The Adrenergic Pathway and Heart Failure

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ABSTRACT

Heart failure represents the endpoint to many triggering cardiovascular pathologies. However, there are molecular and biochemical features that remain common to the failing heart, despite the varying etiologies. Principal among these is heightened activation of the sympathetic nervous system and associated enhancement of adrenergic signaling pathways via the catecholamines, norepinephrine and epinephrine. During heart failure, several hallmark alterations in the adrenergic system contribute to loss of cardiac function. To specifically study these changes in a physiologically relevant setting, we and others have utilized advances in genetically engineered mouse technology. This chapter will discuss the many transgenic and knockout mouse models that have been developed to study the adrenergic system in the normal and failing heart. These models include genetically manipulated alterations of adrenergic receptors, linked heterotrimeric G proteins, and the regulatory G protein-coupled receptor kinases (GRKs). Among the more-interesting information gained from these models is the finding that inhibition of a particular GRK — GRK2 or β adrenergic receptor kinase 1 (βARK1) — is a potential novel therapeutic strategy to improve function in the setting of heart failure. Furthermore, we will discuss recent transgenic research that proposes an important role for hypertension in the development of heart failure. Overall, genetically engineered mouse models pertaining to this critical myocardial signaling system have provided novel insight into heart function under normal conditions and during states of dysfunction and failure.

I. Heart Failure and Sympathetic Nervous System Signaling

More than 500,000 new cases of heart failure are reported each year in the United States alone, making it one of the world's most-prolific diseases. The principal function of the heart is to provide enough oxygenated blood to meet the body's metabolic demands through cardiac output. Although the heart is adaptable to many physiological conditions, various etiologies can perturb its function, leading to ventricular dysfunction and ultimately failure. The initial response of the heart to excessive stress is to enlarge morphologically to a state known as cardiac hypertrophy. Classically, cardiac hypertrophy is defined as the physiological response of the heart to an increased workload. This hypertrophy may serve as compensatory and aid in preventing progressive deterioration of cardiac function (Grossman *et al.*, 1975; Chein, 1999). However, often, the stress will

overwhelm the system, sending the hypertrophied heart into failure. As the disease progresses, the heart dilates and thins, becoming too weak to maintain adequate cardiac output. During these hypertrophic and failing processes, there is sustained heightened activation of the renin-angiotensin system and the sympathetic nervous system in an attempt by the body to maintain cardiac output and systemic blood pressure (Esler *et al*., 1997). Recent evidence suggests that the signaling pathways stimulated during the hypertrophic process, if left unchecked, participate in the pathogenesis of heart failure and may be more important in this disease process than the actual stress placed on the heart (Esposito *et al.*, 2002; Rockman *et al.*, 2002).

The importance of sympathetic activity, via the catecholamines norepinephrine and epinephrine, in heart failure progression and mortality is well established (Cohn *et al.*, 1984). At a cellular level, the catecholamines act upon the heart by binding to the adrenergic receptors (ARs), which are members of the superfamily of proteins known as the G protein-coupled receptors (GPCRs) (Caron and Lefkowitz, 1993). In the heart, norepinephrine principally binds to the α_{1B}- and β_1AR, while epinephrine is a ligand for both β_1- and β_2AR (Caron and Lefkowitz, 1993). The β_1AR is the most-abundant βAR in the human heart, approaching 75% of the total number of receptors (Brodde, 1993). The βARs are coupled primarily to the heterotrimeric G protein, Gs, to stimulate adenylyl cyclase activity. This association generates intracellular cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activation, which regulate cardiac contractility and heart rate (Bristow *et al*., 1989). β_2ARs also can couple to the G protein, Gi, which can inhibit adenylyl cyclase activity and stimulate novel mitogen-activated protein kinase (MAPK) pathways in the heart through Gα and G$\beta\gamma$ subunits (Rockman *et al.*, 2002). Alternatively, binding of norepinephrine to the α_{1B}AR elicits phospholipase C (PLC) activity via activation of the G protein, Gq, which is the principal G protein signaling pathway implicated in the hypertrophic response of the heart (Molkentin and Dorn, 2001).

