Acta Pharmacologica Sinica (2012) 33: 335–341
 1671-4083/12 $32.00
www.nature.com/aps

Review

β-Adrenergic receptor subtype signaling in heart: From bench to bedside

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β-Adrenergic receptor (βAR) stimulation by the sympathetic nervous system or circulating catecholamines is broadly involved in peripheral blood circulation, metabolic regulation, muscle contraction, and central neural activities. In the heart, acute βAR stimulation serves as the most powerful means to regulate cardiac output in response to a fight-or-flight situation, whereas chronic βAR stimulation plays an important role in physiological and pathological cardiac remodeling.

There are three βAR subtypes, β_1AR, β_2AR and β_3AR, in cardiac myocytes. Over the past two decades, we systematically investigated the molecular and cellular mechanisms underlying the different even opposite functional roles of β_1AR and β_2AR subtypes in regulating cardiac structure and function, with keen interest in the development of novel therapies based on our discoveries. We have made three major discoveries, including (1) dual coupling of β_2AR to G_s and G_i proteins in cardiomyocytes, (2) cardioprotection by β_2AR signaling in improving cardiac function and myocyte viability, and (3) PKA-independent, CaMKII-mediated β_1AR apoptotic and maladaptive remodeling signaling in the heart. Based on these discoveries and salutary effects of β_1AR blockade on patients with heart failure, we envision that activation of β_2AR in combination with clinically used β_1AR blockade should provide a safer and more effective therapy for the treatment of heart failure.

Keywords: β-adrenergic receptor; heart failure; signal transduction; cardiovascular system

Acta Pharmacologica Sinica (2012) 33: 335–341; doi: 10.1038/aps.2011.201; published online 30 Jan 2012

Introduction

Heart failure (HF) is a syndrome characterized by the insufficient pumping of blood to meet the need of the body. It is a chronic and severely debilitating disease with people older than 65 composed more than 75% of all cases[1]. Regardless of the cause, the failing heart usually ends up in a viscous cycle of progressive functional decline. Owing to its high prevalence, morbidity, mortality and significant health-care costs, HF represents a major current health problem in China and its prevalence is in an upward trend as atherothrombotic diseases, which often lead to HF, will be the first cause of death in the world by 2020[2].

In congestive HF, both the activities of the sympathetic nervous system and the renin-angiotensin system (RAS) are increased[3]. Initially, the increased activity of these neurohormonal systems serves to compensate for the reduced blood pressure and cardiac output. But long term exposure to high levels of circulating catecholamines and angiotensin increases the workload of the heart, and causes maladaptive cardiac remodeling and myocyte death[4–6]. Many of these effects appear to be mediated by the signal transduction cascades of the receptors involved.

β-Adrenergic receptor (βAR) and angiotensin receptor belong to the superfamily of G protein-coupled receptors (GPCRs) or seven transmembrane receptors. GPCRs constitute the most ubiquitous of plasma membrane receptors. They are involved in the regulation of many important physiological functions and also serve as the most important drug targets[7]. Over the past 25 years, βAR antagonists (β-blockers), angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs), alone or in combination, have been used to treat HF conditions. Their use ameliorates the deterioration of left ventricular function, improves symptoms and hemodynamics, and decreases the mortality rate and the need for hospitalization[8–11]. However, these therapeutic agents have limited effectiveness in some patient populations and they also have some adverse effects. Therefore, there is a compelling need to develop new treatments that can improve clinical outcomes.

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Received 2011-11-16 Accepted 2011-12-19

Subtype-specific βAR signaling in the heart

βARs exist as three subtypes, β_1, β_2, and β_3, and the former two are important in the regulation of excitation-contraction coupling of myocardium. β_1AR is the predominant receptor subtype expressed in the heart. Its stimulation results in the activation of the G_s-adenylyl cyclase (AC)-cAMP-protein kinase A (PKA) signaling cascade. In ventricular myocytes, the phosphorylation of PKA substrates including phospholamben, *L*-type calcium channel, ryanodine receptor, cardiac troponin I, and cardiac myosin-binding protein C results in the increase in calcium transient and contractility. In pacemaker cells, PKA-mediated phosphorylation of membrane ion channels and Ca^{2+} handling proteins increases Ca^{2+} cycling and pacing rate. Similarly, β_2AR also has a functional role in cardiomyocyte contraction[12]. But unlike β_1AR which couples only to G_s, β_2AR also couples to pertussis toxin (PTX)-sensitive G_i proteins[13]. The β_2AR-G_i signaling has negative effects on AC activity, cAMP synthesis, PKA activation, and the inotropic response mediated by G_s.

