

## REVIEW ARTICLE

## $\beta$ -Adrenergic receptor signaling in cardiac function and heart failure

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### INTRODUCTION

Congestive heart failure (CHF) is a major public health problem. Nearly 5 million Americans are afflicted with CHF and about 550,000 new cases are diagnosed each year (1). CHF is a pathophysiological condition in which the heart is unable to pump enough blood to meet the metabolic needs of the body. It is the endpoint for several cardiovascular diseases including coronary artery disease, myocardial infarction, hypertension, cardiomyopathy, congenital heart defects, myocarditis, and valvular heart disease resulting from rheumatic fever or endocarditis. Regardless of the etiology, compensatory and adaptive changes occur in the heart to preserve cardiac output. Some of these changes are mediated by enhanced sympathetic nervous system activity (2). Increased catecholamine outflow from this system induces sustained elevated activation of the  $\beta$ -adrenergic receptors ( $\beta$ -ARs), which results in abnormalities in the  $\beta$ -AR signaling system that may ultimately lead to the pathogenesis of CHF (3).

In this review, I will discuss (i)  $\beta$ -AR subtypes in the heart; (ii) the functional role of  $\beta$ -AR signaling in CHF; and (iii) the recent studies in genetically engineered mice to elucidate the functional effects and therapeutic potential of critical genes in the cardiac  $\beta$ -AR signal transduction pathways.

### $\beta$ -ARs IN THE HEART

The  $\beta$ -ARs belong to the superfamily of membrane proteins known as G-protein-coupled receptors (GPCRs) (4). GPCRs are characterized by a conserved core structure with extracellular amino terminus, intracellular carboxyl terminus and seven transmembrane  $\alpha$ -helices, which are connected by three extracellular and three intracellular loops. They transduce extracellular signals from endogenous hormones and neurotransmitters, ambient physical and

chemical stimuli, as well as exogenous therapeutic agents. GPCRs are involved in regulation of a vast array of physiological processes including sensory perception, cell growth, metabolism and hormonal homeostasis.

The transmembrane signaling by GPCRs is initiated by the binding of ligands such as hormones or neurotransmitters (Figure 1). Ligand binding induces a conformational change in GPCRs that causes coupling with heterotrimeric G-proteins (5). G-proteins consists of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits and GPCR coupling leads to the exchange of G-protein-bound GDP for GTP and the dissociation of the G-protein into active  $G\alpha$  and  $G\beta$  subunits to mediate downstream signaling. Based on their amino acid sequences and function,  $G\alpha$  subunits are grouped into four subfamilies -  $G\alpha_s$ ,  $G\alpha_i$ ,  $G\alpha_q$  and  $G\alpha_{12}$  (6). Subunits of the diverse G-proteins differentiate the cellular signal by modulating the activity of various effector molecules such as adenylyl cyclase (AC) or phospholipase C- $\beta$ . These effector molecules regulate the concentrations of second messengers in the cell, activating a number of different downstream signaling molecules.

There are four subtypes of  $\beta$ -ARs- $\beta_1$ -AR,  $\beta_2$ -AR,  $\beta_3$ -AR and the  $\beta_4$ -AR (6). The  $\beta_1$ -AR is found primarily in the heart and comprises 75-80% of the  $\beta$ -ARs found in the heart (Figure 2). The  $\beta_2$ -AR is expressed in the lungs, kidneys and blood vessels as well as the heart and comprises 20-25% of cardiac  $\beta$ -ARs. The  $\beta_3$ -AR is found primarily in the adipose tissue, and minimally in the heart. The  $\beta_4$ -AR is considered a low affinity state of  $\beta_1$ -AR, which awaits genetic and pharmacologic characterization. Epinephrine and norepinephrine serve as the primary agonists for all  $\beta$ -ARs. However, recent data have revealed significant differences in the signaling pathways and cellular responses of the  $\beta$ -AR subtypes (7).