Following agonist occupation of ARs, these GPCRs become substrates for regulation via G protein-coupled receptor kinases (GRKs), which phosphorylate activated receptor (Inglese *et al*., 1993). This phosphorylation facilitates binding of β-arrestins, which sterically interferes with further coupling to G proteins, thus desensitizing and uncoupling the signal. The principal GRKs involved in intracellular signaling within the heart are GRK2 (or β adrenergic receptor kinase 1, βARK1), GRK3, and GRK5, all of which have specific GPCR selectivity *in vivo* in the heart (Eckhart *et al*., 2000). As will be detailed later, GRK activity in the heart appears to play a critical role, especially in heart failure. The generalized signaling pathways, their regulation, and outcomes in the heart are depicted in Figure 1.

Importantly, in human heart failure, chronic activation of the sympathetic nervous system has adverse implications and can accelerate cardiac pathology

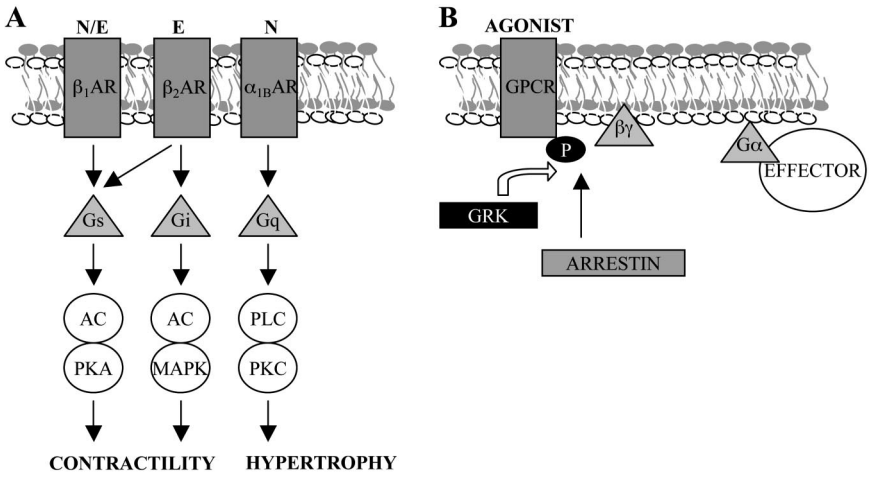

FIG. 1. (A) Schematic representation of the signaling pathways through the various adrenergic receptors (ARs) in the heart. (B) Schematic representation of the process of desensitization of G protein-coupled receptors (GPCRs). Following agonist binding to the GPCR, the associated heterotrimeric G protein splits into G$\beta\gamma$ and Gα. The Gα elicits a signal through the cell via effector molecules. In addition, the receptor is now a substrate for phosphorylation by the appropriate G protein-coupled receptor kinase (GRK). This phosphorylation facilitates arrestin binding, which sterically interferes with further G protein activation, thus desensitizing and uncoupling the signal. Abbreviations: N, norepinephrine; E, epinephrine; PKA, protein kinase A; MAPK, mitogen-activated protein kinase; PKC, protein kinase C.

(Cohn *et al*., 1984). Constant stimulation of ARs by catecholamines leads to selective β_1AR downregulation (Bristow et al., 1982,1993; Ungerer et al., 1993). However, both the β_1AR and the β_2AR are markedly uncoupled from their G proteins and effector systems (Bristow *et al.*, 1982,1989). The latter appears to be due to increased levels and GRK activity of βARK1 (Ungerer *et al*., 1993,1994). In addition, Gαi is significantly upregulated, to dampen adenylyl cyclase activation (Feldman *et al*., 1988). Overall, these molecular adrenergic changes in the failing human heart (summarized in Table I) lead to a marked attenuation of cardiac βAR signaling.

