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, β₁AR, β₂AR and β₃AR, in cardiac myocytes. Over the past two decades, we systematically investigated the molecular and cellular mechanisms underlying the different even opposite functional roles of β₁AR and β₂AR 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 β₂AR to Gs and Gi proteins in cardiomyocytes, (2) cardioprotection by β₂AR signaling in improving cardiac function and myocyte viability, and (3) PKA-independent, CaMKII-mediated β₁AR apoptotic and maladaptive remodeling signaling in the heart. Based on these discoveries and salutary effects of β₁AR blockade on patients with heart failure, we envision that activation of β₂AR in combination with clinically used β₁AR 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
Subtype-specific βAR signaling in the heart

βARs exist as three subtypes, β₁, β₂, and β₃, and the former two are important in the regulation of excitation-contraction coupling of myocardium. β₂AR is the predominant receptor subtype expressed in the heart. Its stimulation results in the activation of the Gₛ-adenyl cyclase (AC)-cAMP-protein kinase A (PKA) signaling cascade. In ventricular myocytes, the phosphorylation of PKA substrates including phospholamban, 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 Ca²⁺ handling proteins increases Ca²⁺ cycling and pacing rate. Similarly, β₂AR also has a functional role in cardiomyocyte contraction[12]. But unlike β₁AR which couples only to Gi, β₂AR also couples to pertussis toxin (PTX)-sensitive Gᵢ proteins[13]. The β₂AR-Gᵢ signaling has negative effects on AC activity, cAMP synthesis, PKA activation, and the inotropic response mediated by Gₛ.

Importantly, persistent stimulation of β₁AR and β₂AR exhibits distinct outcomes under certain pathological circumstances such as HF. Specifically, persistent stimulation of β₁AR triggers cardiomyocyte apoptosis by a Ca²⁺/calmodulin-dependent kinase II (CaMKII)-dependent, but PKA independent mechanism[14]. Furthermore, the β₁AR-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 β₁AR activation, persistent β₂AR stimulation is cardioprotective. The cardioprotective effect of persistent β₂AR signaling is largely mediated by β₂AR-Gᵢ coupling, which activates the Gₛ₇-phosphoinositol 3-kinase (PI3K)-Akt cell survival pathway[18]. Although beneficial in terms of cardiomyocyte viability, the protective effect of β₂AR comes at the cost of compromised contractile support.

Heart failure-associated alterations in βAR signaling

During HF, β₁AR is persistently downregulated at the mRNA and protein levels[19, 20]. Its density on the plasma membrane is reduced by 50%, while that of β₂AR has no such change[21]. The resulting change in the ratio of β₁/β₂AR 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 β₂AR signaling in the disease condition. In the failing heart, the selective downregulation of β₁AR is often associated with an upregulation of Gₛ and an enhanced β₂AR-Gᵢ signaling[22, 23]. Importantly, the β₂AR-mediated contractile response is cross-inhibited by the enhanced β₂AR-Gᵢ signaling in the failing heart. Thus, the enhanced β₂AR-Gᵢ signaling contributes to the dysfunction of both β₁AR- and β₂AR-Gᵢ signaling in the failing heart[24-27].

In addition, the signaling efficiency of β₁AR 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 Gₛ 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 Gₛ₇ 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 Gₛ₇ from the β-arrestin-associated receptor, and (d) the β-arrestin-dependent internalization of the receptor (endocytosis)[31]. While β₁AR stays at a similar level in the failing heart, its coupling efficiency to Gₛ is markedly reduced[21]. Desensitization of βARs leads to reduced Gₛ₇-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 Gₛ 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 β₂AR-coupled Gₛ signaling in mammalian cells. First, early work has shown that β₂AR-induced activation of ERK1/2 in HEK293 cells is mediated by a Gₛ₇-dependent mechanism, and that phosphorylation of β₂AR by PKA is a prerequisite for the switch of the receptor coupling from Gₛ to Gₛ₇[42]. Second, our recent studies[43] have demonstrated that elevated β₂AR phosphorylation by GRK2 acerbates the Gₛ signaling, whereas inhibition of GRK2 activity profoundly suppresses the β₂AR-Gₛ₇ 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 β₂AR-coupled Gₛ signaling in the failing heart. Indeed, disruption of Gₛ signaling with PTX or inhibition of GRK2 with a peptide inhibitor, βARK-c-t, can restore cardiac contractile response to βAR stimulation in multiple HF models[46-49].

