Distinct β-adrenergic receptor subtype signaling in the heart and their pathophysiological relevance

ZHENG Ming1, HAN Qi-De1, XIAO Rui-Ping1,2,*
1The Institute of Molecular Medicine, Peking University, China; 2Laboratory of Cardiovascular Science, NIA, NIH, Baltimore, MD 21224

Abstract: In the heart, stimulation of β-adrenergic receptors (βAR) serves as the most powerful means to increase cardiac contractility and relaxation in response to stress or a “fight-or-flight” situation. However, sustained β-adrenergic stimulation promotes pathological cardiac remodeling such as myocyte hypertrophy, apoptosis and necrosis, thus contributing to the pathogenesis of chronic heart failure. Over the past decade, compelling evidence has demonstrated that coexisting cardiac βAR subtypes, mainly β1AR and β2AR, activate markedly different signaling cascades. As a result, acute β1AR stimulation activates the Gs-adenylyl cyclase-cAMP-PKA signaling that can broadcast throughout the cell, whereas β2AR-evoked cAMP signaling is spatially and functionally compartmentalized, due to concurrent Gi activation. Chronic stimulation of β1AR and β2AR elicits opposing effects on the fate of cardiomyocytes: β1AR induces hypertrophy and apoptosis; but β2AR promotes cell survival. The cardiac protective effect of β2AR is mediated by a signaling pathway sequentially involving Gs, Gβγ, PI3K and Akt. Unexpectedly, β1AR-induced myocyte hypertrophy and apoptosis are independent of the classic cAMP/PKA pathway, but require activation of Ca2+/calmodulin-dependent kinase II (CaMK II). The outcomes of cardiac-specific transgenic overexpression of either βAR subtype in mice have reinforced the fundamentally different functional roles of these βAR subtypes in governing cardiac remodeling and performance. These new insights regarding βAR subtype stimulation not only provide clues as to cellular and molecular mechanisms underlying the beneficial effects of βAR blockers in patients with chronic heart failure, but also delineate rationale for combining selective β1AR blockade with moderate β2AR activation as a potential novel therapy for the treatment of chronic heart failure.

Key words: β-adrenergic receptors; heart; signal transduction
Overview

As a prototypical member of G protein-coupled receptor (GPCR) superfamily, β-adrenergic receptor (βAR) stimulation activates the classic Gs-adenylyl cyclase (AC)-cAMP-PKA signaling pathway, which, in turn, mediates phosphorylation of target proteins involved in metabolic regulation, growth control, muscle contraction, and cell survival or death. In the heart, βAR stimulation provides the most powerful mechanism to augment cardiac function in response to stress or exercise. A hallmark of βAR-mediated regulation of cardiac function is to increase cardiac contractility (a positive inotropic effect), accelerate cardiac relaxation (a positive lusitropic effect), and increase heart rate (a positive chronotropic effect). Prolonged exposure of βAR to an agonist, however, leads to a decrease in receptor responsiveness, i.e., desensitization, as is the case for most GPCRs. When this occurs in the context of diminished responsiveness to a diverse array of other agonists for Gs-coupled GPCRs (heterologous desensitization), it generally results from a negative feedback regulation by the second messenger-activated protein kinases, e.g., PKA-mediated phosphorylation. In contrast, chronic exposure to an agonist may also result in a diminished response to only that particular agonist, i.e., homologous or agonist-specific desensitization, which is generally mediated by G protein-coupled receptor kinase (GRK) family (GRK1-6), particularly GRK2 also known as βAR kinase 1 (βARK1).

Chronic heart failure (CHF) due to diverse etiologies is characterized by elevated circulating catecholamine levels and a concurrent reduction in βAR density and desensitization, leading to markedly diminished β-adrenergic contractile response. The prevailing interpretation from the early 1960’s to early 1980’s was that the reduced βAR contractile response was a factor involved in the development of CHF, i.e., a cause of heart failure[3]. This interpretation has waned, however, due to the initially counter-intuitive finding that many βAR agonists or phosphodiesterase inhibitors increase mortality despite their short-term positive inotropic effects; more surprising was the observation that some βAR blocking agents successfully improve cardiac function and decrease the mortality of CHF patients[2]. Nevertheless, some βAR blockers cannot prolong the survival of CHF patients, or even worsen cardiac function and increase mortality of those patients. These paradoxical observations have greatly motivated both the basic and clinical research communities to delineate fundamental mechanisms underlying βAR-mediated cardiovascular regulation.

As a new twist, it has been discovered that coexisting cardiac βAR subtypes, mainly β1AR and β2AR, activate different signaling pathways and fulfill strikingly distinct, sometimes even opposing, physiological and pathological roles in the heart. This review will focus on such βAR subtype-specific signaling pathways and their relevance to the heterogeneous clinical outcomes of different βAR blocking agents. Based upon the materials reviewed, rationale with respect to novel βAR-targeting therapeutic regimes is also proposed.