Interestingly, genetic polymorphisms in ARs have been identified and may influence individual characteristics of heart failure (Green *et al*., 1993; Podlowski *et al*., 2000; Small *et al.*, 2002). For example, individuals with an Ile164 allele for the β_2AR have significantly reduced survival (Green *et al*., 1993) and lower exercise capacity (Wagoner *et al*., 2000). In addition, polymorphisms within the β_1AR, combined with a deletion mutant found within the human α_{2c}AR gene, can act synergistically to increase the risk of heart failure in the black population (Small *et al*., 2002). These genetic studies, as well as recent heart failure drug

TABLE I
Changes in the βAR Signaling Pathway in Human Heart Failure

Molecule	Change	References
β_1ARs	↓, uncoupled	Bristow *et al.*, 1982, 1993; Ungerer *et al.*, 1993, 1994
β_2ARs	NC, uncoupled	Bristow *et al.*, 1993; Ungerer *et al.*, 1993, 1994
βARK1	↑ mRNA + activity	Ungerer *et al.*, 1993
GRK3	NC	Ungerer *et al.*, 1994
GRK5	Not studied	—
β Arrestin 1 and 2	NC	Ungerer *et al.*, 1993
Gαi	↑	Feldman *et al.*, 1988
Gαs	NC	Feldman *et al.*, 1988

[Abbreviations: AR, adrenergic receptor; GRK, G protein-coupled receptor kinase; NC, no change.]

trials, have indicated that there is still much to learn about alterations in the βAR system during cardiac failure. This includes recent studies demonstrating significant improvement in survival with βAR antagonist therapy in people with moderate and severe heart failure (Packer *et al*., 1996,2001). This is contraindicative to the short-term effects of β blockers (Epstein and Braunwald, 1966). The recent use of genetically engineered mice has provided unique experimental models for the study of cardiac adrenergic signaling alterations and the function of the normal and failing heart.

II. Mouse Models to Study the Cardiac Adrenergic System

A. LOSS AND GAIN OF AR EXPRESSION

1. The β_1AR

Gene-targeted knockout mice with disruption and ablation of the β_1AR gene generally are embryonically lethal. These mice recently have been reviewed in detail (Rohrer, 1998). Although surviving β_1AR knockout mice have normal heart rates, their response to exercise is abrogated (Rohrer *et al*., 1996). Interestingly, despite the presence of β_2ARs, there was no response to β-agonist stimulation, suggesting that β_1AR is responsible for catecholamine-induced alterations in heart rate in the mouse (Rohrer *et al*., 1996).

Using the α myosin heavy chain (αMHC) promoter to target gene expression to adult ventricular myocardium, mice that overexpress the β_1AR in the heart have been generated (Englhardt *et al*., 1999). These mice, with 5- to 15-fold overexpression compared to endogenous βAR levels, exhibit a pathology that is consistent with chronic sympathetic stimulation, with a phenotype of dilated cardiomyopathy and heart failure (Englhardt *et al*., 1999). As will be detailed, this is in striking contrast to phenotypes observed with myocardial β_2AR overexpression.

2. The β_2AR

In contrast to the β_1AR knockout mice, gene disruption of the β_2AR does not appear to significantly alter cardiac physiology (Rohrer, 1998). This suggests that, under normal conditions, the β_2AR plays no major role in murine cardiac physiology. However, the β_2AR can significantly alter cardiac physiology when overexpressed. Transgenic αMHC-β_2AR mice generated by the Lefkowitz laboratory had greater than 200-fold overexpression of endogenous βARs. These mice possessed a biochemical and physiological phenotype that mimicked maximal βAR myocardial signaling and function (Milano *et al*., 1994b). Surprisingly, even though these mice have enhanced heart rates and contractility from a young age, there is minimal pathology present, even in mice greater than 1 year of age (Koch *et al*., 2000). Furthermore, other transgenic mice with lower levels of overexpression (i.e., 30- to 50-fold) have similar characteristics (Turki *et al*., 1996). However, Liggett and colleagues (2000) have reported that a "transgene dose-response" for the β_2AR often can have delayed deleterious consequences, similar to what is observed with minimal β_1AR overexpression.