Importantly, cardiac-specific transgenic overexpression of a mutant β₂AR lacking PKA phosphorylation sites (PKA-TG), but not the wild type β₂AR (WT TG) or a mutant β₂AR 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 Gₛ signaling with PTX
restores cardiac function in HF associated with increased β2AR to G_{i} coupling induced by removing PKA phosphorylation of the receptor and in GRK2 transgenic mice, indicating that enhanced phosphorylation of β2AR by GRK and resultant increase in G_{i}-biased β2AR signaling play an important role in the development of HF[43]. Altogether, our recent studies have demonstrated that enhanced β2AR phosphorylation by GRK leads the receptor to G_{i}-biased signaling which, in turn, contributes to the pathogenesis of HF, marking G_{i}-biased β2AR 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 β2AR-coupled G_{i} signaling is responsible for the defects of both β2AR and β2AR 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[56–58] is mediated, at least in part, by attenuating GRK-dependent G_{i}-biased β2AR 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[6, 9]. In the molecular level, β-blockade may normalize βAR system through the upregulation of β1AR[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 β2AR in non-cardiac tissues such as the respiratory system and blood vessels. These problems have been partially resolved with the introduction of selective β1AR 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[50].

Apart from being a non-selective β-blocker, carvedilol also has several properties, such as α1-adrenergic blockade, anti-oxidant, anti-proliferative, anti-endothelin and anti-arrhythmogenic 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 β2AR-mediated, G protein-independent, and β-arrestin-dependent mechanism[62]. Moreover, among 20 β-blockers tested, only atenolol and carvedilol could induce the β1AR-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 β1AR 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_{a} and G_{βγ} 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^{1L}-R^{*} 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[68, 69, 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 β2AR antagonist ICI-118551 has been suggested to directly produce a negative inotropic effect by acting as an agonist for the G_{i}-coupled β2AR in myocytes from failing human heart[71].
have screened a panel of β2AR agonists, including zinterol, found that fenoterol fully reversed the diminished β2AR-coupled Gs signaling to couple to different G-proteins. It was further the first evidence to show that different agonists can activate β2AR-Gi signaling.

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 Gi are raised.

In a recent study using a cardiomyocyte model,[49], we have screened a panel of β2AR 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 β2AR agonists but not that of fenoterol. These data indicates that while most β2AR agonists activate both Gi and Gs, fenoterol selectively activates Gi. 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 β2AR-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 β2AR-Gi species in conditions that favor β2AR-Gi 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.[73–79] 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 β1AR blockade in a myocardial infarction induced rat model of dilated cardiomyopathy.[73]. These studies suggest that selective activation of the β2-AR-coupled Gi signaling may provide a useful therapeutic target for the treatment of congestive HF. We envision that new Gi-biased βAR 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 Gi signaling, as evidenced by the absence of PTX-sensitivity of its contractile response and its inability to activate Gi-dependent ERK1/2 signaling, S,R-fenoterol exhibited a robust PTX-sensitivity in these responses, suggesting that the S,R-isomer enables β2AR to activate both Gi and Gs. The same conclusion holds true for other fenoterol derivatives. For instance, S,R-methoxyfenoterol, but not R,R-methoxyfenoterol, activated β2AR-coupled Gi 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 β1AR blockade with metoprolol in a rat model of dilated cardiomyopathy,[79] 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 β1AR 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.

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Classical paradigm

Biased agonism

Functional selectivity

Gα

Gβγ

βArr

Signal 1 Gs

Signal 2 Gi

Signal 3 βArr

GPCRs

Biological effects

Biological effects'

Biological effects 1, 2, ..., n

Magnitude

x%

y%

z%

Percentage
effects for human congestive HF. Topics related to the translation of this novel treatment regimen have been discussed extensively in another recent review[83], which also contains a pathway map for βAR subtype signaling described in this article.

If suppression of β2AR-Gi signaling or enhancement of β2AR-Gs signaling is beneficial in HF, the next question is: what is the difference between β2AR-Gi signaling and β1AR-Gi signaling? In a recent elegant study[83], Mangmool and co-authors have elucidated the molecular mechanism of CaMKII activation by β2AR. They found that stimulation of β2AR 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 β2AR or β1AR suggested that β-arrestin binding to the carboxyl-terminal tail of β2AR 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 β2AR but not β1AR activates CaMKII significantly. As CaMKII 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 arrhythmias in HF[86], this explains why activation of β2AR-Gi signaling is usually not accompanied with the adverse effects observed in β1AR stimulation.

