. Of interest, the  $\alpha 1D$  also is functional in mouse coronary arteries,<sup>47,48</sup> further reinforcing the similarities between mouse and human cardiac  $\alpha 1$ -ARs.

### III. The abundance of myocardial $\alpha 1A$ - and $\alpha 1B$ -ARs is maintained in heart failure

Heart failure is characterized by persistent marked elevations in catecholamines and the response of ARs to chronic stimulation is central to the pathobiology of the failing myocardium.  $\beta 1$ -ARs are down-regulated and dysfunctional in heart failure,<sup>10,49</sup> and copious evidence demonstrates that excessive stimulation of cardiac  $\beta$ -ARs in the setting of heart failure mediates harmful processes to include cell death, fibrosis, and adverse remodeling. Many large clinical trials indicate that drugs that antagonize  $\beta$ -ARs (i.e.  $\beta$ -blockers) decrease mortality due to heart failure<sup>50</sup> and these medications are an essential component of contemporary heart failure therapy.<sup>51</sup>

In contrast, the abundance and beneficial functions of cardiac  $\alpha 1$ -ARs are maintained or augmented in the failing heart. Multiple studies indicate that  $\alpha 1$ -ARs constitute 2-23% (mean = 11%) of all AR binding in non-failing human heart tissue. However, in myocardium from failing human hearts,  $\alpha 1$ -ARs are 9-38% (mean = 25%) of all ARs.<sup>7,52-55</sup>

Importantly,  $\alpha 1$ -ARs also appear to maintain their function in the setting of chronic agonist exposure and heart failure. Chronic treatment of NRVMs with norepinephrine leads to upregulation of  $\alpha 1A$ -ARs without desensitization of  $IP_3$  synthesis or cardiomyocyte growth.<sup>56</sup> In non-failing human myocardium,  $\beta$ -AR stimulation induces significantly greater inotropy than  $\alpha 1$ -AR stimulation; however in failing human heart muscle  $\alpha 1$ - and  $\beta$ -AR stimulation can produce equal degrees of positive inotropy.<sup>57,58</sup>

Analysis of  $\alpha 1$ -AR subtype profiles in failing and non-failing hearts reveals that the abundance of  $\alpha 1A$  mRNA is increased by 40% in the setting of heart failure,<sup>7</sup> and that  $\alpha 1A$  mRNA abundance positively correlates with left ventricular contractile function.<sup>59</sup>  $\alpha 1A$  and  $\alpha 1B$  binding levels are both maintained at nearly identical levels in failing human hearts, as are levels of the  $\beta 2$ -AR, whereas the  $\beta 1$ -AR is markedly down-regulated (Figure 3).<sup>7</sup> Consequently, the relative importance of  $\alpha 1$ -ARs and  $\beta 2$ -ARs is magnified in the failing human heart.

These findings recapitulate earlier and ongoing studies in rodent hearts. Total cardiac  $\alpha 1$ -AR abundance and function are unaffected by hypertrophy or heart failure in rat models.<sup>56,60</sup>  $\alpha 1A$  mRNA abundance is increased in rats subjected to pressure-loading, as well as in NRVMs exposed to hypertrophic agonists,<sup>56</sup> similar to humans with HF.<sup>7,59</sup> Recent studies suggest also that  $\alpha 1A$  expression is maintained or increased in failing mouse hearts (Myagmar et al, unpublished data). Thus it appears that both the non-failing and failing mouse heart accurately model human  $\alpha 1$ -AR subtype abundance.

The mechanisms underlying the preservation of  $\alpha 1$ -AR levels and function in heart failure are partly known.  $\beta 1$ -AR desensitization occurs through GRK2, the abundance and function of which is upregulated in human and rat heart failure, similar to upregulation of GRK5.<sup>61,62</sup> GRK2 and GRK5 have little if any effect on  $\alpha 1$ -ARs,<sup>63-65</sup> and both GRK2 and GRK5 are expressed in many myocardial cell types.<sup>62</sup> In contrast, GRK3 is expressed exclusively in myocytes and selectively regulates  $\alpha 1$ -ARs.<sup>62,63</sup> GRK3 abundance is unchanged in heart

