New Insight Into the Role of Enhanced Adrenergic Receptor-Effecter Coupling in the Heart

Arthur M. Feldman, MD, PhD; Charles McTiernan, PhD

The ability of the heart to augment the perfusion of vital organs and skeletal muscles during stress is predicated on its ability to increase contractility in response to adrenergic neurohormones that are released from postsynaptic nerve terminals within the heart and/or from the adrenal gland. These neurohormonal signals must then be detected and processed by a complex of proteins located within the sarcolemmal membrane, resulting in the conversion of the adrenergic signal to an alteration in the biomechanical properties of individual myocytes. This sarcolemmal protein complex consists of adrenergic receptors (ARs), guanine nucleotide–binding regulatory proteins, and the effector enzyme adenylyl cyclase. Myocardial contractility is augmented by the interaction of adrenergic agonists with β1- and/or β2-adrenergic receptors (βARs) located on the surface of the sarcolemmal membrane (see review in Reference 1). These 2 βAR subtypes are coupled to adenylyl cyclase activation by the stimulatory guanine nucleotide–binding protein (Gs). Interaction of the agonist-βAR complex with the heterotrimetric G protein catalyzes the release of GDP from the α-subunit of the G protein (αs), allowing the binding of GTP and the subsequent activation of adenylyl cyclase by αs-GTP. This activation persists until intrinsic GTPase activity of αs hydrolyzes the nucleotide, resulting in an inactive αs-GDP moiety. G-protein–mediated activation of adenylyl cyclase effects the synthesis of the intracellular second messenger cAMP and the resulting phosphorylation of a cAMP-dependent protein kinase (PKA). Once phosphorylated, PKA is then able to effect positive inotropic and chronotropic responses by phosphorylating a group of intracellular proteins, including phospholamban, and the L-type voltage-dependent calcium channel while at the same time enhancing lusitropy via alterations in the sensitivity of troponin for Ca2+.

α-Subunits of some G proteins also appear to effect myocyte ion channel activity independent of adenylyl cyclase activation and in so doing can also regulate myocyte function.

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In the human heart, activation of β1-AR results in a marked increase in contractility. Furthermore, there is strong evidence that β2-AR also couples to a positive inotropic response. A similar inotropic response is seen in neonatal but not adult rodent myocytes. However, investigators have recently suggested that β2-ARs can regulate cardiac contractility independently of cAMP in both rodents and humans. Moreover, recent studies in murine myocytes demonstrate that β2-AR couples to both Gs and the inhibitory guanine nucleotide–binding regulatory protein (Gi), resulting in an absence of inotropic responsiveness unless Gi is inhibited.

As seen with many cellular pathways that impart critically important homeostatic control, the inotropic activity of the receptor–G-protein–adenylyl cyclase complex (RGC) is highly regulated. Indeed, in the presence of an agonist, there is a rapid waning of response due to desensitization of the RGC complex. This desensitization was initially attributed to sequestration within the cellular milieu; however, recent studies have demonstrated that an important component of receptor desensitization is phosphorylation of activated receptors by members of the G protein–coupled receptor kinase (GRK) family. βAR kinase-1 (βARK1: GRK2) is a GRK that specifically phosphorylates both β1- and β2-ARs when they are in the activated form, resulting in desensitization. Interestingly, regulation of βARK expression is ligand induced: expression is significantly increased on exposure to agonist and substantially decreased in the presence of β-adrenergic blockade. Although βARK plays an important role in short-term desensitization, long-term exposure to agonists is associated with a decrease in the mRNA encoding the βARs, a phenomenon that appears to be due at least in part to changes in mRNA stability. The activity of the RGC complex can also be modulated at the level of the G proteins. Changes at both the transcriptional and posttranslational levels can result in an alteration in either G-protein activity or stability. These changes in G-protein expression or function can be initiated by exposure to a variety of neurohormones, including catecholamines and proinflammatory cytokines.