The molecular mechanism of the cardioprotective effect of β2AR-Gs signaling in HF is unclear. One possibility is the crosstalk of the Gs-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 β1AR and β2AR in regulating myocyte viability and myocardial remodeling with a cardiac protective effect of β2AR stimulation and a detrimental effect of β1AR stimulation. Unlike the sole Gi coupling of β1AR, β2AR couples to both Gi and Gs signaling pathways with the Gi coupling negating the Gs-mediated contractile support. In the failing heart, enhanced expression and activity of GRK2 and Gi proteins further promote Gs-biased β2AR signaling, thus blunting both β1AR- and β2AR-mediated cardiac reserve function, resulting in cardiac maladaptive remodeling and failure. These findings defined the β2AR-Gi signaling as an essential link between pathologic upregulation of GRK and the development of HF. Since GRK2 and resultant Gi-biased β2AR signaling are pathogenic factors of HF, Gi-biased β2AR agonists may present an important therapeutic strategy for the treatment of HF caused by various etiologies.

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References
1. Rich MW. Epidemiology, pathophysiology, and etiology of congestive heart failure in older adults. J Am Geriatr Soc 1997; 45: 968–74.
2. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, et al. Executive summary: Heart disease and stroke statistics — 2010 update: A report from the American Heart Association. Circulation 2010; 121: 948–54.
3. Levine TB, Francis GS, Goldsmith SR, Simon AB, Cohn JN. Activity of the sympathetic nervous system and renin-angiotensin system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. Am J Cardiol 1982; 49: 1659–66.
4. Hasenfuss G, Mulieri LA, Allen PD, Just H, Alpert NR. Influence of isoproterenol and ouabain on excitation-contraction coupling, cross-bridge function, and energetics in failing human myocardium. Circulation 1996; 94: 3155–60.
5. Teerlink JR, Pfeffer JM, Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. Circ Res 1994; 75: 105–13.
6. Blaufarb IS, Sonnenblick EH. The renin-angiotensin system in left ventricular remodeling. Am J Cardiol 1996; 77: 8C–16C.
7. Overington JP, Al-Lazikani B, Hopkins AL. How many drug targets are there? Nat Rev Drug Disc 2006; 5: 993–6.
8. McMurray JJ, Pfeffer MA. Heart failure. Lancet 2005; 365: 1877–89.
9. Waagstein F, Bristow MR, Swedberg K, Camerini F, Fowler MB, Silver MA, et al. Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group. Lancet 1993; 342: 1441–6.
10. Beckwith C, Munger MA. Effect of angiotensin-converting enzyme inhibitors on ventricular remodeling and survival following myocardial infarction. Ann Pharmacother 1993; 27: 755–66.
11. Lee VC, Rhew DC, Dylan M, Badamgarav E, Braunstein GD, Weingarten SR. Meta-analysis: angiotensin-receptor blockers in chronic heart failure and high-risk acute myocardial infarction. Ann Intern Med 2004; 141: 693–704.
12. Xiao RP, Hohl C, Altschuld R, Jones L, Livingston B, Ziman B, et al. β2-adrenergic receptor-stimulated increase in cAMP in rat heart cells is not coupled to changes in Ca2+ dynamics, contractility, or phospholamban phosphorylation. J Biol Chem 1994; 269: 19151–6.
13. Xiao RP, Ji X, Lakagga EG. Functional coupling of the β2-adrenergic receptor to a pertussis toxin-sensitive G protein in cardiac myocytes. Mol Pharmacol 1995; 47: 322–9.
14. Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH, et al. Linkage of β1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. J Clin Invest 2003; 111: 617–25.
15. Sucharov CC, Mariner PD, Nunley KR, Long C, Leinwand, L, Bristow MR. A β1-adrenergic receptor CaM kinase II-dependent pathway mediates cardiac myocyte fetal gene induction. Am J Physiol Heart Circ Physiol 2006; 291: H1299–308.
16. Bisognano JD, Weinberger HD, Bohlmeier TJ, Pende A, Raynolds MV, Sastravaha A, et al. Myocardial-directed overexpression of the human β1-adrenergic receptor in transgenic mice. J Mol Cell Cardiol 2000; 32: 817–30.
17. Engelhardt S, Hein L, Wiesmann F, Lohse MJ. Progressive hypertrophy and heart failure in β1-adrenergic receptor transgenic mice. Proc Natl Acad Sci U S A 1999; 96: 7059–64.
18. Zhu WZ, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, Xiao RP. Dual modulation of cell survival and cell death by β2-adrenergic signaling.
in adult mouse cardiac myocytes. Proc Natl Acad Sci U S A 2001; 98: 1607–12.

