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REVIEW

Biased β_2-adrenoceptor signalling in heart failure: pathophysiology and drug discovery

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The body is constantly faced with a dynamic requirement for blood flow. The heart is able to respond to these changing needs by adjusting cardiac output based on cues emitted by circulating catecholamine levels. Cardiac β-adrenoceptors transduce the signal produced by catecholamine stimulation via G_s proteins to their downstream effectors to increase heart contractility. During heart failure, cardiac output is insufficient to meet the needs of the body; catecholamine levels are high and β-adrenoceptors become hyperstimulated. The hyperstimulated β_1-adrenoceptors induce a cardiotoxic effect, which could be counteracted by the cardioprotective effect of β_2-adrenoceptor-mediated G_i signalling. However, β_2-adrenoceptor-G_i signalling negates the stimulatory effect of the G_s signalling on cardiomyocyte contraction and further exacerbates cardiodepression. Here, further to the localization of β_1- and β_2-adrenoceptors and β_2-adrenoceptor-mediated β-arrestin signalling in cardiomyocytes, we discuss features of the dysregulation of β-adrenoceptor subtype signalling in the failing heart, and conclude that G_i-biased β_2-adrenoceptor signalling is a pathogenic pathway in heart failure that plays a crucial role in cardiac remodelling. In contrast, β_2-adrenoceptor-G_s signalling increases cardiomyocyte contractility without causing cardiotoxicity. Finally, we discuss a novel therapeutic approach for heart failure using a G_s-biased β_2-adrenoceptor agonist and a β_1-adrenoceptor antagonist in combination. This combination treatment normalizes the β-adrenoceptor subtype signalling in the failing heart and produces therapeutic effects that outperform traditional heart failure therapies in animal models. The present review illustrates how the concept of biased signalling can be applied to increase our understanding of the pathophysiology of diseases and in the development of novel therapies.

LINKED ARTICLES

This article is part of a themed section on Chinese Innovation in Cardiovascular Drug Discovery. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-23

Abbreviations

ACEI, ACE inhibitors; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; ct, carboxy terminus; EGFR, epidermal growth receptor; Epac, exchange protein directly activated by cAMP; G_i, inhibitory G protein; GRK, GPCR kinase; G_s, stimulatory G protein; HF, heart failure; PKA, cAMP-dependent protein kinase; SNS, sympathetic nervous system

Tables of Links

TARGETS	
GPCRs[a]	**Enzymes**[d]
β_1-adrenoceptor	Adenylyl cyclase (AC)
β_2-adrenoceptor	Akt (PKB)
Angiotensin receptors	CaMKII
Nuclear hormone receptors[b]	Epac
Aldosterone receptor	ERK
Catalytic receptors[c]	GRK2
EGFR	PKA
	PI3K

LIGANDS	
Carvedilol	Fenoterol
Digoxin	Metoprolol

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*, 2013a,b,c,d).

Introduction

Cardiovascular disease is the number one cause of death globally (World Health Organization, 2011). Coronary artery disease is the most prevalent form of cardiovascular disease and is the cause of heart attack (myocardial infarction), an acute illness with very high mortality and morbidity. Given that adult cardiomyocytes cannot re-enter the cell cycle, the death of cardiomyocytes as a result of the blockade of a major coronary artery will permanently weaken cardiac performance. The workload of the remaining cardiomyocytes has to increase to maintain a sufficient cardiac output. A series of compensatory responses are usually triggered, which in many cases lead to structural changes in the heart itself. The process once started will progress to a serious chronic illness called heart failure (HF). Thus, survivors of heart attacks are predisposed to HF, a potentially fatal condition manifested by a progressive decline of cardiac function. HF is also a common converging point of various late-stage cardiovascular conditions, such as cardiomyopathy, valvular heart disease and hypertension. Age is an important risk factor for HF as more than 75% of all cases are composed of people older than 65 (Rich, 1997). Patients diagnosed with HF have a mean survival rate of 50% in 5 years inspite of medical interventions. In 2011, HF was one of the top 10 most expensive conditions seen during inpatient hospitalizations in the United States, with aggregate inpatient hospital costs of more than $10.5 billion (Torio and Andrews, 2013). Medications for the management of HF with left ventricular dysfunction commonly include β-blockers and ACE inhibitors (ACEI) which have been used clinically for more than 25 years. However, a large and growing population of patients respond poorly to this standard treatment (Owan *et al.*, 2006). Therefore, HF is a serious public health problem in many societies with an urgent need for better treatment options.