Importantly, persistent stimulation of β_1AR and β_2AR exhibits distinct outcomes under certain pathological circumstances such as HF. Specifically, persistent stimulation of β_1AR triggers cardiomyocyte apoptosis by a Ca^{2+}/calmodulin-dependent kinase II (CaMKII)-dependent, but PKA independent mechanism[14]. Furthermore, the β_1AR-activated CaMKII signaling, but not the PKA pathway, is involved in catecholamine-induced cardiomyocyte hypertrophy *in vitro*[15] and maladaptive cardiac remodeling *in vivo*[16, 17]. In contrast to the cardiotoxic effects of persistent β_1AR activation, persistent β_2AR stimulation is cardioprotective. The cardioprotective effect of persistent β_2AR signaling is largely mediated by β_2AR-G_i coupling, which activates the $G_{\beta\gamma}$-phosphoinositol 3-kinase (PI3K)-Akt cell survival pathway[18]. Although beneficial in terms of cardiomyocyte viability, the protective effect of β_2AR comes at the cost of compromised contractile support.

Heart failure-associated alterations in βAR signaling

During HF, β_1AR is persistently downregulated at the mRNA and protein levels[19, 20]. Its density on the plasma membrane is reduced by 50%, while that of β_2AR has no such change[21]. The resulting change in the ratio of β_1/β_2AR from an 80:20 distribution in the healthy heart to a ratio of 60:40 in the failing heart may indicate the prominent role of β_2AR signaling in the disease condition. In the failing heart, the selective downregulation of β_1AR is often associated with an upregulation of G_i and an enhanced β_2AR-G_i signaling[22, 23]. Importantly, the β_1AR-mediated contractile response is cross-inhibited by the enhanced β_2AR-G_i signaling in the failing heart. Thus, the enhanced β_2AR-G_i signaling contributes to the dysfunction of both β_1AR- and β_2AR-G_s signaling in the failing heart[24–27].

In addition, the signaling efficiency of β_1AR is also markedly reduced in the failing heart as a result of desensitization[28]. This is attributed, in part, to a significant increase in the expression level of G protein coupled receptor kinase 2 (GRK2)[29], the prototypical member of the GRK family. The process of βAR desensitization involves a series of events, including (a) the translocation of GRK2 to the plasma membrane facilitated by the free $G_{\beta\gamma}$ subunits liberated from the activated heterotrimeric G proteins[30], (b) the phosphorylation of the serine or threonine residues on the C-terminal tail of βARs by GRK2, (c) the recruitment of β-arrestins to the phosphorylated receptor, the physical displacement of $G_{s\alpha}$ from the β-arrestin-associated receptor, and (d) the β-arrestin-dependent internalization of the receptor (endocytosis)[31]. While β_2AR stays at a similar level in the failing heart, its coupling efficiency to G_s is markedly reduced[21]. Desensitization of βARs leads to reduced G_s-mediated responses such as cAMP production and positive inotropic effect. Although receptor downregulation and desensitization are considered to be protective responses against excessive sympathetic stimulation during HF[32, 33], the resultant abnormality in βAR signaling may lead to the activation of signaling pathways that are involved in cardiac remodeling, such as the PI3K cascades[34].

Indeed, in humans or animal models with HF, chronic catecholamine elevation causes marked dysregulation of βARs, resulting in various molecular abnormalities, including the upregulation of GRK2[29, 35] and G_i proteins[22, 23, 36]. Upregulation of both of these proteins have been implicated as causal factors in the development of HF. In particular, GRK2 is the most abundant and best-characterized GRK in the heart[37]. GRK2 expression and activity are markedly elevated and play a central role in the HF-associated defect in βAR signaling[38] and cardiac dysfunction[39]. Myocardial ischemia and hypertension in humans and animal models have also been associated with elevated GRK2 expression and activity[40, 41]. These previous studies have defined GRK2 upregulation as an early common event in cardiac maladaptive remodeling and HF.