failure,<sup>61,62</sup> which might partly explain the fact that heart failure does not change  $\alpha$ 1-AR levels and function. Interestingly, transgenic mice with cardiac-specific expression of a GRK3 peptide inhibitor (GRK3ct, analogous to the well-studied GRK2ct) have increased contractility without fibrosis and enhanced  $\alpha$ 1-AR signaling at baseline. After transverse aortic constriction, GRK3ct transgenic mice are protected from systolic dysfunction, despite equal induction of ventricular hypertrophy and  $\beta$ -myosin heavy chain induction.<sup>66,67</sup> These findings imply that increased  $\alpha$ 1-AR signaling can be beneficial, and collectively mirror the beneficial phenotype of mice with modest  $\alpha$ 1A-AR overexpression.<sup>68</sup>

#### IV. Blocking $\alpha$ 1-ARs is associated with heart failure in clinical trials

$\alpha$ 1-AR antagonists, or “ $\alpha$ -blockers”, are used widely for the treatment of benign prostatic hypertrophy (BPH) and occasionally in the treatment of hypertension. ALLHAT, the largest clinical trial of anti-hypertensive medications, included an arm in which roughly 24,000 men and women with high blood pressure received the non-selective  $\alpha$ 1-blocker, doxazosin. The incidence of heart failure in this group was twice as high as in the other arms of the trial and the Data Safety Monitoring Board discontinued the doxazosin arm prematurely due to safety concerns.<sup>69</sup> Of note, in 2002 13.2 million prescriptions for  $\alpha$ -blockers were dispensed nationally, and the release of ALLHAT had little near-term effect on the use of these medications.<sup>70,71</sup>

Smaller studies also corroborate the association between  $\alpha$ 1-AR blockade and worsening heart failure. In the V-HeFT trial, use of the  $\alpha$ 1-blocker prazosin was associated with a trend toward increased mortality, in contrast to the beneficial effects of the other vasodilator medications tested.<sup>72</sup> A recent analysis also indicated an increased risk for heart failure hospitalizations in patients taking  $\alpha$ -blockers without concomitant  $\beta$ -blockade.<sup>73</sup>

Further loss-of-function evidence suggesting the benefit of  $\alpha$ 1-AR activation comes from multiple trials of sympatholysis for the treatment of heart failure. The Moxonidine Safety and Efficacy trial (MOXSE)<sup>74</sup> and the Moxonidine Congestive Heart Failure trial (MOXCON)<sup>75</sup> studied the efficacy of the sympatholytic agent moxonidine in the treatment of heart failure. Both trials found that moxonidine was very effective in decreasing plasma NE levels, yet both also observed an excess of mortality in the sympatholysis group. The mortality signal was strong enough to prompt premature discontinuation of enrollment in MOXCON.

The BEST trial examined the effects of bucindolol, a  $\beta$ -blocker with sympatholytic properties, on heart failure patients. In contrast to trials using other  $\beta$ -blockers for the treatment of heart failure, the bucindolol group experienced higher mortality.<sup>76</sup> It is controversial whether pharmacogenetic factors play a role in this result with bucindolol.<sup>77-82</sup> With reference to the evidence presented in this review, we suggest that one possible explanation for sympatholytic-associated harm in heart failure is the withdrawal of adaptive pathways mediated by  $\alpha$ 1-AR activation.

The findings from these studies collectively challenge the prevailing paradigm that sympathetic activation is inherently and categorically harmful in heart failure.

#### V. Knockout (KO) mouse models demonstrate cardioprotective effects of $\alpha$ 1A- and $\alpha$ 1B-ARs

$\alpha$ 1-Antagonists with the aforementioned adverse effects in clinical trials might have had “off-target” effects. However,  $\alpha$ 1-AR KO mice provide convincing evidence that  $\alpha$ 1-signaling is required for cardiac protection.