Distinct βAR-subtype modulation of cardiac Ca2+ handling

The traditional view of βAR signal transduction is that stimulation of the receptor activates the Gs-AC-cAMP cascade, leading to PKA-dependent phosphorylation of a set of regulatory proteins. In the heart, PKA phosphorylates a multitude of Ca2+ handling proteins, including sarcolemmal L-type Ca2+ channels, sarcoplasmic reticulum (SR) membrane proteins, phospholamban (PLB, an important SR Ca2+ pump regulator) and ryanodine receptors (RyRs, SR Ca2+ release channels), and some myofilament components (troponin I and C protein). As recently reviewed[3], β1AR stimulation affects the phosphorylation of sarcolemmal L-type Ca2+ channels and aforementioned regulatory proteins remote from the sarcolemma, thus increasing the amplitudes and kinetics of the intracellular Ca2+ transient and contraction[4-10]. In contrast, a large body of evidence has demonstrated that β2AR stimulation specifically modulates sarcolemmal L-type Ca2+ channels without affecting the aforementioned intracellular regulatory proteins in cardiomyocytes from many mammalian species, including rat and dog[4-10], although in the human heart, β2AR stimulation increases cellular cAMP and...
PKA-dependent phosphorylation of intracellular regulatory proteins (PLB, TnI and C protein), similar to β₁AR stimulation\[11,12\]. Single-channel recordings have revealed that the stimulatory effect of βAR on L-type Ca²⁺ channels is highly restricted to subsurface membrane microdomains in the vicinity of L-type Ca²⁺ channels, whereas β₂AR-mediated cAMP/PKA signaling can diffuse throughout the cell\[13,14\]. Thus, β₂AR stimulation induces an increase in the intracellular Ca²⁺ transient and a positive inotropic effect without appreciable effects on the Ca²⁺ transient decay time or contractile relaxation in adult rat cardiac myocytes\[4\-10\].

Another interesting difference between β₁AR and β₂AR in regulating intracellular Ca²⁺ cycling is that stimulation of β₁AR, but not β₂AR, increases the likelihood for the occurrence of spontaneous intracellular Ca²⁺ oscillations (Ca²⁺ waves). This was first observed in adult rat ventricular myocytes\[4\], and was confirmed by studies in the German shepherd sudden death model\[15,16\]. This finding may have important clinical implications, since spontaneous Ca²⁺ oscillations can activate depolarizing membrane currents, leading to Ca²⁺-dependent cardiac arrhythmia and sudden death. Thus, β₂AR stimulation may be able to provide inotropic support without the risk of causing arrhythmias, whereas β₁AR stimulation is known to be potentially arrhythmogenic.

**β₂AR/G_i signaling compartmentalizes β₁AR/G_s-mediated cAMP signaling**

Studies over the past decade have unraveled a fundamental difference between βAR subtypes in their G protein coupling. In the heart of mammalian species, including human, stimulation of native cardiac β₂AR evokes both G₁ and pertussis toxin (PTX)-sensitive G₁ signaling pathways, whereas β₁AR activates exclusively the G₁ signaling cascade\[10,13,17-21\]. Several lines of evidence indicate that the β₂AR-G₁ signaling is essential to the spatial and functional confinement of β₂AR-G₁-mediated cAMP/PKA signaling. First, disrupting G₁ function with PTX-mediated ribosylation enables β₂AR to stimulate remote L-type Ca²⁺ channels\[13\] and potentiates β₂AR-mediated positive inotropic effect in adult rodent ventricular myocytes\[17,18\]. Second, in PTX-treated cells, β₂AR stimulation leads to a robust PKA-dependent phosphorylation of PLB and myofilament proteins, resulting in a de novo relaxant effect that is not usually elicited by cardiac β₂AR signaling in these species\[9,10\]. Third, inhibition of phosphatidylinositol 3-kinase (PI3K), similar to PTX treatment, enables β₂AR-activated cAMP/PKA signaling to reach intracellular substrates, as manifested by a marked increase in phosphorylation of PLB, a de novo relaxant effect and an overtly enhanced positive inotropic effect\[21\]. Blocking G₁ or G₁₉₉₉₉₉ signaling completely prevents the potentiating effects induced by PI3K inhibition, suggesting that the pathway restricting β₂AR-cAMP signaling sequentially involves G₁, G₁₉₉₉₉₉₉, and PI3K\[21\]. Thus, in addition to its pivotal roles in cell growth and cell survival (see below), PI3K constitutes a key downstream event of acute β₂AR-G₁ signaling that confines and negates the concurrent β₁AR/G₁-mediated cAMP signaling (Fig. 1).

Another candidate mechanism underlying the compartmentalization of cAMP/PKA signaling in response to β₂AR stimulation is the structural restriction of PKA diffusion by specific A-kinase anchoring proteins (AKAPs)\[22\]. In adult rat cardiomyocytes, the positive inotropic and lusitropic effects of the nonselective βAR agonist, isoproterenol, are significantly augmented by disrupting AKAP-mediated PKA anchoring using adenovirus-mediated gene transfer of Ht31, a peptide that binds the PKA regulatory subunit type I with high affinity\[23\]. It has been also shown that direct interaction of β₂AR with some AKAPs (such as gravin and AKAP79/150) is essential for agonist-induced β₂AR trafficking and desensitization\[24-27\]. Whether AKAPs participate in the spatial and functional compartmentalization of β₂AR-stimulated cAMP signaling, and if so, whether AKAPs are causally related to the G₁₉₉₉₉₉₉₉-G₁₉₉₉₉₉₉₉-PI3K pathway to confine cAMP/PKA signal is presently unknown.