When stimulated, cardiomyocyte  $\beta_1$ -AR primarily binds to the G stimulatory (Gs) protein. The  $G\alpha$  subunit of the Gs protein ( $G\alpha_s$ ) activates AC, which generates the second messenger cyclic adenosine monophosphate

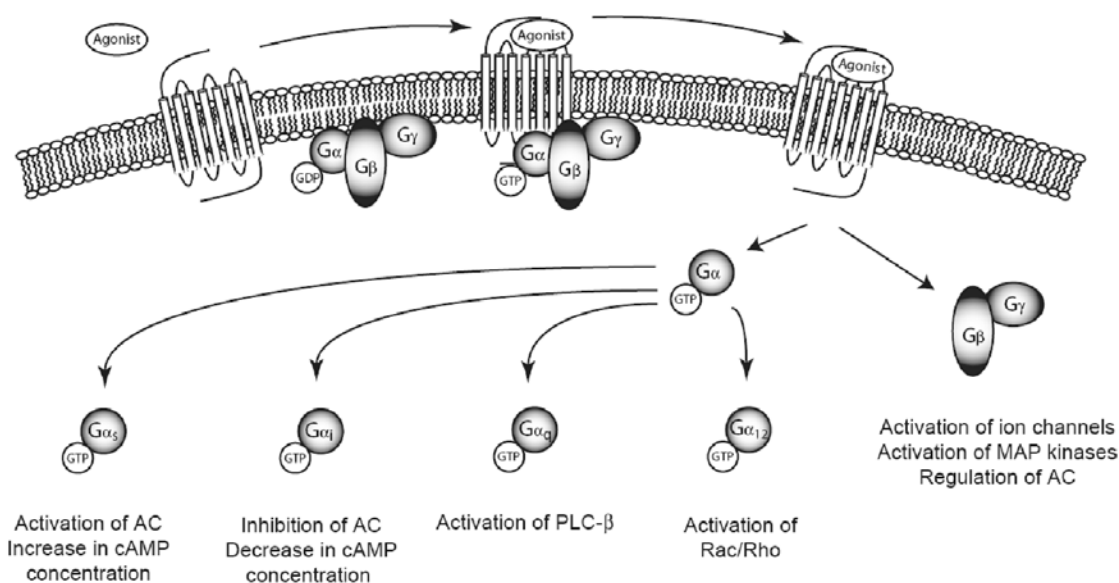
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(cAMP); heightened cAMP levels activate cAMP-dependent protein kinase A (PKA). Activated PKA then phosphorylates troponin I, the L-type  $\text{Ca}^{2+}$  channel and phospholamban (PLB), resulting in greater contractility (8). In addition to Gs, the  $\beta_2$ -AR couples to pertussis toxin - sensitive G inhibitory ( $G_i$ ) protein.  $G_i$  coupling releases the activated  $G_i\alpha$  subunit, which inhibits AC activity, and the  $G_i\beta\gamma$  subunit, which causes downstream activation of the mitogen-activated protein kinases (MAPK).  $G_i\alpha$  coupling also activates the cytosolic effector molecule phospholipase A2 (cPLA2), which causes cAMP-independent enhancement of calcium signaling and cardiac contraction. Recent studies indicate that  $\beta_2$ -AR-cPLA2 pathway substitutes for a deficient  $\beta_1$ -AR-Gs-cAMP pathway; decreased  $\beta_1$ -AR-AC coupling promotes the  $\beta_2$ -AR-cPLA2 pathway, whereas efficient  $\beta_1$ -AR-AC coupling suppresses it by the activity of PKA (9). The effect of cardiac  $\beta_2$ -AR activation varies depending on the species, and the developmental or pathophysiological state of the heart (9). The ability of  $\beta$ -ARs to couple with different G-proteins allows for the receptors to activate multiple signaling pathways and initiate diverse cellular responses.