### Va. Characterization of $\alpha$ 1-AR KO mice

The findings from loss-of-function human trials are corroborated by studies in mice lacking cardiac  $\alpha$ 1-ARs, with evidence indicating distinct beneficial roles for both the  $\alpha$ 1A and  $\alpha$ 1B. Broadly speaking, the  $\alpha$ 1A appears to regulate  $\alpha$ 1-mediated cardioprotection, the  $\alpha$ 1B might mediate catecholamine-induced physiological hypertrophy, and the  $\alpha$ 1D contributes to regulation of blood pressure, as reviewed in detail.<sup>83</sup>

Mice lacking the  $\alpha$ 1A (on a mixed FVB/N-129 background) have low blood pressure and normal heart size.<sup>18</sup>  $\alpha$ 1B-AR knockout mice ( $\alpha$ 1B-KOs) on a mixed N-129/B6 background have normal heart size,<sup>15,84</sup> but a reduced cardiac hypertrophic response to subpressor infusion of an  $\alpha$ 1-agonist.<sup>84</sup> Furthermore,  $\alpha$ 1B-KOs on a congenic C57BL/6 background have small hearts.<sup>85</sup> Regardless of genetic background,  $\alpha$ 1B-KO mice have normal blood pressure, but a blunted pressor response to infusion of adrenergic agonist.<sup>15,84-87</sup>  $\alpha$ 1D-KO mice have normal hearts, but reduced blood pressure and decreased coronary vasoconstriction in response to phenylephrine.<sup>47,87,88</sup>

Systemic knockout of both the  $\alpha$ 1A and  $\alpha$ 1B ( $\alpha$ 1AB-KO) eliminates all myocardial  $\alpha$ 1-AR binding.<sup>8</sup> Surprisingly, the  $\alpha$ 1AB-KO mice (congenic B6 background) are normotensive, indicating that one or more of the other mechanisms controlling blood pressure can compensate for absence of  $\alpha$ 1-ARs.<sup>8</sup> However, cardiac growth cannot be compensated, in that adult  $\alpha$ 1ABKO male mice have small heart, indicating impaired physiological cardiac hypertrophy after weaning. This “small heart” phenotype is not seen in female mice, which have smaller hearts than do males. Overall contractility measured by echocardiography is normal in the smaller  $\alpha$ 1AB-KO hearts, but stroke volume, heart rate, and cardiac output are reduced, and the  $\alpha$ 1AB-KO mice have decreased exercise tolerance.<sup>8</sup>

### Vb. KO models show that myocardial $\alpha$ 1-ARs are cardioprotective

$\alpha$ 1AB-KO mice also are more susceptible to injury, as evidenced by increased mortality and dilated cardiomyopathy leading to severe heart failure, in the setting of pressure-loading due to transverse aortic constriction.<sup>8,89,90</sup> Pressure-loaded  $\alpha$ 1-ABKO hearts have worse fibrosis than their wild type littermates, and increased cardiac cell death due in part to enhanced apoptosis. Cultured  $\alpha$ 1-ABKO cardiac myocytes exhibit increased necrosis and apoptosis when treated with NE, isoproterenol, doxorubicin or H<sub>2</sub>O<sub>2</sub>.<sup>90-92</sup> The enhanced susceptibility to cell death is rescued by adenoviral reintroduction of the  $\alpha$ 1A, but not the  $\alpha$ 1B, and requires activation of ERK 1/2.<sup>92</sup> Collectively, these in vivo and in vitro findings demonstrate an essential cardioprotective role for the  $\alpha$ 1A in cardiac myocytes, mediated through ERK signaling.

## VI. Gain-of-function experiments demonstrate beneficial effects of cardiac $\alpha$ 1-AR activation

Gain-of-function experiments, largely using well-characterized agonists, demonstrate broadly cardioprotective effects of activating myocardial  $\alpha$ 1-ARs. These adaptive processes include physiological hypertrophy, i.e. hypertrophy with preserved or increased contractile function, positive inotropy, protection from cell death, and ischemic preconditioning. In many cases, it remains unclear which  $\alpha$ 1-AR subtype regulates these cardioprotective effects, though the  $\alpha$ 1A has been implicated in a number of studies.