**Opposing roles of β₁AR and β₂AR stimulation in regulating the fate of cardiomyocytes**

Apoptosis has been implicated in cardiac ischemic and reperfusion injury and is involved in the transition from cardiac hypertrophy to decompensated heart failure in humans and animal models\[28-32\]. A low rate of myocyte apoptosis is sufficient to induce CHF. In a transgenic mouse model expressing low levels of activated caspase-8, apoptotic rates of only 23/10⁴ are adequate to induce a lethal dilated cardiomyopathy over several months\[33\]. These studies clearly establish that apoptosis plays an important causal role in the evolution of CHF, and suggest that inhibition of myocyte death may provide a target for new therapies.

Pharmacological evidence suggests that β₁AR and β₂AR may exert different effects on the fate of cardiac myocytes, since β₂AR blockade can exaggerate norepi-
nephrine-induced apoptosis in cultured adult rat cardiac myocytes[34, 35]. To avoid complicated interactions between βAR subtypes, genetically “pure” β₁AR or β₂AR experimental settings have been created using gene-targeted mice lacking either β₁AR or β₂AR, or mice lacking both subtypes (β₁β₂ double-knockout) in conjunction with adenoviral gene transfer of either β₁AR or β₂AR[36, 37]. These studies have demonstrated that stimulation of β₁AR leads to cardiac apoptosis, whereas stimulation of β₂AR activates concurrent proapoptotic and antiapoptotic signals, with the net effect being cell survival[38]. In fact, the β₂AR survival signaling not only counteracts the concurrent Gₛ-AC-cAMP-PKA signaling, but also protects cardiac myocytes against a wide range of apoptotic insults, including hypoxia or reactive oxygen species (ROS) in rat neonatal cardiomyocytes[39].

In vivo studies in several gene-targeted mouse models in which various components of the signal transduction pathways of β₁AR or β₂AR have been manipulated have reinforced the fundamental differences between these βAR subtypes in their signaling pathways and functional roles in regulating the fate of cardiac myocytes. In transgenic mouse models, chronic stimulation of each βAR subtype elicits distinctly different phenotypes and prognosis in terms of cardiac remodeling and heart failure. Overexpression of cardiac β₁AR by a factor of 5-40 leads to cardiac hypertrophy, myocyte apoptosis, fibrosis within a few weeks after birth, heart failure, and even premature death within several months[40, 41]. Likewise, cardiac-specific overexpression of Gₛ by 5-fold also causes cardiomyopathy, increases myocyte apoptosis, and results in premature death in the transgenic mice at the age of 16-months[42, 43]. In sharp contrast, overexpression of the cardiac β₂AR by a factor of 200 does not produce hypertrophy or heart failure, at least up to the age of 1 year[44]. Subsequent studies of β₂AR overexpression suggest that the duration and degree of receptor overexpression are important determinants for the prognosis of β₂AR transgenic mice[45, 46]. A relatively low level of β₂AR overexpression (30-fold) is able to improve cardiac function and normalize cardiac hypertrophy in Gₛₜₖₜ overexpression mice[45], whereas high levels of β₂AR expression (such as 350~1000 times over the normal level) result in pathological phenotypes and premature death of these transgenic mice at 25 weeks of age[45, 46]. Most recently, these observations have been corroborated by in vivo studies in mice lacking either β₁AR (β₁KO)[47]...
or β2AR (β2KO)48. In response to isoproterenol infusion for 14 days, β2KO mice experience greater mortality, increased myocyte apoptosis, and more severe clinical signs of heart failure compared to WT animals, suggesting that selective β2AR activation in vivo exhibits a cardiac protective effect, while β2AR stimulation is cardiac toxic49.

**Sustained β2AR stimulation protects cardiomyocytes via the Gs signaling pathway**

The opposing effects of β1AR and β2AR on cardiac cell survival and cell death largely stem from their distinct G protein coupling, particularly the β2AR-Gi coupling. This perception is based on the following lines of evidence. First, β2AR stimulation is able to induce myocyte apoptosis only under conditions in which Gi is inactivated by PTX-mediated ribosylation38. Second, in cultured adult rat myocytes, β2AR blockade enhances the apoptotic effect of norepinephrine in a PTX-sensitive manner34. Third, β2AR, but not β1AR, activates a Gs-Gbg-P13K-Akt cell survival signaling pathway; and more importantly, inhibition of this pathway converts β2AR signaling from survival to apoptotic136. Furthermore, the effect of β2AR stimulation to protect cardiomyocytes from hypoxia and ROS is also mediated by the Gs-P13K-Akt signaling pathway39. Thus, the β2AR-Gs-Gbg-P13K-Akt signaling pathway not only counteracts Gi-mediated apoptosis, but also protects myocytes from various apoptotic insults (Fig.1). During sustained βAR stimulation, the dual G protein coupling of the βAR is shifted towards Gi by PKA-mediated phosphorylation of the receptor50 and may serve as an adaptive mechanism to protect myocardium against catecholamine-induced cardiotoxic effects.