Emerging evidence suggests that activation of GRK2 as well as PKA is essentially involved in the activation of the β_2AR-coupled G_i signaling in mammalian cells. First, early work has shown that β_2AR-induced activation of ERK1/2 in HEK293 cells is mediated by a G_i-dependent mechanism, and that phosphorylation of β_2AR by PKA is a prerequisite for the switch of the receptor coupling from G_s to G_i[42]. Second, our recent studies[43] have demonstrated that elevated β_2AR phosphorylation by GRK2 acerbates the G_i signaling, whereas inhibition of GRK2 activity profoundly suppresses the β_2AR-G_i coupling. Since GRK2 upregulation occurs prior to the onset of HF and contributes to the development of HF[44, 45], enhanced GRK2 activation may play an important role in the exacerbated β_2AR-coupled G_i signaling in the failing heart. Indeed, disruption of G_i signaling with PTX or inhibition of GRK2 with a peptide inhibitor, βARK-ct, can restore cardiac contractile response to βAR stimulation in multiple HF models[46–49].

Importantly, cardiac-specific transgenic overexpression of a mutant β_2AR lacking PKA phosphorylation sites (PKA-TG), but not the wild type β_2AR (WT TG) or a mutant β_2AR lacking GRK sites (GRK-TG), led to exaggerated cardiac response to pressure overload, as manifested by markedly exacerbated cardiac maladaptive remodeling and failure, and early mortality[43]. Furthermore, inhibition of G_i signaling with PTX

restores cardiac function in HF associated with increased β_2AR to G_i coupling induced by removing PKA phosphorylation of the receptor and in GRK2 transgenic mice, indicating that enhanced phosphorylation of β_2AR by GRK and resultant increase in G_i-biased β_2AR signaling play an important role in the development of HF[43]. Altogether, our recent studies have demonstrated that enhanced β_2AR phosphorylation by GRK leads the receptor to G_i-biased signaling which, in turn, contributes to the pathogenesis of HF, marking G_i-biased β_2AR signaling as a primary event linking pathological upregulation of GRK to cardiac maladaptive remodeling, failure and cardiodepression. It is also noteworthy that, as is the case in the failing heart, enhanced β_2AR-coupled G_i signaling is responsible for the defects of both β_1AR and β_2AR signaling in the GRK2 transgenic mice[43], and that the previously reported beneficial effects of βARK-ct in improving the function of the failing heart[38, 39, 50-52] is mediated, at least in part, by attenuating GRK-dependent G_i-biased β_2AR signaling.

Carvedilol paradox

In clinical settings, long-term use of β-blockers improves clinical symptom of HF. Treatment with β-blockers improves left ventricular contractile function in the failing heart and reverses cardiac remodeling[8, 9]. In the molecular level, β-blockade may normalize βAR system through the upregulation of β_1AR[53] and the restoration of receptor sensitivity by decreasing the expression of GRK2[50]. However, the effects of different β-blockers are not identical. The use of subtype non-selective β-blockers in early years has caused some major side effects including bronchial and blood vessel constriction[54, 55]. This is largely due to the inhibition of β_2AR in non-cardiac tissues such as the respiratory system and blood vessels. These problems have been partially resolved with the introduction of selective β_1AR antagonists, such as atenolol, metoprolol, bisoprolol and nebivolol. Recent clinical trials have indicated that only 3 out of 16 β-blockers are beneficial in terms of cardiovascular survival[9, 56–58], with carvedilol emerging as the best[59].