### Vla. Pharmacologic $\alpha$ 1-AR activation induces physiological cardiac hypertrophy

The experimental use of adrenergic agonists has been central to advancing our understanding of the function of  $\alpha$ 1-ARs in the heart. NE, epinephrine, and phenylephrine (PE) have been used in cell, animal, and human models for decades. The first report of

functional  $\alpha$ 1-ARs in cardiac myocytes described hypertrophy and an increase in beating frequency of NRVMs upon incubation with NE and the  $\beta$ -blocker, propranolol.<sup>93,94</sup> Treatment of NRVMs with  $\alpha$ 1-AR agonists remains a widely utilized model for experimental cardiac myocyte hypertrophy, and the hypertrophic response has been confirmed in adult cardiac myocytes and myocytes of multiple species.<sup>95-100</sup>  $\alpha$ 1-AR activation induces hypertrophy characterized by upregulation of the “fetal gene” program, to include atrial natriuretic peptide,<sup>101,102</sup>  $\alpha$ -skeletal actin,<sup>103,104</sup> and  $\beta$ -myosin heavy chain.<sup>105-107</sup> In vitro agonist experiments have elucidated the complex signaling pathways that mediate  $\alpha$ 1-induced cellular hypertrophy, identifying over 70 downstream signaling partners to include PKC isoforms,<sup>106,108,109</sup> PKD,<sup>110,111</sup> ERK 1/2,<sup>8</sup> p90RSK,<sup>112,113</sup> and HDACs.<sup>114,115</sup>

Though fetal gene induction generally is considered inherently detrimental, it is unclear whether upregulation of this gene program is causative or merely a marker of pathology. In fact, numerous beneficial roles have been identified for the natriuretic peptides,<sup>116</sup> and any pathogenicity of  $\alpha$ -skeletal actin and  $\beta$ -myosin heavy chain can be questioned.<sup>117-119</sup> Evidence even suggests that  $\beta$ -myosin heavy chain induction is not a marker for myocyte hypertrophy per se.<sup>118</sup>

In vivo agonist studies substantiate the biologic significance of  $\alpha$ 1-AR induction of cardiac myocyte hypertrophy. Infusion of a sub-pressor dose of NE causes cardiac hypertrophy with preserved or increased contractile function in mouse, cat, and dog models.<sup>22,84,120-123</sup> Importantly, the increase in cardiac mass is not accompanied by fibrosis or cell death. Collectively these findings define the in vivo response to  $\alpha$ 1-AR agonist infusion as physiological, rather than pathological, cardiac hypertrophy.

#### **Vib. Myocardial $\alpha$ 1-ARs cause positive inotropy, particularly in the failing heart**

Though induction of hypertrophy is the most broadly studied of  $\alpha$ 1-AR effects, the first physiological response to  $\alpha$ 1-AR stimulation detected in humans was increased inotropy. In early reports,  $\alpha$ 1-AR agonists induced an inotropic response ex vivo in preparations from human atria<sup>124</sup> and ventricles,<sup>125,126</sup> as well as in vivo.<sup>127,128</sup> The contractile response to  $\alpha$ 1-AR activation is preserved or enhanced in failing human myocardium,<sup>52,58,129</sup> in contrast to the  $\beta$ -AR response, which is markedly diminished.<sup>10</sup>

Animal models generally confirm the positive inotropic response to  $\alpha$ 1-AR stimulation,<sup>130-132</sup> and experiments in mice reveal critical details. Ex vivo perfused hearts have a positive inotropic response to  $\alpha$ 1-AR stimulation,<sup>48</sup> and chronic  $\alpha$ 1-AR activation is required for normal contractile performance in perfused mouse hearts.<sup>89</sup> Interestingly, myocytes and ventricular trabeculae from the mouse right ventricle have a negative inotropic response to  $\alpha$ 1-AR activation, as contrasted with mostly positive responses in left ventricular myocytes and myocardium.<sup>133-135</sup> The overall net positive response in the intact heart is likely explained by the greater contribution of the left ventricle. A surprising finding is that heart failure switches the  $\alpha$ 1-AR inotropic response in mouse right ventricular trabeculae from negative to positive,<sup>136</sup> a potential adaptive response to the pulmonary hypertension of heart failure. Given the central role of impaired contractile function in the pathophysiology of heart failure, the augmentation of inotropy through  $\alpha$ 1-AR activation clearly constitutes an adaptive response.