There appears to be significant Gi coupling in αMHC-β_2AR mice, as demonstrated by studies utilizing the Gi inhibitory pertussis toxin (Xiao *et al*., 1999). These data contribute to recent findings demonstrating that signaling via β_1AR and β_2AR in the heart is fundamentally different (Rockman *et al*., 2002). The overall positive effects seen with transgenic β_2AR overexpression suggest that the use of genetic engineering to replace lost βARs with the β_2AR in the failing heart may be therapeutic (Maurice *et al*., 1999). Consistent with this, we have found that cardiac β_2AR overexpression can "rescue" a mouse model of decompensated hypertrophy and heart failure due to cardiac Gαq overexpression (Dorn *et al*., 1999). However, αMHC-β_2AR mice were unable to rescue other mouse models of cardiomyopathy (Rockman *et al*., 1998b; Freeman *et al*., 2001). Moreover, β_2AR overexpression leads to functional deterioration of the heart, following induction of pressure overload (Du *et al*., 2000).

3. The α_{1B}AR

Two lines of mice have been generated using the αMHC promoter and the α_{1B}AR. These mice express either the wild-type receptor or a constitutively active mutant of the α_{1B}AR (CAMα_{1B}AR) (Akhter *et al*., 1997; Milano *et al*, 1994a). The CAMα_{1B}AR mice exhibit significant myocardial hypertrophy, suggesting that α_{1B}AR activation can induce cardiac changes independent of hemodynamic influences (Milano *et al*., 1994a). In contrast, mice overexpressing the wild-type α_{1B}AR do not develop an increase in heart size, despite displaying some biochemical characteristics of hypertrophy (Akhter *et al*., 1997). These mice, however, show a reduced tolerance to chronic α_{1B}AR stimulation, indicating that they are primed for a hypertrophic response (Iaccarino *et al*., 2001). Interestingly, the αMHC-α_{1B}AR transgenic mice have a decreased response to βAR stimulation (Akhter *et al*., 1997), which appears to be mediated via an observed increase in βARK1 expression and activity (Akhter *et al*., 1997; Iaccarino *et al*., 2001) and an activation of the sympathetic nervous system (Iaccarino *et al*., 2001). Thus, this mouse model has led to an elucidation of molecular cross-talk between the α_{1B}AR and the βAR systems in the heart. Finally, transgenic mice expressing the α_{1B}AR under the control of its isogenic promoter exhibit myocardial hypertrophy and have a surprising loss of sympathetic activity (Zusic *et al*., 2001).

B. GENETIC ALTERATION OF CARDIAC G PROTEIN EXPRESSION

1. Gαs

Transgenic mice overexpressing the stimulatory G protein Gαs in the heart have been generated and characterized (Gaudin *et al*., 1995). These mice exhibit enhanced responsiveness to catecholamines and develop cardiomyopathy as they age (Geng *et al*., 1999), in a model reminiscent of human heart failure. Interestingly, the phenotype can be rescued by chronic administration of a βAR blocker (Asai *et al*., 1999), suggesting that this phenotype, at least partially, mimics chronic sympathetic nervous system activation and enhanced βAR signaling.

2. Gαi

Targeted disruption of Gαi_2 or Gαi_3 (the major Gi subtypes in myocardium) in mice revealed that there appears to be no significant role for Gi signaling in basal cardiac function or in the response to βAR stimulation in the normal heart (Jain *et al*., 2001). In contrast to these knockout results, expression of a novel Gi-coupled receptor in the heart resulted in a large decrease in myocardial force, suggesting that defects in the Gi signaling pathway may contribute to the

development of cardiac pathology (Redfern *et al*., 1999; Baker *et al*., 2001). These results are consistent with the upregulation of Gαi, contributing to human heart failure and the uncoupling of the βAR system (Feldman *et al*., 1988).

3. Gαq

Dorn and colleagues have described transgenic mice overexpressing Gαq in the heart (D'Angelo *et al*., 1997). αMHC-Gq mice with 4-fold overexpression have cardiac hypertrophy and alterations in all of its molecular markers (D'Angelo *et al*., 1997). These animals, like the α_{1B}AR overexpressors, display abrogated αAR function. Crossbreeding the Gq mice with transgenic mice that had 200-fold overexpression of the β_2AR worsened the Gq phenotype (Dorn *et al*., 1999). However, a line of β_2AR mice with only 30-fold overexpression of the receptor rescued the cardiac hypertrophy (Dorn *et al*., 1999), suggesting that selective, controlled βAR enhancement may be beneficial. At higher levels of Gq expression in the transgenic mice, severe heart failure and early death was observed, with a component of increased myocyte apoptosis (Adams *et al*., 1998).