Although the heart depends on adrenergic drive to enhance function during times of stress, investigators suggested that long-term exposure to adrenergic drive might be maladaptive on the basis of the finding of (1) a direct relationship between norepinephrine expression and mortality and (2) an inability of the failing human heart to appropriately augment myocardial contractility in response to challenge with an adrenergic agonist. That alterations in βAR-effector coupling are important in the development of adrenergic insensitivity was first recognized when investigators demonstrated that heart failure in humans was characterized by a selective and nearly 50% downregulation of β1-AR. As a result of this receptor downregulation, the ratio of β1:β2-receptors was shifted from ~80:20 in nonfailing heart to 60:40 in failing ventricular

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myocardium. Subsequent studies demonstrated that this downregulation was related to a decrease in β2-receptor mRNA17 and could be initiated by exposure to adrenergic agonists.18 However, heart failure in humans was also associated with other substantive changes in the RGC complex, including uncoupling of β2-AR from adenylly cyclase, increased functional activity of αGn, and enhanced expression of βARKs.19

Although downregulation of RGC coupling was associated with diminished contractile response, investigators proposed that enhanced exposure to catecholamines could be cardiotoxic, and thus, receptor downregulation might be an adaptive rather than a maladaptive phenomenon. This hypothesis was based on the recognition of an inverse relationship between norepinephrine levels and survival in patients with congestive heart failure (CHF).14 Additional support for this hypothesis comes from laboratory data demonstrating the ability of catecholamines to negatively affect the biology and structure of the myocyte, as evidenced by downregulation of adrenergic signaling systems, stimulation of apoptosis in adult rat ventricular myocytes in vivo, and myofibrillar degeneration.20 Furthermore, despite favorable hemodynamic effects, endogenous production of catecholamines, as occurs in some disease states and with stress,21 can be cardiotoxic in humans, and the therapeutic use of β-adrenergic agonists has been shown to accelerate disease progression and shorten life expectancy in patients with heart failure. Most importantly, both selective and nonselective β-blockade has provided salutary effects on morbidity and mortality in patients with CHF,22 presumably due to reversal of intrinsic systolic dysfunction via a time-dependent biological effect on the myocardium. Interestingly, recent studies have demonstrated the presence of autoantibodies directed against β2-AR in some patients with dilated cardiomyopathy. In vitro, these autoantibodies can enhance myocardial contractility but also downregulate adrenergic receptors after longer-term treatment. Thus, even in the absence of enhanced adrenergic drive, immune-mediated alterations in receptor-effector coupling might precipitate the development of cardiac failure.

To better understand the role of the many components of the RGC complex in cardiovascular homeostasis, investigators have recently taken advantage of transgenic technology that has been developed over the past decade. In studies evaluating the RGC complex, transgenic manipulation of various components provides the novel opportunity to enhance or impede signaling in an agonist- or antagonist-independent manner. Not surprisingly, overexpression of β2-AR resulted in increased basal myocardial adenylly cyclase activity, enhanced atrial contractility, and increased left ventricular function in vivo, changes that were consistent with those seen in wild-type animals maximally stimulated with the adrenergic agonist isoproterenol.23 However, transgenic mice overexpressing β2-AR also demonstrated maximal enhancement of myocardial relaxation due to a selective decrease in the amount of phospholamban protein, a constitutive inhibitor of sarcoplasmic reticulum Ca2+-ATPase activity.24 Consistent with the results in studies of mice overexpressing β2-AR, transgenic mice overexpressing a βARK inhibitor (and therefore having enhanced β1-AR and β2-AR effector enzyme coupling) also demonstrated increased myocardial contractility.7 Mice overexpressing βARK demonstrated attenuation of isoproterenol-stimulated myocardial contractility, diminished myocardial adenylly cyclase activity, and uncoupling of βARs from downstream signal-transduction pathways when the levels of βARK overexpression were similar to those seen in failing human hearts.7 Importantly, neither the mice overexpressing β2-AR nor those overexpressing the βARK inhibitor developed abnormalities in myocardial function or morphology despite the heightened adrenergic signaling.

In this issue of Circulation,25 Akhter and colleagues present an elegant study that provides additional evidence that supports the role of βARK in heart muscle adrenergic responsiveness and more importantly suggests that βARK might provide a novel target for future therapeutic strategies. Using the heart as an “in vivo reaction vessel,” Akhter et al created hybrid transgenic mice harboring cardiac-specific overexpression of both βARK and a βARK inhibitor (βARKct peptide). The coexpression of βARKct inhibited both the elevated myocardial βARK1 activity as well as the abnormal βAR responsiveness that characterized mice overexpressing only βARK. Thus, βARKct effectively normalized both the biochemical and physiological pathology effected by βARK overexpression. Several other technologies could putatively have been used to address a similar question. For example, inhibitor proteins or dominant negatives could be overexpressed by driving gene expression with an adenoviral vector, or alternatively, pharmacological inhibitors could be used to selectively inhibit a known protein. However, both of these techniques have marked limitations. Adenoviruses may be rapidly cleared by immunologic reactions, allowing only a limited window in which to study the effects of gene overexpression. In addition, gene-transfer techniques effect changes in only a subpopulation of myocytes. Pharmacological inhibitors, if available, often lack specificity or have ancillary properties that abrogate accurate interpretation of results. Therefore, the novel technique used in the report by Akhter et al provides a mechanism for persistently and selectively abrogating the effects of βARK overexpression in vivo and confirms the hypothesis that βARK might provide a new therapeutic target. As noted by Akhter et al, additional support for a role for βARK inhibition in the therapy of CHF comes from recent studies in which the hybrid transgenic mouse technique was used to overexpress βARKct in a line of cardiomyopathic mice harboring mutations in the gene encoding the muscle-specific LIM protein MLP (MLP−/−).26 Overexpression of βARKct rescued the myopathic phenotype in these transgenic mice, which, like the βARK transgenic mice, demonstrated increases in βARK1 levels consistent with those found in failing human heart.