The inotropic response to  $\alpha$ 1-AR stimulation likely arises from multiple mechanisms. Myofilament calcium sensitivity is increased,<sup>137</sup> as is calcium entry.<sup>138-140</sup>  $\alpha$ 1-AR activation also leads to phosphorylation of myosin light chain and troponin I by myosin light chain kinase and PKC.<sup>136,141</sup>

### Vic. $\alpha$ 1-AR activation promotes cardiac myocyte survival

The pathobiology of heart failure is characterized by increased cell death through both necrosis and apoptosis, mediated in part by chronic catecholamine surge.<sup>142</sup> Abundant evidence identifies  $\beta$ 1-ARs as the mediators of enhanced cell death, whereas  $\alpha$ 1-ARs do not appear to participate in either necrotic or apoptotic cell death. In fact, in vitro studies offer convincing evidence that  $\alpha$ 1-ARs even counteract toxic  $\beta$ 1-AR pathways to preserve cell viability.

In NRVMs,  $\alpha$ 1-AR agonists block apoptosis due to the  $\beta$ -AR specific agonist isoproterenol,<sup>143,144</sup> and in adult rat cardiac myocytes, NE-induced apoptosis is inhibited by a  $\beta$ -blocker (propranolol) but not an  $\alpha$ -blocker (prazosin).<sup>143</sup> Pharmacologic  $\alpha$ 1-AR activation also protects against cell death due to numerous other experimental insults to include hypoxia,<sup>145</sup> serum starvation,<sup>145</sup> and doxorubicin.<sup>146</sup>

$\alpha$ 1-AR anti-apoptotic signaling involves multiple pathways. Studies using NRVMs indicate phosphorylation of Bcl-2<sup>145</sup> and Bad after  $\alpha$ 1-AR activation,<sup>147</sup> potentially leading to stabilization of the mitochondrial membrane. ERK 1/2 is a frequent downstream partner of  $\alpha$ 1-ARs, and also regulates cytoprotective effects in the setting of agonist treatment.<sup>92,143,144,148</sup> Phenylephrine (PE)-mediated cytoprotection requires  $\alpha$ 1-AR regulation of the GATA-4 and NFAT transcription factors.<sup>146,149</sup>

In vivo models using  $\alpha$ 1-AR agonists reinforce the in vitro findings. Chronic infusion of  $\alpha$ 1-AR agonists causes physiological hypertrophy without evidence of cell death in multiple animal models.<sup>22,84,120-123</sup> These early studies used agonists that bind all 3  $\alpha$ 1-AR subtypes, thus it has been unclear whether the salutary effects resulted from activation of the  $\alpha$ 1A, the  $\alpha$ 1B, or some combination thereof. However, recent evidence points toward the  $\alpha$ 1A as the primary cardioprotective AR subtype. Continuous infusion of a subpressor dose of the  $\alpha$ 1A-selective agonist A61603 prevents death and heart failure in mice injected with the chemotherapeutic agent, doxorubicin.<sup>150,151</sup> These results echo the findings from experiments using cardiac myocytes from  $\alpha$ 1-ABKO mice in which adenoviral reconstitution of the  $\alpha$ 1A, but not the  $\alpha$ 1B, abrogates enhanced myocyte death due to doxorubicin,  $\beta$ -AR stimulation, and H<sub>2</sub>O<sub>2</sub>.<sup>92</sup>

### VId. $\alpha$ 1-ARs mediate ischemic preconditioning in the heart

Abundant evidence also supports a role for  $\alpha$ 1-ARs in ischemic preconditioning. Agonist studies in multiple species including rat,<sup>152,153</sup> mouse,<sup>154</sup> dog,<sup>155-157</sup> pig,<sup>158</sup> and rabbit<sup>159</sup> indicate that myocardial  $\alpha$ 1-AR activation protects against ischemic injury. In particular, the  $\alpha$ 1A is implicated in the preconditioning response in a number of different models. Transgenic mice overexpressing a constitutively active  $\alpha$ 1A show complete protection from ischemic injury, whereas  $\alpha$ 1B transgenics develop injury comparable to wild type mice.<sup>160</sup> A novel transgenic rat model also indicates that overexpression of the  $\alpha$ 1A in the rat heart reduces infarct size after coronary ligation by mediating the second window of ischemic preconditioning.<sup>161</sup>