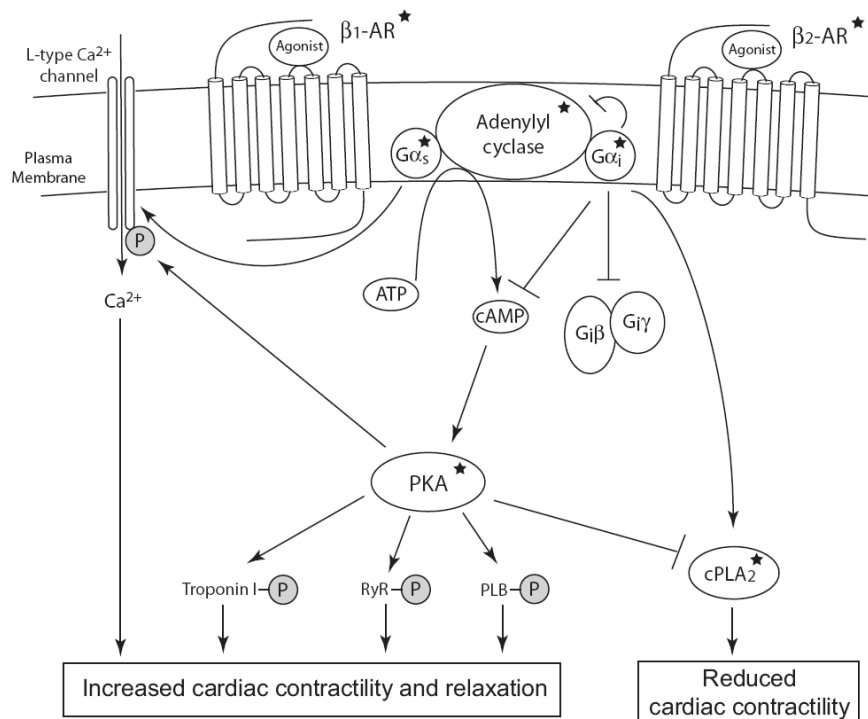
The  $\beta$ -AR signaling system, like all GPCR systems, has a number of post-agonist stimulation regulatory mechanisms that serve to prevent overstimulation (10). These processes, together known as receptor desensitization, act as a negative feedback to the  $\beta$ -AR signal, under conditions of extended agonist

stimulation, by repressing the intensity of  $\beta$ -AR signaling and decreasing the total number of receptors in the cell. The first of these processes is the deactivation of trimeric G-proteins. Soon after the dissociation of the coupled G-protein into active  $G\gamma$  and  $G\beta\gamma$  subunits, conformational changes occur that cause re-formation of the inactive G-protein. The rapid termination of the G-protein signal maintains the dose-dependent nature of  $\beta$ -AR signal propagation and resets the protein for future signaling. Although apparently contradictory,  $\beta$ -AR desensitization (discussed later) much like chronic receptor stimulation, contributes to the pathogenesis of CHF (3).

Continuous catecholamine stimulation overwhelms the short-term active/inactive cycling of the G-protein signal and triggers further negative feedback regulation of GPCR activity. Activation-dependent regulation of receptors, also known as homologous desensitization, proceeds within minutes of agonist stimulation (11). Homologous desensitization begins when the  $G\beta\gamma$  subunit of the activated G-protein binds with the active form of a G-protein-coupled receptor kinase (GRK). GRK2, also known as  $\beta$ -AR kinase 1 ( $\beta$ ARK1), is translocated to the stimulated receptor after binding with the activated  $G\beta\gamma$  subunit, where it phosphorylates the agonist-occupied  $\beta$ -AR (8).  $\beta$ ARK1 is the most prominent cardiac GRK; GRK3 and GRK5, which are also capable of phosphorylating  $\beta$ -ARs, are present in cardiac myocytes in minimal levels. Phosphorylation of the receptor occurs at the C-terminus of the receptor