4. Gαq Inhibition

Due to the importance of Gq signaling in the development of cardiac hypertrophy, our laboratory set out to selectively inhibit this pathway in the heart. To achieve this, a specific peptide inhibitor consisting of the last 54 amino acids of the Gq (GqI) was developed and studied (Akhter *et al*., 1998). This GqI peptide targets the receptor-Gq interface, competitively inhibiting Gαq activation while not affecting Gs or Gi signaling (Akhter *et al*., 1998). Transgenic mice expressing the GqI peptide in the heart were shown to have attenuated responses to Gq-coupled receptor stimulation (Akhter *et al*., 1998). When these animals were subjected to an experimental model of pressure overload cardiac hypertrophy, expression of the GqI peptide in the heart significantly inhibited development of the hypertrophic phenotype (Akhter *et al*., 1998). Thus, this study identified Gq activation as the final common trigger for pressure overload hypertrophy. More recently, these mice have shown resistance to heart failure following chronic hypertrophic stimulus (Esposito *et al*., 2002), suggesting that Gq-class specific inhibition is a novel strategy to prevent ventricular dysfunction in conditions of chronic hypertrophic stress.

C. MANIPULATION OF CARDIAC GRK EXPRESSION

1. βARK1

The importance of βARK1 (GRK2) in the cardiovascular system is clearly noted by the severe cardiac malformations and embryonic death observed

following βARK1 gene ablation (Jaber *et al*., 1996). The findings suggest a possible role for βARK1 in the normal migration and differentiation of myocardial cells during heart development. Heterozygous βARK1 knockout mice with 50% less βARK1 expression and activity in myocardium have no developmental abnormalities (Rockman *et al*., 1998b). Transgenic mice that overexpress βARK1 in the heart due to the use of the αMHC promoter have an attenuated response to catecholamine stimulation with desensitized βARs (Koch *et al*., 1995). This was a significant finding, as it represents the first demonstration that βARK1 could cause the functional uncoupling of βARs *in vivo*. Furthermore, these mice demonstrate that the upregulation of βARK1 seen in human heart failure may have significance and contribute to the pathogenesis of ventricular dysfunction. Contractile responses to angiotensin II also are abrogated in αMHC-βARK1 transgenic mice, suggesting that βARK1 may be important in other receptor systems in the heart (Rockman *et al*., 1996). Interestingly, βARK1 overexpression has no effect on cardiac α_{1B}AR signaling, demonstrating GRK-GPCR selectivity *in vivo* (Eckhart *et al*., 2000).

2. Inhibition of βARK1

Since βARK1 activity is increased in heart failure and appears to play a role in uncoupling of βARs in the heart, we have studied the physiological consequences of βARK1 inhibition. To do this, a specific peptide inhibitor consisting of the last 194 amino acids of the βARK1 (βARKct) was developed and studied (Koch *et al*., 1993). The βARKct contains the $G_{\beta\gamma}$ binding domain and competes with endogenous βARK1 for $G_{\beta\gamma}$-mediated membrane translocation, a process required for βARK1 activation on activated GPCRs (Koch *et al*., 1993). When the βARKct was expressed in the hearts of transgenic mice under the control of the αMHC promoter, cardiac physiology was altered in reciprocal fashion to that seen with βARK1 overexpression (Koch *et al*., 1995). The βARKct mice have enhanced cardiac function at baseline and an augmented response to catecholamines (Koch *et al*., 1995). Importantly, using a hybrid transgenic mouse strategy where βARK1 overexpression and βARKct expression occurred *in vivo* simultaneously, we have shown that the βARKct is, indeed, inhibiting cardiac βARK1 activity (Akhter *et al*., 1999).