In addition to the PI3K signaling pathway, studies in cultured adult rat cardiomyocytes have suggested that both βAR subtype types activate p38 mitogen-activated protein kinase (MAPK) in a Gi-dependent manner, and that activated p38 MAPK results in an antiapoptotic effect51. However, in β2β2 double-knockout mouse cardiomyocytes expressing β1AR or β2AR using adenoviral gene transfer, stimulation of either βAR subtype increases p38 MAPK activation through a PKA-dependent signaling pathway rather than by Gi signaling138,52; and inhibition of p38 MAPK cannot block the β2AR antiapoptotic effect. These studies suggest that p38 MAPK activation is not essential for βAR-mediated antiapoptosis138.

Other members of MAPK family, particularly the extracellular signal-regulated protein kinases (ERK1 and ERK2), can also protect cells from apoptosis. Interestingly, in cultured neonatal rat myocytes, although β2AR activates ERK1/2 in a PTX-sensitive fashion, inhibition of ERK signaling pathway cannot prevent a β2AR-mediated antiapoptotic effect59. In contrast, recent studies have reported that in cultured adult rat cardiomyocytes, a βAR antiapoptotic effect is mediated by Gs-dependent activation of ERK, and that this requires a simultaneous inhibition of PKCy53, highlighting the complexity of β2AR cell survival signaling in the heart. Regardless of the exact mechanism, all studies to date have consistently demonstrated that β2AR signaling, unlike that of β1AR signaling, has no cardiac toxic effects in terms of myocyte apoptosis; rather, β2AR stimulation protects heart cells from death.

**Does the classic Gi-AC-cAMP-PKA pathway play an essential role in β2AR-induced cardiomyocyte apoptosis?**

In marked contrast to β2AR stimulation, it has been well established that enhanced β2AR stimulation induces robust cardiac cell death in vivo41 and in cultured cardiac myocytes34,35,38,54. Several early reports point to an essential role of the cAMP/PKA pathway in β2AR-mediated apoptotic heart cell death, since the ability of β2AR to cause myocyte apoptosis could be abolished by a high concentration (20 mmol/L) of widely used PKA inhibitor, H-8934,35. However, the use of this inhibitor to implicate PKA in β2AR signaling can be misleading, because this compound also acts as a potent βAR blocker55. Although the cAMP/PKA pathway mediates β2AR-induced positive inotropic effect, a close inspection of studies to date has revealed no convincing evidence to validate that this is also the case for β2AR-evoked apoptosis in myocardium. For instance, transgenic overexpression of type V or VI adenylyl cyclase in mouse hearts does not cause cell death, although it markedly augments basal PKA activity and cardiac contractility55,57. More ironically, in cultured cardiac myocytes, or within the myocardium in vivo, selective β2AR stimulation elicits a profound cardiac protective effect, in spite of overtly enhanced cAMP formation34,38,39,45,49.

More recent studies have employed the aforementioned genetically well-defined βAR systems in conjunction with more specific methods to inhibit the PKA pathway, including a specific peptide inhibitor, PKI, or an inactive cAMP analogue, Rp-cAMP, to delineate signaling mechanisms mediating β2AR apoptotic effect. These studies have led to the notion that the apoptotic effect of sustained β2AR stimulation is PKA-independent50. This perspective not
only challenges the conventional wisdom that cAMP/PKA is responsible for βAR-induced cardiac detrimental effects, but also may have profound impact on therapeutic strategies to prevent or attenuate βAR-mediated cardiac toxic effects.

**Role of CaMK II in βAR-mediated cardiomyocyte apoptosis**

Intriguingly, sustained βAR stimulation is able to increase intracellular Ca²⁺ and Ca²⁺/calmodulin-dependent kinase II (CaMKII) activity in a PKA-independent manner⁵⁴. Blocking the L-type Ca²⁺ channel, buffering intracellular Ca²⁺, depleting the SR Ca²⁺ store, or inhibiting CaMKII activity fully protects cardiac myocytes against βAR-induced apoptosis⁵⁴. Furthermore, overexpression of a cardiac cytosolic CaMKII isoform, CaMKII-dC, markedly exaggerates βAR apoptotic effect. These findings indicate that CaMKII constitutes a novel PKA-independent linkage of βAR stimulation to cardiomyocyte apoptosis⁵⁴. The essential role of CaMK II in the pathogenesis of CHF is further supported by the fact that in transgenic mice overexpressing CaMKII-dC causes dilated cardiomyopathy, heart failure and premature death of the animals at the age of several months old⁵⁸,⁵⁹. The exact molecular mechanisms underlying PKA-independent activation of CaMKII during sustained βAR stimulation and the linkage between CaMKII and apoptotic cell death remain to be explored.

**βAR, but not β2AR, promotes cardiac hypertrophy via PKA-independent mechanisms**

Cardiac myocytes undergo physiological or pathological growth (hypertrophy) in response to pressure overload, injury, or neurohormonal stimulation. β1AR and β2AR also manifest opposing effects on cardiac cell growth. Stimulation of β1AR, but not β2AR, causes hypertrophy in cultured neonatal and adult rat cardiac myocytes⁶⁰,⁶¹. Moreover, in cultured adult rat ventricular myocytes, βAR stimulation by isoproterenol induces hypertrophic growth only in the presence of β2AR blockade, suggesting that β1AR stimulation may inhibit β1AR-mediated myocyte hypertrophy⁶⁰. This observation is in agreement with the fact that phenotypes of transgenic mice overexpressing cardiac β1AR distinctly differ from those overexpressing β2AR with respect to cardiac remodeling and prognosis. Interestingly, the β1AR hypertrophic effect is neither dependent on activation of cAMP/PKA⁶⁰,⁶² nor ERK activation⁶², but requires an Akt-glycogen synthase kinase 3b (GSK-3b)-GATA4-signaling pathway⁶² and activation of tyrosine kinase⁶⁰.