Although the study by Akhter et al25 suggests that positive modulation of adrenergic receptor coupling and, in particular, inhibition of βARK1 expression might have salutary effects in patients with CHF, transgenic modulation of other components of the RGC complex have resulted in the development of a different phenotype. For example, overexpression of Gαs in transgenic mice, resulting in a 2.8-fold increase in
Gₐᵦ protein, was reported to enhance the rate of adenyl cyclase activation and the relative number of βARs that bound agonist with high affinity without affecting myocardial morphology.²⁷ However, subsequent studies by the same investigators using older mice (≥10 months of age) demonstrated normal baseline contractility but an enhanced response to isoproterenol. Furthermore, in 16-month-old mice, pathological and histological analyses revealed cellular hypertrophy, degeneration, atrophy, and replacement fibrosis.²⁸ Moreover, ECG monitoring demonstrated a high incidence of arrhythmias with increased mortality compared with control mice.²⁹ Indeed, in the older mice, left ventricular ejection fraction was markedly diminished, left ventricular end-diastolic dimension was increased, and baseline heart rates were elevated compared with wild-type controls. Recently, transgenic technology has also been used to create mice overexpressing the human β₁-AR.³⁰ At a young age (4 to 5 months), these mice display myocardial remodeling with myocyte hypertrophy and interstitial replacement fibrosis. However, by 8 months of age, they demonstrate marked increases in left ventricular end-systolic and end-diastolic dimensions consistent with development of a dilated cardiomyopathy.³¹ Thus, although overexpression of β₁-AR or enhanced β₂-AR activity via overexpression of a βARK inhibitor improves left ventricular performance without compromising the normal cardiac morphological phenotype, enhanced expression of other components of the RGC complex has deleterious effects that result in a transition from the wild type to a myopathic phenotype.

The disparate results seen in these transgenic mice overexpressing the various components of the RGC complex raise obvious questions about the physiological effects of RGC pathway inhibition and stimulation in mice. Moreover, they raise interesting questions about the difference between mice and humans and suggest that the RGC complex and its regulation may be far more complex than originally realized. At the clinical level, it appears that β-blockade is beneficial in patients with CHF, whereas β-stimulation is deleterious. However, studies of transgenic mice provide a confusing array of results. Activation of β₂-ARs and inhibition of βARK provide beneficial effects, whereas stimulation of other RGC complex moieties is clearly harmful. Although the origin of these marked differences remains undefined, several explanations might be speculated: (1) differences in the ratios of receptor subtypes in humans and rodents; (2) the presence of unique and undefined substrates for the βARK2 inhibitor; (3) the potential relevance of the recently identified β₁-AR and its possible uncoupling by βARK; (4) differences between β₁-AR coupling with Gα and Gβγ in human and murine hearts; and (5) the possibility that the beneficial effects of β-blockade are due to cellular mechanisms independent of the β₁-AR or β₂-AR. Interestingly, the latter possibility is supported by a recent study that has suggested that the benefits of the β-blocker carvedilol are inversely correlated with norepinephrine levels in patients with CHF.³² In addition, genetic factors that alternatively regulate the various proteins of the RGC complex might play a role in the response of a specific individual to enhance adrenergic drive.

In summary, the marked disparities in phenotype that result after overexpression of the various components of the RGC complex have raised interesting questions regarding the biology and pathophysiology of the RGC complex. Although we must learn more about the intricacies of cardiac signal transduction, the elegant hybrid transgenic technique described by Akhter et al³³ in this issue of Circulation should provide the technology with which investigators can address these important questions.

References


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