The phenotypes of the βARK1 and the βARKct mice are consistent with our hypothesis that this GRK plays a critical role in cardiac function and potentially in cardiac pathologies. Interestingly, heterozygous βARK1 knockout mice also have a phenotype of enhanced cardiac function (Rockman *et al*., 1998b), demonstrating that lowering βARK1 expression or its activity can have profound *in vivo* effects on cardiac contractility. Moreover, hybrid mice that express the βARKct in the heart and are heterogeneous for βARK1 gene ablation have even-greater enhancement of cardiac function (Rockman *et al*., 1998b).

In addition to heart failure, enhanced βARK1 expression and activity has been shown to be indicative of several models of cardiac hypertrophy (Koch *et al.*, 2000). Enhanced βARK1 activity in the hypertrophied heart has been shown to be responsible for the loss of βAR inotropic reserve seen in this pathological condition (Choi *et al.*, 1997). To study the inhibition of βARK1 during hypertrophy, we used a novel transgenic mouse model. The βARKct was targeted to the heart using the cardiac ankyrin repeat protein (CARP) promoter, which turns off during adulthood. However CARP belongs to a family of fetal genes, such as atrial natiuretic factor (ANF), that can be reactivated in adult ventricular myocardium by stress. CARP-βARKct mice lose βARKct expression after 3 weeks of life and adult mice do not have enhanced contractility (Manning *et al*., 2000). However, following induction of pressure overload hypertrophy, expression of the βARKct is seen once again in the myocardium, resulting in improved βAR responsiveness and cardiac function (Manning *et al*., 2000).

3. Cardiac Transgenic Studies with GRK3 and GRK5

Following the profound effects seen with βARK1 manipulation in the hearts of transgenic mice, we studied the physiological consequences of GRK3 and GRK5 overexpression. These two GRKs are found normally in the heart but their overall role in cardiac signaling is not well understood, although GRK5 has been found to be upregulated in some animal models of heart failure (Ping *et al*., 1997; Vinge *et al*., 2001). Unlike βARK1, both GRK3 and GRK5 homozygous knockout mice are viable with no overt cardiac phenotype (Wess, 2000). Overexpression of these GRKs in the heart has, however, led to unexpected and interesting results that have uncovered novel aspects of GRK regulation *in vivo* in the heart. GRK3 (also known as βARK2) previously was thought to be an isozyme of βARK1, since it is highly homologous and appeared to have the same *in vitro* GPCR activity (Benovic *et al*., 1991; Freedman *et al*., 1995). However, when αMHC-GRK3 mice were generated and studied, there were no signaling alterations in the cardiac βAR system (Iaccarino *et al*., 1998a). This was the first demonstration *in vivo* that GRK3 was different from βARK1, with a unique GPCR specificity profile. Further investigation revealed that thrombin signaling in the heart was uncoupled in these mice, demonstrating that the thrombin receptors are *in vivo* substrates for GRK3 (Iaccarino *et al*., 1998a). The difference in these GRKs may lie in the $G_{\beta\gamma}$ binding regions, which is the area between GRK3 and βARK1 that is the most divergent (Muller *et al*., 1997). Thus, there may be selective GPCR-mediated translocation of these GRKs. In hybrid transgenic mice with different GRKs overexpressed along with the α_{1B}AR, it was found that GRK3 is also the primary kinase for desensitization of this AR in the heart (Eckhart *et al*., 2000).

GRK5, which is the second-highest expressing GRK in the heart, is not regulated by $G_{\beta\gamma}$ and thus would be expected to have different receptor substrates in the heart. However, like βARK1 overexpressing mice, αMHC-GRK5 transgenic mice had severely blunted βAR inotropic responses *in vivo* in the heart, demonstrating that this GRK also could desensitize cardiac βARs (Rockman *et al*., 1996). These mice exhibited GPCR substrate selectivity, compared to βARK1, as responses to angiotensin II were not altered, whereas this Gq-coupled receptor system was desensitized in mice overexpressing βARK1 (Rockman *et al*., 1996). GRK5 also has some activity against cardiac α_{1B}ARs, again demonstrating a difference with βARK1 (Eckhart *et al.*, 2000). The overall significance of the findings that GRK5 may be altered in heart failure is not clear at this time but obviously could have important implications.