**Figure 1.** Classical GPCR signaling. Agonist binding to the receptor results in the coupling with G-proteins and exchange of G-protein-bound GDP for GTP. The activated G-protein dissociates into  $G\alpha$  and  $G\beta\gamma$  subunits, both of which independently affect cellular signaling through the activation or inhibition of effectors such as adenylyl cyclase (AC) or phospholipase C-b (PLC-b).  $G\alpha$  subunits are grouped into four subfamilies -  $G\alpha_s$ ,  $G\alpha_i$ ,  $G\alpha_q$  and  $G\alpha_{12}$  - based on their structure and function. The members of stimulatory  $G\alpha_s$  family couple to AC to cause an increase in intracellular cAMP levels, whereas members of  $G\alpha_i$  family inhibit AC and decrease cAMP levels. The members of  $G\alpha_q$  activate PLC-b, whereas members of  $G\alpha_{12}$  family activate Rac and Rho.  $G\beta\gamma$  dimers activate large number of effectors including ion channels, mitogenactivated protein (MAP) kinases and activate or inhibit AC.



**Figure 2.**  $\beta$ -AR-mediated cardiomyocyte contractility. Agonist binding stimulates  $\beta_1$ -AR and results in coupling with and activation of heterotrimeric Gs, which dissociates into  $G_{\alpha_s}$  and  $G_{\beta\gamma}$  subunits. The  $G_{\alpha_s}$  activates both adenylyl cyclase (AC), which increases intracellular cAMP levels and L-type calcium channel, which allows  $Ca^{2+}$  to enter into cardiomyocytes. The cAMP activates PKA, which phosphorylates (P) several substrates including phospholamban (PLB), L-type  $Ca^{2+}$  channels, troponin I and the cardiac ryanodine receptor (RyR) resulting in increased cardiac contractility and relaxation. In addition to Gs,  $\beta_2$ -AR couples to pertussis toxin-sensitive  $G_i$  upon agonist binding. Activated- $G_i$  releases  $G_{\beta\gamma}$  subunit, which inhibits AC and  $G_{\beta\gamma}$  and activates phospholipase A2 (cPLA2) leading to reduced cardiac contractility. The  $\beta_1$ -AR-induced cAMP suppresses the  $\beta_2$ -AR/cPLA2 pathway, via PKA. Asterisks denote activated proteins and  $\text{---|}$  indicates inhibition.

protein and targets it for arrestin-induced functional uncoupling from the G-protein. An alternate internalization pathway involves phosphorylation of the receptor protein through the secondary action of a downstream signaling product of  $\beta$ -AR activation. This process, known as heterologous desensitization, is initiated when the second messenger-regulated kinase PKA phosphorylates the  $\beta$ -AR (12). As opposed to homologous desensitization, heterologous desensitization indiscriminately phosphorylates both stimulated and unstimulated receptors. In both cases, receptor phosphorylation allows for  $\beta$ -arrestin to bind and interfere with future association with G-proteins, functionally uncoupling the receptor (13). Binding to  $\beta$ -arrestin increases the receptor's affinity for adaptor protein (AP)-2 and clathrin (14). Internalization of receptors via clathrin-coated vesicles follows rapidly, within minutes of stimulation (15).

Following internalization, receptors are transported to endosomes. At the endosomes, the receptors can be recycled back to the plasma membrane where they return to a complete functioning state (14). In response to persistent receptor stimulation, the internalized receptor can instead be degraded in the liposome. This process, known as down-regulation, lowers the total number of receptors in the cell to relieve chronic overstimulation. Down-regulation is initiated within

hours to days after consistent receptor internalization.