It is noteworthy that CaMK II is also a critical player in βAR-induced myocyte hypertrophy⁶². In support of an essential role of CaMK II in the development of cardiac hypertrophy, it has been shown that the expression of the hypertrophic marker, atrial natriuretic factor (ANF), is closely correlated with activation of CaMK II in cultured cardiomyocytes and in vivo⁶³,⁶⁴, and that pressure overload selectively upregulates CaMK II in vivo in mice⁶⁵. Hence, the effects of βAR stimulation on cardiac remodeling might be largely mediated by the Ca²⁺/CaMKII signaling pathway independently of the long-suspected cAMP/PKA cascade.

**Differences between βAR and β2AR in their spontaneous activation**

GPCRs, including βARs, exist in equilibrium between two distinct functional and conformational states: an inactive conformation (R) and an active conformation that is capable of activating G proteins (R*). In the absence of ligand, the receptor can undergo a spontaneous transition to the active state; the equilibrium between R and R* sets the level of this basal receptor activation. Thus, an overexpression of a given receptor would be expected to proportionally increase the number of R* state receptors. Agonists shift the receptor population to the R* state, whereas neutral antagonists cause no change in the equilibrium. Some antagonists referred to inverse agonists shift the R*-R equilibrium to the inactive R state.

Myocardial β2ARs exhibit robust spontaneous activation both in vivo and in vitro. Specifically, in transgenic mice, cardiac-specific overexpression of the human β2AR overtly increases basal cardiac AC activity, cAMP accumulation, cardiac contractility, and heart rate in the absence of agonist stimulation⁶⁶-⁶⁹. Similar results have been reproduced with adenoviral gene transfer of β2AR in cultured adult mouse cardiac myocytes⁷⁰,⁷¹. Because the efficacy of pharmacological stimulation of βAR may be limited by receptor desensitization and proarrhythmic effects, moderate overexpression of β2AR in the heart might provide a potential gene therapy to treat chronic heart failure (CHF), as evidenced by the beneficial effects induced by crossing transgenic mice with cardiac-specific overexpression of β2AR by 30-fold with mice overexpressing Gβγ⁴⁹. Since extremely high levels of β2AR overexpression (e.g. 350–1000 fold) fail to rescue the genetic mouse heart failure model and can be detrimental even at early time points⁴⁵,⁴⁶, caution must be exer-
cised when designing therapies to enhance \( \beta \)AR signaling, so that the beneficial level of spontaneous receptor activation is not exceeded.

Although the two-state ternary complex model for the adrenergic receptor is sufficient to explain many aspects of \( \beta \)AR activation, there are several important differences between spontaneously activated \( \beta_2 \) ARs and agonist-stimulated \( \beta_2 \) ARs in terms of their molecular conformations and effector selectivity. For instance, in myocytes from \( \beta_2 \) AR transgenic mice, \( \beta_2 \) AR agonists produce a marked increase in \( I_{\text{cua}} \) whereas ligand-independent constitutive \( \beta_2 \) AR activation increases cardiac contractility without affecting \( I_{\text{cua}} \). Recent studies have also demonstrated that a mutation of Asn293Asp of the human \( \beta_2 \) AR abolishes agonist-induced, but not spontaneous activity of the receptor in COS-7 cells and Chinese hamster ovary cells. Hence, spontaneously activated \( \beta_2 \) AR and agonist-activated \( \beta_2 \) AR may represent functionally distinct conformational states of the receptor. This is in agreement with recent reports that \( \beta_2 \) ARs exhibit multiple active states.

Interestingly, the predominant \( \beta \)AR subtype expressed in myocardium, \( \beta_2 \) AR, exhibits either considerably lower or no spontaneous activity depending on the endpoints examined in different studies. In the \( \beta_1 \) AR and \( \beta_2 \) AR null background, overexpression of \( \beta_1 \) AR to a similar, or even a greater level than that of \( \beta_2 \) AR has virtually no effect on myocyte cAMP accumulation, basal contraction amplitude, or contractile kinetics. These observations are consistent with the results from transgenic mice overexpressing \( \beta_2 \) AR (5–15 fold over wild type). Recent studies have also demonstrated that \( \beta_2 \) AR spontaneous activity is overtly greater (~5 times) than that of \( \beta_1 \) AR under the same experimental conditions in either myocardium or COS-7 cells. Thus, the \( \beta_1 \) AR, unlike \( \beta_2 \) AR, exerts rather weak or no spontaneous activity.