III. βARK1 Inhibition and Rescue of Murine Models of Heart Failure

One interesting area where this research has led us is to investigate whether inhibition of βARK1 activity could be a novel therapeutic strategy for improving function of the failing heart. Over the last few years, this has become possible to study in the mouse, as murine models of cardiomyopathy have been described, many of which have important manifestations of the human condition. These models have been the result of a specific gene deletion in the mouse or cardiac-specific overexpression of a heart failure-inducing transgene. Powerful information can be generated by cross-breeding αMHC-βARKct mice and various heart failure models to test the hypothesis that $G_{\beta\gamma}$-βARK1 inhibition could be beneficial. Simply studying the cardiac phenotype of these novel hybrid mice could give an answer and provide information on the role of βARK1 and GRK activity in the pathogenesis of the various heart failure etiologies, specific for the different models. We have studied six different heart failure models with βARKct mice and, for the most part, have seen overwhelming rescue. Table II summarizes our findings over the last 3–4 years using this novel genetic approach; some of the more-important findings are detailed in the next paragraph.

The first murine heart failure model rescued by any genetic manipulation was done by us with βARKct animals (Rockman *et al*., 1998a). The model of heart failure was due to the gene knockout of the muscle LIM protein (MLP), a cytoarchitectural protein and conserved regulator of myogenic differentiation (Rockman *et al.*, 1998a). The improvements made by the βARKct in this model of dilated cardiomyopathy ($MLP^{-/-}$) included restoring cardiac chamber dilation, increasing basal contractility, and enhancing βAR function (Table II). A second model of heart failure with different characteristics of disease and rescue was due to cardiac-targeted overexpression of calsequestrin (CSQ), a high-capacity calcium-binding protein (Harding *et al*., 2001). These mice have much more-severe disease than the $MLP^{-/-}$ animals and experience early mortality, as

TABLE II
Murine Models of Heart Failure and Rescue Status with Cardiac βARKct Expression

Murine model	Phenotypic change with βARKct	Reference
$MLP^{-/-}$ knockout	Functional rescue with restored βAR responsiveness	Rockman *et al.*, 1998a
Transgenic cardiac CSQ overexpression	Functional rescue and cardiac dimensions with improved survival	Harding *et al.*, 2001
Transgenic cardiac expression of a mutant myosin heavy chain (HCM)	Functional rescue, prevention of hypertrophy, and dimensions and improved exercise tolerance	Freeman *et al.*, 2001
Transgenic cardiac overexpression of MCP-1	Prevention of hypertrophy	Khouri *et al.*, 2002
Transgenic cardiac overexpression of dominant-negative mutant of CREB ($CREB_{A133}$)	Restoration of βAR signaling, no functional or mortality rescue	Eckhart *et al.*, 2002a
Transgenic cardiac overexpression of Gαq	No rescue	Dorn *et al.*, 1999

[Abbreviations: $MLP^{-/-}$, homozygous knockout of muscle LIM protein; CSQ, calsequestrin; MCP-1, monocyte chemotactic protein-1; CREB, cyclic AMP-responsive element binding protein.]

all mice are dead within 20 weeks of life. Thus, in this study, we were able to carry out a survival test. βARKct expression nearly doubled the life-span of these heart-failure mice (Harding *et al.*, 2001). In addition to survival, the βARKct prevented excessive ventricular deterioration and improved cardiac function (Harding *et al.*, 2001). Furthermore, combination of β-blocker therapy (metoprolol) and βARKct expression was synergistic in improving survival (Harding *et al.*, 2001). These data are particularly interesting, given the clinical promise of β-blocker therapy in human heart failure (Packer *et al.*, 1996,2001). Studies from our laboratory have also shown that chronic βAR blockade by carvedilol decreases the expression of βARK1 in the heart and reduces cardiac GRK activity (Iaccarino *et al.*, 1998b).