Apart from being a non-selective β-blocker, carvedilol also has several properties, such as α_1-adrenergic blockade, anti-oxidant, anti-proliferative, anti-endothelin and anti-arrythmogenic effects[60, 61], which may explain its higher efficacy. Interestingly, carvedilol has been found to be the only one among 16 blockers that activated ERK by a β_2AR-mediated, G protein-independent, and β-arrestin-dependent mechanism[62]. Moreover, among 20 β-blockers tested, only atenolol and carvedilol could induce the β_1AR-mediated transactivation of EGFR and this effect is also β-arrestin-dependent[63]. It has been implicated that this effect may contribute to the special therapeutic effect of carvedilol. In this regard, recent studies have shown that β-arrestin-dependent, G protein-independent activation of EGFR via β_1AR confers cardioprotection in mice chronically stimulated with catecholamine[64]. These data suggest that a ligand can antagonize the G protein-dependent activity of a GPCR and at the same time stimulates signaling pathways in a G protein-independent β-arrestin-dependent fashion[65]. They are also of great relevance to our discussion in the next section about the application of this principle in the development of novel therapeutic agents.

Biased βAR signaling and drug discovery

In the classical paradigm of GPCR signaling, ligand binding leads to conformational change of the receptor from an inactive state R into a single activated state R* that results in the coupling of the receptor to heterotrimeric G proteins. Receptor coupling facilitates the exchange of the bound GDP with GTP in the α subunit of the G protein complex. This triggers dissociation of the complex into G_α and $G_{\beta\gamma}$ subunits. They go on to activate their respective effectors such as AC, phospholipases and ion channels. These receptor mediated reactions often generate signaling molecules called second messengers which activate or inhibit other components of the cellular machinery. Thus, receptor stimulation produces a multitude of cellular responses via the activation of the signal transduction pathways downstream of G proteins. Agonist efficacy, a measure of the ability of an agonist to activate this cascade, quantitatively defines the agonist as partial or full. In this scheme, antagonist is defined as a ligand which binds to the receptor but produces no receptor activation and thus has the ability to block agonist-stimulated G protein activation. This unidirectional understanding of agonist efficacy is contradictory to the aforementioned findings that a ligand for a single GPCR can be an antagonist for the G protein-dependent signals and also an agonist for the β-arrestin-dependent signals[62, 63].

Over the past fifteen years, more and more evidence has accumulated indicating that a ligand for a given GPCR does not simply possess a single defined efficacy. Rather, a ligand possesses multiple efficacies, depending on the downstream signal transduction pathways analyzed. Moreover, GPCR can be differentially activated to target a specific subset of signal transduction pathways by the so-called "biased ligand". In particular, research has revealed that GPCR can be stimulated to produce a β-arrestin-dependent but G protein-independent signal, which differs both spatially and temporally from the β-arrestin-mediated signal stemmed from receptor desensitization[66]. It is believed that the β-arrestin-biased ligand activates the alternative signaling pathway by stabilizing the receptor in a distinct active conformation R*'. Thus, in this new paradigm, GPCR may be stabilized by different ligands in distinct active conformations R^{*1}-R^{*n} each capable of activating a diverse array of signal transduction pathways and responses (Figure 1). This concept, described as functional selectivity, collateral/pluridimensional efficacy, or biased agonism, has major implications for pharmacological therapeutics[65, 67–70].

To add another layer of complexity to this scheme, the signal trafficked by a biased agonist is context-dependent, too. Not only does the selectivity of a ligand towards different signaling pathways change in different cell types, the change in the levels of cytosolic reactants of GPCR also has an impact on the functional selectivity of a ligand. For example, the specific β_2AR antagonist ICI-118551 has been suggested to directly produce a negative inotropic effect by acting as an agonist for the G_i-coupled β_2AR in myocytes from failing human heart[71].