The pathophysiology of HF commonly involves initiation of hormonal factors that stimulate a wide variety of membrane-bound receptors. Many of these receptors, including angiotensin receptors and β-adrenoceptors, are members of the GPCR superfamily, which play essential roles in the regulation of cardiovascular function. Understanding the signalling mechanisms of these receptor systems is the key to the development of medications beyond β-blockers, angiotensin receptor blockers and ACEI. In particular, a recently described phenomenon named 'functional selectivity' has been highly regarded as a new avenue for drug discovery based on GPCR signal transduction (Kenakin, 2007; Mailman, 2007; Urban *et al.*, 2007; Violin and Lefkowitz, 2007; Woo and Xiao, 2012). However, GPCR signal transduction is dauntingly complex with multiple intracellular signalling cascades operating integrally to produce an orchestrated biological response. Even for a single β_2-adrenoceptor, the prototypical member of the GPCR superfamily purified (Caron and Lefkowitz, 1976) and cloned (Dixon *et al.*, 1986) about 30 years ago, we still have much to learn about its signalling after more than 20 years of research. Here, we review some recent developments in β_2-adrenoceptor signalling with special emphasis on the translational implications of biased β_2-adrenoceptor signalling in the context of HF. In this review, we focus on the biased signalling of the β_2-adrenoceptor with regard to its coupling to different G-protein subtypes. The β_2-adrenoceptor is also known to transduce the G protein-independent β-arrestin-dependent signalling. Reviews discussing the different types of biased signallings of the β-adrenoceptors are available (Christensen *et al.*, 2010; Evans *et al.*, 2010; Woo and Xiao, 2012).

Physiological function of β-adrenoceptors in the heart

The mammalian heart expresses three subtypes of β-adrenoceptors (β_1, β_2 and β_3). In a normal human heart, the β_1- and β_2-adrenoceptors play predominant roles in enhancing excitation-contraction coupling. As shown in Figure 1,

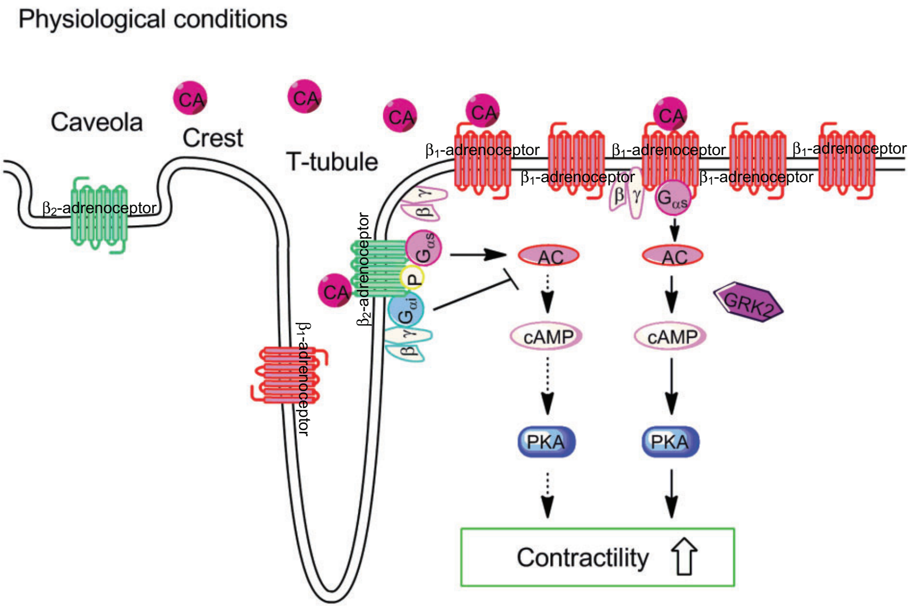

Figure 1

Cardiac β-adrenoceptor signalling in physiological conditions. Under physiological conditions, cardiomyocytes express β_1- and β_2-adrenoceptors at a 4:1 ratio. While β_1-adrenoceptors are distributed across the entire cell surface, β_2-adrenoceptors are localized at the T-tubules and the caveolae. In response to catecholamine (CA) stimulation, the β_1-adrenoceptor couples to the heterotrimeric G protein G_s, leading to its activation and dissociation into the $G_{\alpha s}$ subunit and $G_{\beta\gamma}$ dimers. The activated $G_{\alpha s}$ subunit activates AC which then produces cAMP to activate PKA. PKA phosphorylates effector proteins to increase cardiomyocyte contractility. The β_2-adrenoceptor couples to both G_s and G_i proteins. It also responds to CA stimulation and mediates a mild contractile response via the G_s-AC-cAMP-PKA signalling cascade. The inotropic effect is mild because β_2-adrenoceptor also activates G_i proteins which inhibit the production of cAMP by AC.

the stimulation of β-adrenoceptors by catecholamines such as adrenaline and noradrenaline activates the canonical G_s-AC-cAMP-PKA signalling cascade. In cardiomyocytes, the activated PKA phosphorylates multiple cellular proteins which concertedly increase calcium mobilization across different cellular compartments. It also sensitizes contractile proteins to cytosolic calcium ion levels. In sinoatrial nodal cells, PKA increases the automaticity of the calcium clock. The overall physiological effect of cardiac β-adrenoceptor stimulation is an increase in heart contractility (inotropic effect) and heart rate (chronotropic effect). The relative densities of β_1- and β_2-adrenoceptors are significantly greater in the sinoatrial node than in the atrium. Thus, total β_1- and β-adrenoceptor densities are >3-fold higher in the sinoatrial node than adjacent atrial myocardium, reflecting their specialized roles in regulating cardiac rate and rhythm. The β_1-subtype is predominant in both regions. The β_2-subtype, however, is >2.5-fold more abundant in the sinoatrial node than in the atrial myocardium. The relatively high β_2-adrenoceptor density in the human sinoatrial node is consistent with physiological studies that implicate this receptor in regulating cardiac chronotropism (Rodefeld *et al.*, 1996).