### $\beta$ -AR SIGNALING IN HEART FAILURE

As the major component of the interface between the sympathetic nervous system and the cardiovascular system, the  $\beta$ -AR signaling pathway emerges as a key actor during the progression of heart failure. Prominent irregularities in  $\beta$ -AR signaling changes include a reduction of the  $\beta_1$ -AR levels of up to 50% (interestingly  $\beta_2$ -AR levels remain constant), a sharp increase (up to 200%) of  $G_{\alpha_1}$  levels, and significantly elevated  $\beta$ ARK1 activity (8). All of these adjustments reflect severely diminished  $\beta$ -AR signaling, likely the result of sustained, elevated catecholamine levels.

Cardiac hypertrophy often directly precedes the onset of CHF. Excessive stress activates a complex network of interacting pathways that initiate myocardial hypertrophy to preserve cardiac function. Conventional wisdom held that cardiac hypertrophy is an adaptive process that improves ventricular function in the face of a growing workload. However, as the cardiac efficiency of the hypertrophied heart further diminishes, the sympathetic nervous system is increasingly activated in an attempt to maintain cardiac output and systemic blood pressure (16). Furthermore, many studies indicate that sustained cardiac hypertrophy is closely associated with reduced contractility and an increased risk of heart

failure (17). Also, significant desensitization of  $\beta$ -ARs and other  $\beta$ -AR signaling irregularities are shared characteristics in the development of cardiac hypertrophy and the progression of heart failure. Transgenic mice that lack a hypertrophic response mechanism suffer less cardiac dysfunction than normal mice when facing long-term mechanical stress (18). In light of this evidence, a new paradigm has emerged in which it is argued that the signaling pathways stimulated during the hypertrophic process may actually play a greater role in the pathogenesis of heart failure than the original stress on the heart.

According to this model, heightened stimulation by the sympathetic nervous system elicits a hypertrophic response in the heart that is initially beneficial but becomes maladaptive when sustained. The hypertrophic response causes the activation of a number of signaling pathways, at a time when consistently elevated catecholamine levels are already overstimulating the  $\beta$ -AR signaling pathways. Sustained activation of the  $\beta$ -AR system combined with biochemical changes produced by hypertrophic processes strongly activate desensitization and down-regulation pathways, which ultimately lead to diminished  $\beta$ -AR function and loss of contractility.

#### LESSONS FROM TRANSGENIC MOUSE MODELS

Over the last decade and a half, cardiovascular research has been revolutionized through the use of genetically engineered mouse models. Mouse lines have been developed to model every aspect of cardiac pathology. Furthermore, the use of murine  $\alpha$ -myosin heavy chain ( $\alpha$ MyHC) gene promoter in ventricular myocytes has enabled cardiac-specific gene expression. The  $\alpha$ MyHC promoter, which is inactive until around birth, avoids developmental complications arising from the specific transgene overexpression (15). The ability to genetically manipulate molecular components of the  $\beta$ -AR signaling system in murine heart failure models provides a powerful experimental system for uncovering potential roles of various elements of this system in the pathophysiology of cardiac failure.

#### $\beta_1$ -AR

The  $\beta_1$ -AR has been studied in both gain and loss of expression studies.  $\beta_1$ -AR knockout mice were largely embryonically lethal, while the surviving mice showed no response to catecholamine stimulation despite the presence of  $\beta_2$ -ARs (19). Cardiomyocyte-targeted overexpression of the  $\beta_1$ -AR using the  $\alpha$ MyHC promoter generated mice with 5 to 15-fold overexpression of  $\beta_1$ -ARs. The  $\beta_1$ -AR overexpressing mice developed dilated cardiomyopathy and heart failure at young age, which is similar to the pathology

caused by chronic catecholamine overstimulation (20). This data reinforced the view that increased  $\beta$ -AR signaling, as happens in chronic receptor stimulation, is ultimately detrimental to cardiac function.