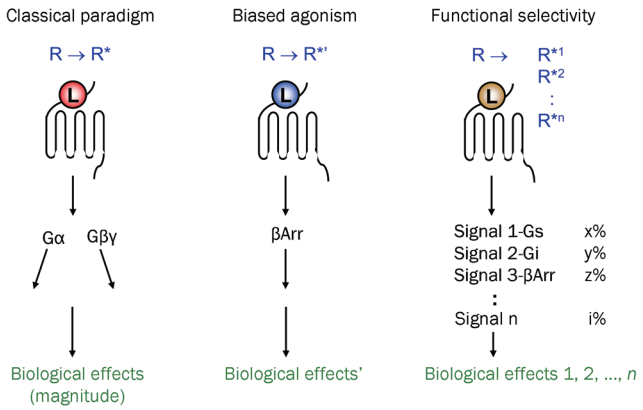

Figure 1. Development of the receptor theory. In the classical paradigm, ligands have linear efficacies, referring to their abilities to stabilize the receptor into a single active state. The emerging concept of biased agonism suggests that a biased ligand may stabilize the receptor into a distinct active state that does not activate G proteins but activates β-arrestins. In the concept of functional selectivity, receptors may exist in multiple active conformations as stabilized by different ligands, and each of these conformations gives rise to different downstream signals and biological effects. βArr, β-arrestin; L, ligand; R, inactive conformation of GPCR; R*, active conformation of GPCR.

This effect is not due to the blocking of the endogenous catecholamines and is also different in principle from an inverse agonistic effect also described for this ligand[72]. It is because this negative inotropic effect of ICI-118551 is PTX-sensitive, is observable at receptor levels with or without overexpression manipulation, and only becomes apparent under the conditions when the levels of G_i are raised.

In a recent study using a cardiomyocyte model[49], we have screened a panel of β_2AR agonists, including zinterol, salbutamol, and procaterol for their receptor-mediated contractility stimulatory activities and the sensitivities of these effects towards PTX. We have found that PTX augmented the contractile responses of most β_2AR agonists but not that of fenoterol. These data indicates that while most β_2AR agonists activate both G_s and G_i, fenoterol selectively activates G_s. This is the first evidence to show that different agonists can activate a receptor to couple to different G-proteins. It was further found that fenoterol fully reversed the diminished β_2AR-mediated inotropic effect in cardiomyocytes isolated from failing spontaneous hypertensive rat hearts even in the absence of PTX. This study is particularly valuable in that fenoterol was identified to be a unique agonist capable of selectively stabilizing the coupled β_2AR-G_s species in conditions that favor β_2AR-G_i coupling. It also reveals the therapeutic potential of fenoterol in the treatment of HF.

The effectiveness of fenoterol in treating HF conditions has been demonstrated in a number of follow-up *in vivo* studies[73–76]. Prolonged use of fenoterol not only improves cardiac function, but also retards cardiac maladaptive remodeling, and that the overall beneficial effects of fenoterol are greater than the salutary effects of β_1AR blockade in a myocardial infarction induced rat model of dilated cardiomyopathy[73]. These studies suggest that selective activation of the β_2-AR-coupled G_s signaling may provide a useful therapeutic target for the treatment of congestive HF. We envision that new G_s-biased β_2AR agonists, such as fenoterol and its derivatives, may be developed into drugs to improve the structure and function of the failing heart.

Fenoterol contains two chiral centers and can exist as four stereoisomers. We have synthesized a cohort of fenoterol derivatives including the *R,R*-, *R,S*-, *S,R*-, and *S,S*-isomers[77, 78]. While the pharmaceutical preparation of fenoterol is a racemic mixture of its *R,R*- and *S,S*-enantiomers, our recent studies have shown that the *R,R*-enantiomer is the only active isomer in receptor binding and cardiomyocyte contraction assays[77, 78]. It has been known for a century that stereoisomers of catecholamines differ in their potency and efficacy. However, the molecular basis for the differences in the efficacies of GPCR ligand stereoisomers has remained poorly defined. We have, therefore, used some of these fenoterol derivatives to examine the hypothesis that the stereochemistry of an agonist determines functional selectivity of a given receptor coupling to different G protein(s) and resultant activation of subset(s) of downstream signaling pathways[79]. We found that while R,R-fenoterol failed to activate G_i signaling, as evidenced by the absence of PTX-sensitivity of its contractile response and its inability to activate G_i-dependent ERK1/2 signaling, S,R-fenoterol exhibited a robust PTX-sensitivity in these responses, suggesting that the S,R-isomer enables β_2AR to activate both G_s and G_i. The same conclusion holds true for some fenoterol derivatives. For instance, S,R-methoxyfenoterol, but not R,R-methoxyfenoterol, activated β_2AR-coupled G_i signaling in cardiomyocytes[79]. Thus, in addition to receptor subtype and phosphorylation status, the different stereoisomers of an agonist selectively activate distinct receptor-G protein interactions and downstream signaling events. This finding is important because it is the first account to show that even the subtle chemical differences within a ligand stereoisomer pair are sufficient to stabilize GPCR conformations with distinct G-protein coupling properties, highlighting how important it is to carefully examine both the "active" and the "inactive" stereoisomer to understand the exact mechanism of action and cellular effects of a GPCR ligand[80].