19 Bristow MR, Ginsburg R, Fowler M, Minobe W, Rassmussen R, Zera P, et al. β₁ and β₂-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β₁-receptor down-regulation in heart failure. Circ Res 1986; 59: 297–309.

20 Bristow MR, Minobe WA, Raymonds MV, Port JD, Rassmussen R, Ray PE, et al. Reduced β₁-receptor messenger RNA abundance in the failing human heart. J Clin Invest 1993; 92: 2737–45.

21 Bristow MR, Hersherberger RE, Port JD, Minobe W, Rassmussen R. β₁- and β₂-adrenergic-receptor-mediated adenylyl cyclase stimulation in nonfailing and failing human ventricular myocardium. Mol Pharmacol 1989; 35: 295–303.

22 Bohm M, Eschenhagen T, Gierschik P, Larisch K, Lensche H, Mende U, et al. Radioimmunochemical quantification of Gαi in right and left ventricles from patients with ischaemic and dilated cardiomyopathy and predominant left ventricular failure. J Mol Cell Cardiol 1994; 26: 133–49.

23 Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, et al. Increase of the 40 000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest 1988; 82: 189–97.

24 Lokuta AJ, Maertz NA, Meethal SV, Potter KT, Kamp TJ, Valdivia HH, et al. Increased nitrification of sarcoplasmic reticulum Ca²⁺-ATPase in human heart failure. Circulation 2005; 111: 988–95.

25 Sato M, Gong H, Terracciano CM, Ranu H, Harding SE. Loss of β-adrenoceptor response in myocytes overexpressing the Na⁺/Ca²⁺ exchanger. J Mol Cell Cardiol 2004; 36: 43–8.

26 Xiao RP, Balke CW. Na⁺/Ca²⁺ exchange linking β₂-adrenergic G, signaling to heart failure: associated defect of adrenergic contractile support. J Mol Cell Cardiol 2004; 36: 7–11.

27 Zhu W, Zeng X, Zheng M, Xiao RP. The enigma of β₂-adrenergic receptor G, signaling in the heart: the good, the bad, and the ugly. Circ Res 2005; 97: 507–9.

28 Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, et al. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. N Engl J Med 1982; 307: 205–11.

29 Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of β-adrenergic receptor kinase and β₁-adrenergic receptors in the failing human heart. Circulation 1993; 87: 454–63.

30 Pitcher JA, Inglese J, Higgins JB, Ariza JL, Casey PJ, Kim C, et al. Role of β subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science 1992; 257: 1264–7.

31 Lefkowitz RJ. G protein-coupled receptors. Ill. New roles for receptor kinases and β-arrestins in receptor signaling and desensitization. J Biol Chem 1998; 273: 18677–80.

32 Faux MD, Ernsberger P, Valtrner D, Hoffman RD, Lewis W, Strachan R, et al. Strain-dependent β₁-adrenergic receptor function influences myocardial responses to isoproterenol stimulation in mice. Am J Physiol Heart Circ Physiol 2005; 289: H30–6.

33 Liggett SB, Cresci S, Kelly RJ, Syed FM, Matkovich SJ, Hahn HS, et al. A GRK5 polymorphism that inhibits β-adrenergic receptor signaling is protective in heart failure. Nat Med 2008; 14: 510–7.

34 Shioi T, Kang PM, Douglas PS, Hampe J, Ybaele CM, Lawatts J, et al. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. EMBO J 2000; 19: 2537–48.

35 Ungerer M, Parrutti G, Bohm M, Puzicha M, DelBlasi A, Erdmann E, et al. Expression of β-arrestins and β-adrenergic receptor kinases in the failing human heart. Circ Res 1994; 74: 206–13.

36 Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T, et al. Coupling of β₁-adrenergic receptor to G, proteins and its physiological relevance in murine cardiac myocytes. Circ Res 1999; 84: 43–52.

37 Hata JA, Koch WJ. Phosphorylation of G protein-coupled receptors: GPCR kinases in heart disease. Mol Interv 2003; 3: 264–72.

38 Choi DJ, Koch WJ, Hunter JJ, Rockman HA. Mechanism of β-adrenergic receptor desensitization in cardiac hypertrophy is increased β-adrenergic receptor kinase. J Biol Chem 1997; 272: 17223–9.