#### $\beta_2$ -AR

Experiments using transgenic mouse models have made it clear that  $\beta_1$ -AR and  $\beta_2$ -AR are not identical signaling molecules. In contrast to  $\beta_1$ -AR knockouts, the  $\beta_2$ -AR knockout mice do not suffer from developmental defects and show no significant differences in cardiovascular phenotype when compared with wild-type mice (21). The  $\beta_2$ -AR knockout mice also differ from  $\beta_1$ -AR knockouts in that they show typical response to catecholamine stimulation. This indicates that the  $\beta_1$ -AR is the signaling conduit for the catecholamine-induced changes in cardiac contractility.

Mice with cardiac-specific overexpression of  $\beta_2$ -ARs displayed enhanced basal contractility and cardiac function with minimal pathology (22). Adenoviral-mediated gene transfer of  $\beta_2$ -AR to myocytes from a rabbit heart failure model rescued  $\beta$ -AR signaling (23). Crossing transgenic mice overexpressing moderate amounts (30-fold above wild-type levels) of cardiac  $\beta_2$ -AR with transgenic mice overexpressing  $G\alpha_q$  improved cardiac function and hypertrophy in the  $G\alpha_q$  overexpression heart failure model (24). These results are encouraging for the prospective use of  $\beta_2$ -AR overexpression as a therapeutic approach. However, conflicting data clouded the issue as  $\beta_2$ -AR overexpression has failed to rescue other heart failure models (25) and has proven deleterious in the face of pressure overload (26). More information about appropriate levels of  $\beta_2$ -AR expression levels is needed to clarify the potential therapeutic value of  $\beta_2$ -AR gene transfer.

#### $\beta$ ARK1

The prevalence of  $\beta$ ARK1 activity in the progression of maladaptive hypertrophy and cardiovascular disease has made it an object of extensive research. The role of  $\beta$ ARK1 was investigated by studying the contrast between hearts of transgenic mice overexpressing  $\beta$ ARK1 and those expressing a peptide inhibitor of  $\beta$ ARK ( $\beta$ ARKct).  $\beta$ ARKct, a peptide expressing the terminal 194 amino acids of  $\beta$ ARK1, contains the  $G\beta\gamma$ -binding domain and competes with endogenous  $\beta$ ARK1 for  $G\beta\gamma$  subunits necessary for  $\beta$ ARK1 activity (27). Overexpression of  $\beta$ ARK1 in transgenic mice showed a diminished  $\beta$ -AR response to catecholamine stimulation. This mirrors the augmented  $\beta$ ARK1 activity observed in human heart failure. In stark contrast, the  $\beta$ ARKct mice had enhanced cardiac function under normal conditions and an augmented

response to catecholamine stimulation (28). While homozygous  $\beta$ ARK1<sup>-/-</sup> mice were embryonic lethal, heterozygous  $\beta$ ARK1<sup>+/-</sup> mice had a cardiac phenotype of enhanced contractility similar to the  $\beta$ ARKct transgenic animals (29, 30).

To determine the significance of increased  $\beta$ ARK activity in the progression of heart failure, a number of studies were conducted involving the overexpression of  $\beta$ ARKct in mice from various existing heart failure models. Remarkably, in each case, it was shown that cardiac overexpression of  $\beta$ ARKct leads to prevention of progressive deterioration in cardiac function, prevention of hypertrophy, improved exercise tolerance, and correction of  $\beta$ -AR dysfunction (31). Furthermore, adenoviral delivery of  $\beta$ ARKct to larger animal models of heart failure has shown therapeutic effects (32). Taken together, these studies indicate that inhibition of  $\beta$ ARK1 activity can preserve  $\beta$ -AR signaling and lead to an improved response to catecholamine stimulation. This suggests that inhibition of  $\beta$ ARK1 activity via introduction of  $\beta$ ARKct or other small molecule inhibitors is a viable therapeutic approach for disease states characterized by increased  $\beta$ ARK1 activity.