This finding also has important clinical implications. In particular, it has been shown that long-term (1 year) treatment with racemic fenoterol enhances the beneficial effect of β_1AR blockade with metoprolol in a rat model of dilated cardiomyopathy[75], and the combined (fenoterol+metoprolol) therapy is as good as the clinical combination (metoprolol+ACEI) treatment with respect to mortality, and exceeds the latter with respect to cardiac remodeling and myocardial infarct expansion[76]. It will be interesting to study the effects of different fenoterol derivatives[77, 78, 81] alone or in combination with β_1AR blocker or RAS inhibitor in this model. Continued efforts on this research line may lead to the development of potential novel therapies with greater selectivity, efficacy and fewer side

effects for human congestive HF. Topics related to the translation of this novel treatment regimen have been discussed extensively in another recent review[82], which also contains a pathway map for βAR subtype signaling described in this article.

If suppression of β_2AR-G_i signaling or enhancement of β_2AR-G_s signaling is beneficial in HF, the next question is: what is the difference between β_2AR-G_s signaling and β_1AR-G_s signaling? In a recent elegant study[83], Mangmool and co-authors have elucidated the molecular mechanism of CaMKII activation by β_1AR. They found that stimulation of β_1AR induces the formation of a β-arrestin-CaMKII-Epac1 complex, allowing its recruitment to the plasma membrane, and whereby promotes cAMP-dependent activation of CaMKII. Further studies using chimeric receptors with switched carboxyl-terminal tails of β_1AR or β_2AR suggested that β-arrestin binding to the carboxyl-terminal tail of β_1AR promotes a conformational change within β-arrestin that allows CaMKII and Epac to remain in a stable complex with the receptor. These results demonstrate that only β_1AR but not β_2AR activates CaMKII significantly. As $CaMKII_\delta$ is a common intermediate of diverse death stimuli-induced apoptosis in cardiomyocytes[84], is required for the transition from pressure overload-induced cardiac hypertrophy to HF[85], and promotes life-threatening arrythmias in HF[86], this explains why activation of β_2AR-G_s signaling is usually not accompanied with the adverse effects observed in β_1AR stimulation.

The molecular mechanism of the cardioprotective effect of β_2AR-G_s signaling in HF is unclear. One possibility is the crosstalk of the G_s-AC-cAMP-PKA cascade to the tyrosine kinase receptor-mediated Akt phosphorylation[87–89].

Concluding remark

In summary, recent studies have revealed opposing functional roles of β_1AR and β_2AR in regulating myocyte viability and myocardial remodeling with a cardiac protective effect of β_2AR stimulation and a detrimental effect of β_1AR stimulation. Unlike the sole G_s coupling of β_1AR, β_2AR couples to both G_s and G_i signaling pathways with the G_i coupling negating the G_s-mediated contractile support. In the failing heart, enhanced expression and activity of GRK2 and G_i proteins further promote G_i-biased β_2AR signaling, thus blunting both β_1AR- and β_2AR-mediated cardiac reserve function, resulting in cardiac maladaptive remodeling and failure. These findings defined the β_2AR-G_i signaling as an essential link between pathologic upregulation of GRK and the development of HF. Since GRK2 and resultant G_i-biased β_2AR signaling are pathogenic factors of HF, G_s-biased β_2AR agonists may present an important therapeutic strategy for the treatment of HF caused by various etiologies.

Acknowledgements

This work was supported by the National Basic Research Program of China (2012CB518000) and the National Major Scientific Research Program of China (2012CB910400).

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