39 Perrino C, Naga Prasad SV, Schroder JN, Hata JA, Milano C, Rockman HA. Restoration of β-adrenergic receptor signaling and contractile function in heart failure by disruption of the BarK3/phosphoinositide 3-kinase complex. Circulation 2005; 111: 2579–87.

40 Ungerer M, Kessebohmer KH, Kronstein K, Lohse MJ, Richardt G. Activation of β-adrenergic receptor kinase during myocardial ischemia. Circ Res 1996; 79: 455–60.

41 Gros R, Benovic JL, Tan CM, Feldman RD. G-protein-coupled receptor kinase activity is increased in hypertension. J Clin Invest 1997; 99: 2087–93.

42 Daaka Y, Luttrell LM, Lefkowitz RJ. Switching of the coupling of the β₂-adrenergic receptor to different G proteins by protein kinase A. Nature 1997; 390: 88–91.

43 Zhu W, Petrashevskaya N, Ren S, Zhao A, Chakir K, Gao E, et al. G-biased βAR signaling links GRK2 upregulation to heart failure. Circ Res 2011; doi:10.1161/CIRCRESAHA.111.253260.

44 Rockman HA, Chien KR, Choi DJ, Iaccarino G, Hunter JJ, Ross J, et al. Expression of a β-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. Proc Natl Acad Sci U S A 1998; 95: 7000–5.

45 Tachibana H, Rengo G, Gao E, Ebert SN, Dorn GW 2nd, Koch WJ. Reduction of sympathetic activity via adrenal-targeted GRK2 gene deletion attenuates heart failure progression and improves cardiac function after myocardial infarction. J Biol Chem 2010; 285: 16378–86.

46 Chakir K, Daya SK, Alba T, Tunin RS, Dimauno VL, Abraham TP, et al. Mechanisms of enhanced β-adrenergic reserve from cardiac resynchronization therapy. Circulation 2009; 119: 1231–40.

47 Koch WJ, Rockman HA, Samapa P, Hamilton RA, Bond RA, Milano CA, et al. Cardiac function in mice overexpressing the β-adrenergic receptor kinase or a βARK inhibitor. Science 1995; 268: 1350–3.

48 Tachibana H, Naga Prasad SV, Lefkowitz RJ, Koch WJ, Rockman HA. Level of β-adrenergic receptor kinase 1 inhibition determines degree of cardiac dysfunction after chronic pressure overload-induced heart failure. Circulation 2005; 111: 591–7.

49 Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA, et al. Enhanced G, signaling selectively negates β₂-AR but not β₁-AR-mediated positive inotropic effect in myocytes from failing rat hearts. Circulation 2003; 108: 1637–43.

50 Iaccarino G, Tomhave ED, Lefkowitz RJ, Koch WJ. Reciprocal signaling selectively negates β₂AR-butyrate receptor stimulation and blockade. Circulation 1998; 97: 1783–9.

51 White DC, Hata JA, Shah AS, Glower DD, Lefkowitz RJ, Koch WJ. Preservation of myocardial β-adrenergic receptor signaling delays the development of heart failure after myocardial infarction. Proc Natl Acad Sci U S A 2000; 97: 5428–33.

52 Harding VJ, Jones LR, Lefkowitz RJ, Koch WJ, Rockman HA. Cardiac βARK1 inhibition prolongs survival and augments β blocker therapy in a mouse model of severe heart failure. Proc Natl Acad Sci U S A 2001; 98: 5809–14.