As detailed in Table II, cardiac βARKct expression also has rescued other models of heart failure, including one with cardiac-targeted overexpression of a mutant form of the αMHC gene (HCM) that is associated with human hypertrophic cardiomyopathy (Freeman *et al.*, 2001). Interestingly, expression of the βARKct in the Gαq mice had no effect on the Gq phenotype, unlike the β_2AR (Dorn *et al.*, 1999). In the Gq model of decompensated cardiac hypertrophy,

βARK1 is not upregulated, suggesting that the βARKct is acting specifically to inhibit GRKs. However, the exact mechanism of the βARKct may involve sequestration of $G_{\beta\gamma}$ from other signaling pathways, such as those involved in the activation of phosphoinositide-3 kinase (PI3K) (Naga Prasad *et al*., 2000,2001) and $I_{K,Ach}$ channels (Clapham and Neer, 1997). The contribution of these other potential $G_{\beta\gamma}$ effects to the salutary effects of βARKct in heart failure remains to be determined. Finally, the therapeutic benefit of the βARKct may involve enhanced signaling through other GPCRs such as angiotensin II receptors.

IV. Hypertension, the Adrenergic Pathway, and Heart Failure

The American Heart Association suggests that the presence of hypertension or high blood pressure in a patient doubles that person's risk for developing heart failure. In essential hypertension, elevated blood pressure has been associated with increased sympathetic output (Mark, 1990), suggesting that the catecholamines and associated adrenergic pathways may be involved in this pathology. It also implicates hypertension as a potential primary component in the development of heart failure. Indeed, studies have shown that agents that reduce blood pressure, no matter what the mechanism, all appear to eventually reverse hypertrophy (Testa *et al*., 1996; Anker *et al*., 1997).

We previously discussed the importance of Gq signaling in cardiac hypertropy and heart failure. As was detailed, transgenic mice that express the GqI peptide inhibitor of Gq can prevent the development of hypertrophy and heart failure in a pressure-overload model (Akhter *et al*., 1998; Espositio *et al*., 2002). To study the potential contribution of the vascular system and its associated alterations in blood pressure to this hypertrophic response, we developed a line of trangsenic mice that express GqI in vascular smooth muscle cells under the control of the SM22α promoter (Keys *et al*., 2002). Following chronic Gq agonist administration, we observed an attenuation of mean arterial blood pressure and an inhibition of cardiac hypertrophy in the transgenic mice with vascular GqI expression (Keys *et al*., 2002). In contrast — and somewhat unexpectedly — when the GqI was expressed in the heart, neither hypertension nor hypertrophy was inhibited (Keys *et al*., 2002). These findings suggest that, during hypertension, the vascular system is the principal determinant of cardiac hypertrophy, rather than direct stimulation of the heart itself.

Interestingly, impairment of the vascular βAR system has been shown in human and animal models of hypertension (Feldman, 1990; Brodde and Michel, 1992). More specifically, elevations in βARK1 expression have been found in lymphocytes of hypertensive patients (Gros *et al*., 1997,1999). Recently, we generated transgenic mice that express βARK1 in the vascular smooth muscle, again using the SM22α promoter (Eckhart *et al*., 2002b). These mice display attenuated vascular βAR signaling, an increase in mean blood pressure, and

develop cardiac hypertrophy (Eckhart *et al.*, 2002b). This again implicates the adrenergic system in hypertrophy – and, in particular, cardiovascular βARK1 activity — which also proposes a link between hypertension and heart failure.

IV. Conclusion

The development of transgenic mouse models has provided a broader understanding of the physiological impact of individual proteins during heart failure. Overall, through our efforts detailed herein and those from other laboratories around the world, there are currently at least 75 genetically altered mouse models available to study the role of particular signaling systems in the heart (Chu *et al.*, 2002). This review has focused on the adrenergic signaling pathway under normal conditions and during heart failure. It is evident that transgenic mice have given us insight into the role of adrenergic system in the heart that otherwise would not have been possible. In the future, we hope that this knowledge may yield novel therapeutic interventions for the treatment of cardiac disease. In fact, adenoviral-mediated delivery of the βARKct and β_2AR to larger animal models of heart failure has resulted in beneficial effects (White *et al.*, 2000; Shah *et al.*, 2001; Tevaearai *et al.*, 2002), suggesting that gene-therapy strategies may, indeed, target these AR abnormalities in heart failure in the coming years and offer new hope to patients suffering from this disease of epidemic proportions.

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