Regarding the molecular structural basis underlying the difference between these \( \beta \) AR subtypes in their propensity to undergo spontaneous activation, it has been recently shown that both the third intracellular loop and the C-terminus of \( \beta_2 \) AR are involved in the receptor spontaneous activation, and that either domain appears to be sufficient to confer the receptor spontaneous activity. Furthermore, the lack of \( \beta_1 \) AR spontaneous activation is not due to the C-terminal PDZ motif of the receptor, although the PDZ motif prevents the receptor to undergo agonist-induced trafficking and G\(_1\) coupling in cardiomyocytes.

The evolution of wisdom on \( \beta \)AR subtype signaling in chronic heart failure: friend or foe?

From early 1960’s to early 1980’s, it was believed that the attenuation or loss of \( \beta \)AR contractile support was a cause of CHF. This paradigm is supported by the severely diminished contractile response to \( \beta \)AR stimulation, by markedly reduced \( \beta \) AR density (particularly \( \beta_2 \) AR), and by upregulation of \( \beta_1 \) AR desensitization machineries such as \( \beta_2 \) ARK1 and G\(_1\) proteins in CHF with various causes in human and animal models. However, the recent success of \( \beta \)AR blocking agents in the treatment of CHF leads to a totally opposite perception, i.e. enhanced \( \beta \)AR stimulation is cardiac detrimental, such that these desensitization mechanisms may represent cardiac protective adaptions. This current perception is, indeed, substantiated by an inverse relationship between the plasma concentration of catecholamines, in particular, norepinephrine (with higher affinity for \( \beta_2 \) AR than \( \beta_1 \) AR) and the survival in patients with CHF, by the poor outcome of nonspecific sympathomimetic agents in clinical heart failure studies, and by cardiomyopathy and heart failure in animal models induced by catecholamine treatment or transgenic overexpression of \( \beta_2 \) AR. Based on these observations, it is now widely accepted by many heart failure researchers or physicians that inhibition of any type of \( \beta \) AR signaling would be expected to produce beneficial effect in patients with chronic heart failure.

In light of the aforementioned logic, an increase in \( \beta_2 \) ARK1 expression would be expected to improve the function of the failing heart, whereas a reduction in \( \beta_2 \) ARK1 would worsen the performance of the failing heart. But this is not the case! Surprisingly, transgenic mice overexpressing \( \beta_2 \) ARK1 manifest many pathological alterations observed in CHF, including dysfunction of \( \beta \)AR signaling and cardiac hypertrophy. In fact, inhibition of \( \beta_2 \) ARK1 activity by overexpression of a peptide inhibitor (\( \beta_2 \) ARK-ct, which prevents agonist-induced translocation of the kinase to plasma membrane) or reduction in \( \beta_2 \) ARK1 expression, produces no harmful effects, as evidenced by normal life spans and normal or enhanced cardiac performance. Moreover, inhibition of \( \beta_2 \) ARK1 by transgenic overexpression of \( \beta_2 \) ARK-ct can restore the diminished \( \beta \) AR contractile response and largely reverse the impaired cardiac performance in some mouse heart failure models, such as MLP knockout mice and calsequestrin-overexpression mice.

These studies have provided strong evidence challenging the current view that increased \( \beta \) AR signaling is car-
diotoxic and plays a central role in the development of CHF. This concept has also been greatly shaken by recent clinical studies. Moxonidine, a powerful sympathetic agent, increased mortality by >50% in MOXOCON Trial [82]. Similarly, bucindolol, a third generation of βAR blocking agent that blocks β1AR and β2AR (especially presynaptic β2AR, a property is not shared by carvedilol, another member of the third generation of βAR blockers), produces harmful effects in a subpopulation of patients predisposed to adverse effects of β-adrenergic withdrawal, including patients in Class IV advanced heart failure and black patients[83, 84]. These clinical observations suggest that β-adrenergic support is important to the survival in some advanced heart failure patients.

Epidemiologic analysis of βAR subtype signaling in CHF

Human genetic epidemiological studies have demonstrated that enhanced β1AR activation is an obvious risk factor aggravating certain cardiac diseases. The naturally occurring Arg389Gly polymorphism of β2AR with sensitized response to agonist stimulation[85] is associated with increased risk of acute myocardial infarction and heart failure[86, 87]. As an extreme clinical situation, a double adrenergic receptor polymorphism, an α2C deletion-loss of function genotype (α2C De1 322-325, which causes increased norepinephrine release from nerve endings) in conjunction with the gain of function β2AR genotype (β2Arg389Gly) leads to a 10-fold risk of the development of heart failure[88]. It is also noteworthy that the likelihood of the α2C polymorphism is significantly increased in blacks relative to non-blacks, and that this genetic variant might contribute to the worse cardiac performance and prognosis in black CHF patients[88].

In sharp contrast to β1AR polymorphisms, the beneficial effect of β2AR stimulation in the context of heart failure is supported by the analysis of β2AR polymorphisms in patients with CHF. The prognosis of heart failure patients with Ile164 polymorphism (a Thr-to-Ile switch at amino acid 164 with reduced β2AR signaling efficacy) is much worse than that of patients without the β2AR variant[89]. Taken together, these clinical studies have reinforced the notion that different cell signaling of β1AR and β2AR produces opposing effects in the pathogenesis of CHF, consistent with information gleaned from cell biological studies and gene-targeted mouse models.