Activation of PKC is central to  $\alpha$ 1-AR mediated preconditioning,<sup>153,162-164</sup> and further downstream effectors include superoxide dismutase,<sup>165,166</sup> inducible nitric oxide synthase,<sup>154</sup> 5' nucleotidase,<sup>155</sup> and heat shock proteins.<sup>162</sup>

### Vle. $\alpha$ 1-AR transgenic mouse models have variable phenotypes

Studies of mice with transgenic overexpression of  $\alpha$ 1-AR subtypes yield somewhat conflicted results, possibly resulting from disparate constructs (different promoters, different receptor cDNAs), the varying extents to which the receptors are overexpressed, whether the



transgenic mouse is systemic or cardiac-specific, the specific receptor active state conferred by the mutations in constitutively active mutants, and varied sites of transgene integration into the genome. Sex and strain of the transgenic mice studied likely contribute as well, but are not uniformly given in the various papers. Many of the details on each transgenic mouse are tabulated elsewhere.<sup>6</sup>

The disparate findings may also result in part from challenges inherent in the overexpression of GPCRs. In particular, transgenesis does not guarantee that the overexpressed receptor will assume the same conformation as an endogenous receptor in the setting of agonist stimulation. As such, it is conceivable that physiological responses in transgenic GPCR models are governed by aberrant signaling downstream from an overexpressed receptor in an alternate active state, rather than merely an exaggerated representation of endogenous signaling. This concept was demonstrated nicely in mouse cardiomyocytes overexpressing a constitutively active human  $\beta$ 2-AR.<sup>167</sup> With reference to  $\alpha$ 1-AR transgenics, this issue is complicated further by the fact that different  $\alpha$ 1 agonists themselves induce biased signaling at wild type receptors, presumably based on receptor conformation.<sup>168</sup>

Two-fold systemic overexpression of a constitutively active mutant  $\alpha$ 1A enhances ischemic preconditioning.<sup>160</sup> In a separate transgenic model, exaggerated cardiac myocyte-specific overexpression of the wild type  $\alpha$ 1A improves contractility without hypertrophy (148-170 fold overexpression).<sup>169</sup> Sixty-six-fold overexpression of the wild type  $\alpha$ 1A protects against heart failure due to pressure loading and myocardial infarction, but is associated with enhanced fibrosis, apoptosis and death in aged mice.<sup>68,170,171</sup> The latter results are particularly surprising in light of the wealth of evidence from other gain-of-function studies indicating cardioprotection and a lack of fibrosis, and could be confounded by the grossly supraphysiological levels of  $\alpha$ 1A overexpression.

Characterization of multiple  $\alpha$ 1B transgenic mice has been even more confusing. Two-fold systemic overexpression of a constitutively active mutant  $\alpha$ 1B causes cardiac hypertrophy in the setting of normal blood pressure,<sup>172</sup> as does cardiac myocyte-specific 3-fold overexpression of a constitutive active mutant  $\alpha$ 1B,<sup>173</sup> or 70-fold overexpression of a wild type  $\alpha$ 1B.<sup>174</sup> However, another mouse line with 2-fold overexpression of a constitutively active  $\alpha$ 1B has normal heart size with decreased contractile function,<sup>175</sup> and 43-fold myocyte-specific overexpression of the  $\alpha$ 1B produces dilated cardiomyopathy and early death.<sup>176-178</sup>

The only clear commonality between the various  $\alpha$ 1B transgenics is the lack of a blood pressure phenotype. These apparently incompatible findings likely arise from the sources of variability mentioned above, and ultimately offer limited insight regarding the physiological role of the myocardial  $\alpha$ 1B-AR. As there is no available pharmacological compound with  $\alpha$ 1B selectivity, the best available evidence regarding the function of the  $\alpha$ 1B in the heart comes from knockout mouse models that suggest it is involved in normal post-weaning physiological developmental hypertrophy,<sup>8,84,85</sup> and hypertrophy induced by sub-pressor infusion of an  $\alpha$ 1-agonist.<sup>84</sup>