53 Sigmund M, Jakob H, Becker H, Hanrath P, Schumacher C, Eschenhagen T, et al. Effects of metoprolol on myocardial β-adrenoceptors and Gα-proteins in patients with congestive heart failure. Eur J Clin
65 Violin JD, Lefkowitz RJ. β-Arrestin-biased ligands at seven-trans-
66 Ahn S, Shenoy SK, Wei H, Lefkowitz RJ. Differential kinetic and spatial
67 Kenakin T. Principles: Receptor theory in pharmacology. Trends
68 Urban JD, Clarke WP, Zastrow MV, Nichols DE, Kobilka B, Weinstein
69 Mailman RB. GPCR functional selectivity has therapeutic impact. Kenakin T. Collateral efficacy in drug discovery: taking advantage of
70 Ahmet I, Morrell C, Lakatta EG, Talan MI. Therapeutic efficacy of a combination of a β-adrenoreceptor (AR) blocker and β2-AR agonist in a rat model of postmyocardial infarction dilated heart failure exceeds that of a β1-AR blocker plus angiotensin-converting enzyme inhibitor. J Pharmacol Exp Ther 2009; 331: 178–85.
Beigi F, Bertucci C, Zhu W, Chakir K, Wainer IW, Xiao RP, et al. Enantioselective separation and online affinity chromatographic characterization of R,R- and S,S-fenoterol. Chirality 2006; 18: 822–7.
72 Ahmet I, Krawczyk M, Zhu W, Woo AO, Morrell C, Poosala S, et al. Cardioprotective and survival benefits of long-term combined therapy with β1AR agonist and β2-AR blocker in dilated cardiomyopathy post-
myocardial infarction. J Pharmacol Exp Ther 2008; 325: 491–9.
73 Ahmet I, Krawczyk M, Zhu W, Woo AO, Morrell C, Poosala S, et al. Cardioprotective and survival benefits of long-term combined therapy with β1AR agonist and β2-AR blocker in dilated cardiomyopathy post-
myocardial infarction. J Pharmacol Exp Ther 2008; 325: 491–9.
74 Ahmet I, Lekatta EG, Talan M. Pharmacological stimulation of β2-adrenergic receptors (β2AR) enhances therapeutic effectiveness of β1AR blockade in rodent dilated ischemic cardiomyopathy. Heart Fail Rev 2005; 10: 289–96.
75 Ahmet I, Morrell C, Lakatta EG, Talan MI. Therapeutic efficacy of a combination of a β-adrenoreceptor (AR) blocker and β2-AR agonist in a rat model of postmyocardial infarction dilated heart failure exceeds that of a β1-AR blocker plus angiotensin-converting enzyme inhibitor. J Pharmacol Exp Ther 2009; 331: 178–85.
Beigi F, Bertucci C, Zhu W, Chakir K, Wainer IW, Xiao RP, et al. Enantioselective separation and online affinity chromatographic characterization of R,R- and S,S-fenoterol. Chirality 2006; 18: 822–7.
79. Jozwiak K, Khalid C, Tanga MJ, Bertzei-Gurske I, Jimenez L, Kozocas JA, et al. Comparative molecular field analysis of the binding of the stereoisomers of fenoterol and fenoterol derivatives to the β2 adrenergic receptor. J Med Chem 2007; 50: 2903–15.
80. Ahmet I, Lakatta EG, Talan MI. β-arrestin-mediated β1-adrenergic receptor transactivation of the EGFR confers cardioprotection. J Clin Invest 2007; 117: 2445–58.
81. Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, et al. β-blockers alprenolol and carvedilol stimulate β-arrestin-mediated EGFR transactivation. Proc Natl Acad Sci U S A 2008; 105: 14555–60.
82. Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, et al. β-arrestin-mediated β2-adrenergic receptor transactivation of the EGFR confers cardioprotection. J Clin Invest 2007; 117: 2445–58.
83. Violin JD, Lefkowitz RJ. β-Arrestin-biased ligands at seven-trans-
membrane receptors. Trends Pharmacol Sci 2007; 28: 416–22.
84. Ahn S, Shenoy SK, Wei H, Lefkowitz RJ. Differential kinetic and spatial patterns of β-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. J Biol Chem 2004; 279: 35518–25.
85. Metra M, Cas LD, Di Lenarda A, Poole-Wilson PA. β-blockers in heart failure: Are pharmacological differences clinically important? Heart Fail Rev 2005; 9: 123–30.
86. Zhou Q, Xiao J, Jiang D, Wang R, Vembaiyan K, Wang A, et al. Cardioprotection of EGFR confers cardioprotection. J Clin Invest 2007; 117: 2445–58.
87. Chen H, Ma N, Xia J, Liu J, Xu Z. β2-adrenergic receptor subtype signaling in rodent dilated ischemic cardiomyopathy. Circulation 2004; 110: 1083–90.
88. Stuenæs JT, Bolling A, Ingvaldsen A, Rommudstad C, Sønder E, Lin FC, et al. β2-Adrenoreceptor stimulation potentiates insulin-stimulated PKB phosphorylation in rat cardiomyocytes via cAMP and PKA. Br J Pharmacol 2010; 160: 1162–74.
89. Morisco C, Condorelli G, Trimalo V, Bells A, Marrone C, Condorelli G, et al. Akt mediates the cross-talk between β-adrenergic and insulin receptors in neonatal cardiomyocytes. Circ Res 2005; 96: 180–8.