Integrating new perspectives on βAR subtype signaling into novel therapies for CHF

To understand these results from both basic and clinical studies, it is necessary and important to distinguish β1AR signaling from that of β2AR in terms of the causal relationship between βAR stimulation and the development of CHF. In the context of the opposing roles of β1AR and β2AR in regulating myocardial remodeling and function, the beneficial effects of βARK1 inhibition can be interpreted as a result of enhanced cardiac β2AR signaling. This idea is strongly supported by the synergistic effect of βARK-ct and β1AR blockade with metoprolol to prolong the life-span of heart failure mice induced by calsequestrin-overexpression.

In addition to βARK1, G1 signaling is able to functionally counterbalance βAR/Gs-mediated cellular responses. In many types of CHF, the reduction in β1AR and β2AR inotropic responses is often accompanied by an increase in the amount or activity of Gi proteins[90, 91] and a selective down-regulation of β1AR[92, 93], thus leading to a higher β2/β1 ratio and enhanced β2AR/Gi signaling. Although activation of β2AR-coupled Gi protects cardiac myocytes against apoptosis, this may be at the cost of diminished inotropic response. This hypothesis is supported by the observations that PTX treatment restores the diminished βAR inotropic response in a genetic rat heart failure model (spontaneous hypertensive rat, SHR)[94], a rat myocardial infarction heart failure model[95], and in myocytes from failing human hearts[96]. Enhanced Gi signaling is, in fact, specifically involved in the dysfunction of β2AR-induced positive inotropic response in SHR ventricular myocytes, since inhibition of β2AR/Gi signaling by PTX restores β2AR (but not β1AR) contractile response to its full extent, thus abolishing the difference between the failing and normal hearts[94]. These findings are consistent with the notion that β2AR, but not β1AR, dually couples to Gi, and that the Gi coupling offsets the concurrent Gi signaling[17-21].

Inhibiting or avoiding Gi coupling to selectively activate β2AR/Gi-specific signaling might provide a potential target for CHF therapies. However, most β2AR agonists cannot discriminate β2AR-coupled Gi signaling from the Gi signaling. The situation is even worse in chronically failing hearts with elevated Gi signaling, such that β2AR stimulation by most β2AR agonists, including salbutamol, procaterol and zinterol, elicits only a minor contractile response[94]. To unmask the β2AR/Gi signaling from the inhibition by Gi, fenoterol has been recently identified as an apparent β2AR/Gi-selective agonist, as evidenced by the lack of PTX sensitivity of its positive inotropic effect[94]. The ap-
parent \(\beta_1\)AR/Gs signaling-specific agonist, fenoterol, restores the diminished \(\beta_2\)AR-contractile response in myocytes from failing SHR hearts even in the absence of PTX treatment\(^{[84]}\). Similarly, \textit{in vivo} infusion of fenoterol for 8 weeks exhibits a clear beneficial effect in an ischemic rat heart failure model, as manifested by enhanced cardiac output, reversal of cardiac remodeling via inhibiting hypertrophy, perhaps also by reducing mechanical stress due to \(\beta_2\)AR-mediated vasodilatation\(^{[87]}\).

\textbf{\(\beta\)AR blockers as therapy in prevention or amelioration of CHF: do the clinical effects of \(\beta\)AR blockers correlate with subtype-specific \(\beta\)AR signaling?}

Historically, the development of \(\beta\)AR blockers have gone through three different stages, resulting in three distinct generations of \(\beta\)AR blockers in the treatment of CHF. Although all \(\beta\)AR antagonists can block the signal transduction of \(\beta\)ARs, their clinical profiles are strikingly different, as previously reviewed\(^{[1, 2]}\). For example, intolerance to the first generation of \(\beta\)AR blocking drugs was greater than 20\%, due to worsening cardiac contractile function and increased afterload. This class of nonselective \(\beta\)AR blockers, including propranolol and timolol, do not exhibit intrinsic sympathomimetic activity (ISA). However, drug tolerability for the relative \(\beta_1\)AR-selective, second generation compounds (metoprolol, bisoprolol, atenolol and esmolol) is 80–100\%. For the third generation compounds (either \(\beta_1\)AR-selective or non-selective \(\beta/\alpha\) blockers with or without ISA properties, e.g. celiprolol, bucindolol, and carvedilol), drug tolerability is 90–100\% in patients with CHF. Several clinical trials have shown that blockade of myocardial \(\beta_1\)AR by metoprolol is equally effective and beneficial compared to some third generation nonselective \(\beta\)AR blockers, such as carvedilol, which exerts additional inhibitory effects on pathological growth\(^{[98, 99]}\). These data suggest that the therapeutic effects of the second and the third generation of \(\beta\)AR blockers to treat CHF are largely mediated by their \(\beta_1\)AR antagonism. This correlates with the opposing effects of \(\beta_1\)AR and \(\beta_2\)AR stimulation on cardiac remodeling including myocyte apoptosis and hypertrophy, and perhaps also by their distinct arrhythmogenic potential. It is noteworthy that a very recent, large-scale, long-term clinical trial has shown that carvedilol is more beneficial than metoprolol in reducing the mortality in CHF patients (COMET)\(^{[100]}\). The relatively greater beneficial effect of carvedilol compared to metoprolol might also be attributable to the compound’s antagonistic effect on \(\alpha_1\)AR\(^{[101, 102]}\) and antioxidant properties\(^{[101, 104]}\), i.e. carvedilol is a \(\beta_1\)AR blocker with many additional properties.