## VII. Summary

Decades of studies in cells, animals, and humans describe adaptive and protective roles for  $\alpha$ 1-ARs in the heart. These functions are particularly important in the context of chronic adrenergic activation, such as heart failure, wherein  $\beta$ -ARs are downregulated and dysfunctional. Evidence from loss- and gain-of-function studies indicates that myocardial  $\alpha$ 1-ARs not only protect against the development of heart failure, but also maintain their beneficial functions in established heart failure. As detailed in this review, these functions include physiological hypertrophy, enhanced positive inotropy, protection against apoptotic

and necrotic cell death, and ischemic preconditioning.  $\alpha$ 1-ARs are estimated to be 90% unoccupied in chronic heart failure, suggesting that these beneficial pathways could be augmented further by activation with an exogenous agonist.<sup>179</sup> Indeed, this concept was affirmed in a recent small clinical trial in which advanced heart failure patients derived significant clinical benefit from the use of midodrine, a non-selective  $\alpha$ 1-AR agonist.<sup>180</sup>

In mice and humans, the  $\alpha$ 1A and  $\alpha$ 1B are the predominant  $\alpha$ 1-AR subtypes in the myocardium, whereas the  $\alpha$ 1D is expressed in coronary arteries. The  $\alpha$ 1A likely regulates cell survival pathways and ischemic preconditioning, and contributes to contractile function. The function of the  $\alpha$ 1B is less certain, though it appears to regulate post-natal heart size. Activation of the  $\alpha$ 1D leads to coronary vasoconstriction, particularly in the absence of a functioning endothelium. Ongoing translational work will expand upon the recent finding that selective  $\alpha$ 1A activation protects against heart failure in a mouse model,<sup>150,151</sup> and test the hypothesis that cardioselective  $\alpha$ 1-AR activation is a viable therapy for heart failure.

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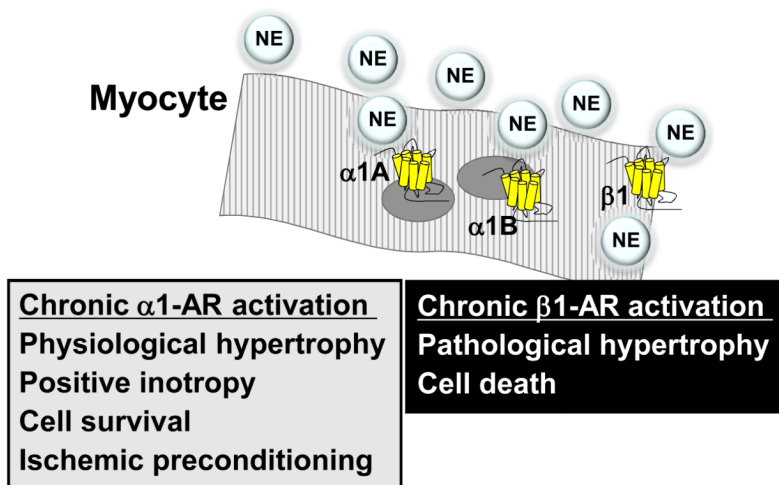