It is important to note that the effects of  $\beta$ ARK1 inhibition may not be completely relegated to the  $\beta$ -AR system as  $\beta$ ARK1 is involved in a number of non- $\beta$ -AR signaling pathways (33). Furthermore, the demonstrated effectiveness of  $\beta$ ARKct expression may not be entirely attributable to  $\beta$ ARK1 inhibition.  $\beta$ ARKct functions by binding to and interfering with activated G $\beta\gamma$  subunits, so it is very possible that inhibition of alternative G $\beta\gamma$  pathways contributes to part of the  $\beta$ ARKct effect. In fact, recent evidence has shown that another G $\beta\gamma$  inhibitor, a N-terminally truncated phosducin, which acts independently of  $\beta_1$ -AR activity, replicates  $\beta$ ARKct's protective effects (34).

### PI3K

Recently, phosphoinositide 3-kinase (PI3K) has begun to garner interest because of its role at the intersection of the hypertrophic and  $\beta$ -AR signaling processes. PI3K, which belongs to a conserved family of lipid kinases that are involved in the regulation of a variety of cellular functions including cell growth, survival, signal transduction and apoptosis, is activated in cardiac myocytes during the hypertrophic response (35). PI3K activity is also closely associated with attenuation of  $\beta$ -AR function resulting in decreased contractility (36, 37). Recent studies demonstrated that  $\beta$ ARK1 interacts with the phosphoinositide kinase (PIK domain) of PI3K to form a cytosolic complex and facilitates agonist-mediated PI3K translocation to the plasma membrane (38). PI3K, via its lipid kinase activity, catalyzes the production of D-3 phosphoinositides, which recruit adaptor proteins essential to receptor internalization.

Overexpression of the PIK domain, which competitively displaces endogenous PI3K and prevents  $\beta$ ARK-PI3K translocation to the membrane, significantly decreased agonist-stimulated  $\beta$ -AR internalization. Recently, we demonstrated that internalization of the receptor also requires the protein kinase activity of PI3K involving tropomyosin phosphorylation (39). Furthermore, overexpression of PI3K $\gamma$ , a catalytically inactive mutant, inhibited agonist-induced  $\beta$ -AR internalization and rescued  $\beta$ -AR function in calsequestrin overexpressing (CSQ) mice, a common heart failure model (40). Restoration of  $\beta$ -AR signaling via PI3K $\gamma$  overexpression resulted in significant improvement in cardiac function and survival, which indicates that inhibition of membrane-targeted PI3K represents a novel therapeutic approach to ameliorate cardiac dysfunction.

### CONCLUSIONS AND NEW DIRECTIONS

In recent years, great progress has been made towards understanding the  $\beta$ -AR system and its role in heart disease. Increasingly, researchers are viewing the myocardial hypertrophic response and concurrent  $\beta$ -AR desensitization as maladaptive responses. However, this evolving notion is counterintuitive to the successful use of  $\beta$ -blockers which favor a reduction of  $\beta$ -AR stimulation to preserve cardiac function. Understanding the differences between  $\beta_1$ -AR and  $\beta_2$ -AR signaling may explain the apparent effectiveness of these  $\beta$ -antagonists in restoring  $\beta$ -AR levels and cardiac function in the failing heart. It is possible that  $\beta$ -blockers function by inhibiting sustained  $\beta_1$ -AR activity and the associated hypertrophic, pro-apoptotic, pro-necrotic effects (24). Another possibility is that  $\beta$ -blockers act to re-sensitize the  $\beta$ -AR system, reversing the abnormalities in  $\beta$ -AR signaling that result from prolonged catecholamine stimulation.

Studies using genetically altered mice have identified G $\beta\gamma$  and PI3K as particularly promising targets for therapeutic intervention. Further investigation of the secondary signaling pathways these molecules are involved in, is needed to fully understand how best to target these molecules for the treatment of CHF. Potential approaches include the use of gene therapy (with  $\beta$ ARKct or PI3K $\gamma$ ) or the development of pharmaceutical inhibitors for heart-specific  $\beta$ ARK1 or PI3K activity.

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