It has been demonstrated both \textit{in vivo} and \textit{in vitro} that treatment with \(\beta_1\)AR antagonists (e.g. atenolol) specifically enhances myocardial inotropic response to \(\beta_1\)AR stimulation by 5–10-fold without affecting \(\beta_2\)AR inotropic effect\(^{[105, 106]}\). Thus, the clinical effect of \(\beta_2\)AR selective antagonists appears to be attributable, at least in part, to a sensitization of myocardial contractile response to \(\beta_2\)AR stimulation. The \(\beta_1\)AR blockade-mediated sensitization of \(\beta_2\)AR signaling is not associated with an elevation in \(\beta_2\)AR density\(^{[105, 106]}\) or with alterations in the expression of G proteins\(^{[107, 108]}\). However, chronic treatment with \(\beta_1\)AR blockers leads to a reduction in \(\beta_2\)AR expression\(^{[109, 110]}\) that may subsequently contribute to the sensitization of \(\beta_2\)AR signaling. This interpretation is supported by the synergy between the beneficial effects of inhibition \(\beta_2\)AR, with \(\beta_1\)AR blocking agents by metoprolol in heart failure mice. Recent studies have shown that chronic treatment of nonselective \(\beta\)AR blockers, e.g. carvedilol, also increases the sensitivity of \(\beta\)AR to agonist stimulation (even in the absence of restoration of \(\beta_2\)AR density) in rodent heart failure models\(^{[111, 112]}\). Since CHF with various causes is associated with a selective down-regulation of \(\beta_1\)AR with little or no change in \(\beta_2\)AR density, it is plausible that carvedilol-induced sensitization of \(\beta_2\)AR may largely reflect sensitized \(\beta_1\)AR signaling. In addition, clinical observations have revealed that \(\beta\)AR blocking agents with \(\beta_2\)AR intrinsic sympathomimetic activity (ISA) are well tolerated in CHF, whereas those with \(\beta_1\)AR ISA are overtly harmful to the heart.

Based on the above discussions, it is reasonable to speculate that the selective down-regulation of \(\beta_1\)AR and the upregulation of \(\beta_2\)AR-coupled Gs signaling in the functionally compensated hypertrophied heart or in the early stages of CHF may represent complimentary cardiac protective mechanisms. This change in the balance of \(\beta_1\)AR and \(\beta_2\)AR signaling may protect against myocyte apoptosis and consequently slow the progression of cardiomyopathy and contractile dysfunction. However, exaggerated \(\beta_2\)AR-Gs signaling blunts the Gs-mediated contractile support, and eventually contributes to the phenotype of decompensated heart failure. Thus, in the context of CHF, desensitization and downregulation of \(\beta_1\)AR may represent a cardiac protective adaptation, whereas desensitization of \(\beta_2\)AR by enhanced Gs signaling should not be simply regarded as a ‘friend’ or ‘foe’, depending on the
functional and morphological status of the heart.

Concluding remarks

Over the past decade, a wealth of information gleaned from classic pharmacological and cell biological approaches and contemporary genetic manipulations coupled with clinical analysis on patients with adrenergic receptor polymorphisms has revealed qualitatively and quantitatively different signaling pathways and opposing functional roles of sustained β1AR and β2AR stimulation in regulating cardiac remodeling, thereby in the pathogenesis of CHF. In particular, sustained β2AR stimulation promotes cardiac hypertrophy and myocyte apoptosis. Emerging evidence suggests that detrimental effects of β1AR stimulation on heart cells are likely mediated by the Ca2+/CaMK II signaling pathway, independently of the long-suspected cAMP/PKA cascade. Moderately enhanced β2AR activation appears to be cardiac protective. Thus, β2AR might be a “friend” in need due to its concurrent antiapoptotic effects and contractile support as well as the lack of arrhythmogenic effect, whereas β1AR is widely recognized as a “foe” in the context of heart failure. These discoveries not only provide evidence for cellular and molecular mechanisms of the beneficial therapeutic effects of some second and third generations of βAR blocking agents, but also suggest the rationale and strategies to design next generation of therapy for treatment of CHF.

One method to accomplish this is to develop strategies to moderately enhance β2AR signaling (through either Gs or Gi) in combination with selective β1AR blockade; an alternative strategy may be to “rest” the β2AR by turning the receptor activity off with inverse agonists and allow the elevated desensitization machinery to return to normal status.

REFERENCES


[39] Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG, Crow MT. The β2-adrenergic receptor delivers an an-
tiapoptotic signal to cardiac myocytes through Gi-dependent coupling to phosphatidylinositol 3'-kinase. Circ Res 2000; 87:1172-1179.


[64] Colomer JM, Means AR. Chronic elevation of calmodulin in the ventricles of transgenic mice increases the autonomic activity of calmodulin-dependent protein kinase [I], which regulates atrial natriuretic factor gene expression. Mol Endocrinol 2000; 14:1125-1136.


[106] Jia H, Monteith S, Brown MJ. Expression of the α₁- and β₁-