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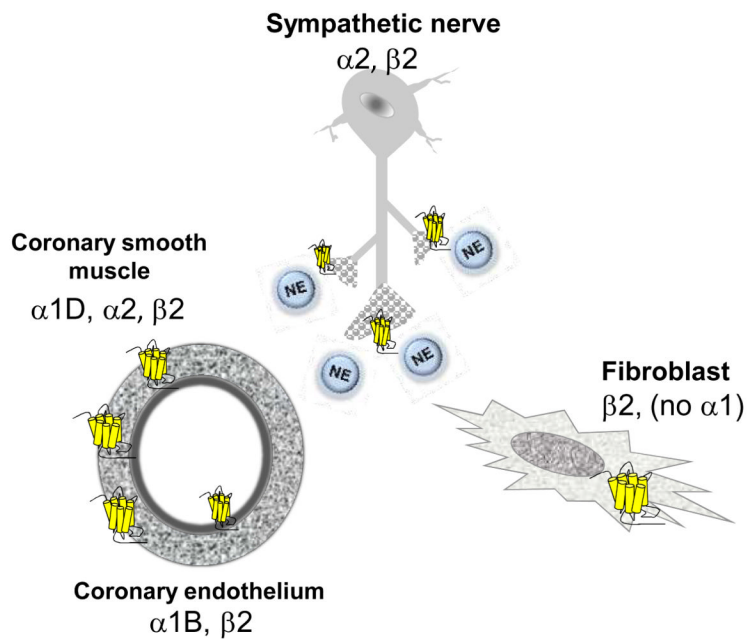
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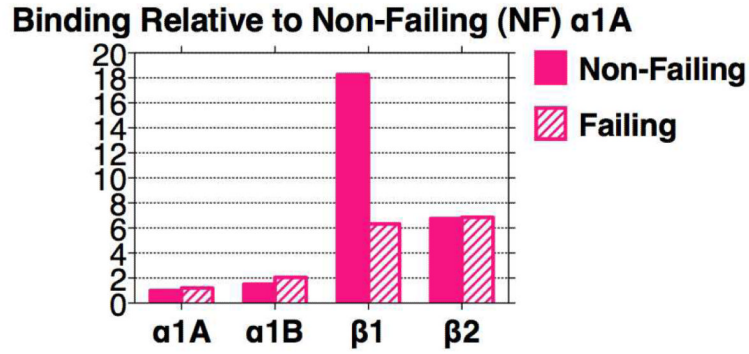
**Figure 1. Adrenergic receptors in the failing cardiac myocyte**

Radioligand binding levels of cardiac myocyte ARs are in the order  $\beta 1 > \beta 2 > \alpha 1B > \alpha 1A > \beta 3$ . Some ventricular myocytes do not express all subtypes.<sup>21</sup> New data localize the  $\alpha 1$ -ARs to the myocyte nucleus.<sup>35,36</sup> The (patho)physiological roles of chronic activation are indicated.



**Figure 2. Adrenergic receptors in cardiac non-myocytes**

Cardiac ARs are also broadly expressed on cells other than cardiac myocytes.  $\alpha_{1D}$ -ARs constrict coronary vascular smooth myocytes.  $\alpha_2$ -ARs constrict coronary vascular smooth muscle cells and inhibit NE release from sympathetic nerves.  $\beta_2$ -ARs stimulate NE release from sympathetic nerves, activate fibroblasts, and relax coronary smooth muscle cells. The roles of ARs in coronary endothelium are less defined.



**Figure 3. Adrenergic receptors in non-failing and failing human myocardium**

The  $\beta 1$ -AR is dominant in the non-failing heart, but is markedly down-regulated in the failing heart, whereas the  $\alpha 1A$ ,  $\alpha 1B$ , and  $\beta 2$  are not. Data are taken from Jensen et al.<sup>7</sup> In that study, receptors in human left ventricular free wall were quantified by radioligand binding, using  $^3H$ -prazosin for  $\alpha 1$ -ARs and  $^{125}I$ -cyanopindolol for  $\beta$ -ARs. Specific binding at the  $^3H$ -prazosin  $K_d$  was 40%. The proportion of the  $\alpha 1A$  subtype was defined by competition with 5-methyl-urapidil; the proportion of  $\beta 1$ , with CGP 20712A; and the proportion of  $\beta 2$ , with ICI-118,551. Non-failing hearts were unused donors, and failing were from transplant. Mean data from 4-6 patients for non-failing and failing are normalized to the value of the  $\alpha 1A$  in non-failing set at 1 (1.44 fmol/mg protein). The preparation used for binding was a complete myocardial lysate, not a “membrane” preparation, so that fmol values relative to protein are low, but no receptors are “tossed out”. The rationale for this approach is that about 60% of total myocardial  $\alpha 1$ -ARs are in a typical 1000g pellet, and only 15% of total remain in a 40,000g “membrane” pellet used typically for binding.<sup>83</sup> Use of “membrane” preparations can cause artifacts, as documented with myocardial  $\beta$ -ARs.<sup>181,182</sup>