Under the control of the CNS, the sympathetic nervous system (SNS) positively modulates cardiac function by promoting the secretion of noradrenaline from the nerve terminals. The activity of the SNS is enhanced by a 'fight-or-flight' trigger when the body's demand for cardiac output is increased. The SNS-catecholamine-β-adrenoceptor axis is the major mechanism by which the heart is driven to work harder. However, long-term activation of the SNS can lead to structural changes in the heart (cardiac remodelling) and may progress to HF.

Dual coupling to G_s and G_i proteins defines β_2-adrenoceptor as a regulator of cardiac function

A fundamental question is whether the existence of different β-adrenoceptor subtypes in the heart represents functional redundancy; the signalling properties of these receptors will reveal the answer. Physiologically, the inotropic response to catecholamine stimulation is mediated mainly by β_1-adrenoceptors because the β_2-adrenoceptor-G_s-mediated cAMP response is inhibited by the co-activated β_2-adrenoceptor-G_i signalling. We have shown that while the β_1-adrenoceptor couples only to G_s proteins, the β_2-adrenoceptor couples to both G_s and G_i proteins (Xiao *et al.*, 1995; 1999) (Figure 1). Numerous studies in rodent cardiomyocytes have confirmed the existence of a strong coupling of β_2-adrenoceptors to G_i proteins. In the normal human heart β_2-adrenoceptors favour coupling to G_s proteins, although coupling to G_i proteins is also detected (Brown and Harding, 1992; Kilts *et al.*, 2000; Molenaar *et al.*, 2007). However, in pathological situations, such as high circulating catecholamine levels during acute Takotsubo syndrome (Gorelik *et al.*, 2013) or high levels of expression of cardiac G_i

proteins during congestive HF, the effect of β_2-adrenoceptor-G_i signalling becomes much more prominent (Bohm *et al*., 1994; Gong *et al*., 2002). Nevertheless, the functional existence of β_2-adrenoceptor-G_i signalling in the chronically failing human heart is still a matter of debate (Kilts *et al*., 2000; Gong *et al*., 2002; El-Armouche *et al*., 2003; Molenaar *et al*., 2007; Hussain *et al*., 2013).

Another distinction between the signallings of the two β-adrenoceptor subtypes is the involvement of Ca^{2+}/calmodulin-dependent kinase II (CaMKII). If β_1-adrenoceptor stimulation is prolonged, the CaMKII signalling pathway is triggered while the PKA pathway subsides (Wang *et al*., 2004). Multiple studies have implicated a pathological role for CaMKII in HF (for reviews, see Anderson *et al*., 2011 and Swaminathan *et al*., 2012). Increases in cardiac CaMKII activity promote cardiomyocyte apoptosis (Zhu *et al*., 2003), cardiac remodelling (Backs *et al*., 2006; Ling *et al*., 2009) and arrhythmias (Wu *et al*., 2002; van Oort *et al*., 2010) (Figure 2B). In the heart, β_2-adrenoceptors respond to catecholamine stimulation and regulate the effect of β_1-adrenoceptors on excitation-contraction coupling by activating G_i signalling. They also protect the cardiomyocytes from the pro-apoptotic stimuli of excessive β_1-adrenoceptor stimulation (Chesley *et al*., 2000; Zhu *et al*., 2001). The β_2-adrenoceptor-G_i signalling prevents excessive activation of the cAMP pathway on the one hand while activating a prosurvival PI3K-Akt signalling cascade on the other (Chesley *et al*., 2000) (Figure 2B). The concept of dual modulation of cardiomyocyte survival and death by the two β-adrenoceptor subtypes (Zhu *et al*., 2001) has been confirmed in various genetic models including transgenic overexpression of β_1-adrenoceptors (Engelhardt *et al*., 1999; Bisognano *et al*., 2000), knockout of β_2-adrenoceptors (Patterson *et al*., 2004; Bernstein *et al*., 2005), gain-of-function mutation of β_1-adrenoceptors (Mialet Perez *et al*., 2003) and loss-of-function mutation of β_2-adrenoceptors (Liggett *et al*., 1998). It is concluded that the existence of β_2-adrenoceptors in the heart is not merely a functional redundancy. The β_2-adrenoceptor is, in fact, the first-line regulator of cardiac function.

Mechanisms of β_2-adrenoceptor-G_i coupling and β-adrenoceptor desensitization

Regarding the mechanism of β_2-adrenoceptor-G_i coupling, Daaka *et al*. (1997) have suggested that phosphorylation of the β_2-adrenoceptor by PKA causes the switching of the receptor coupling from G_s to G_i. Others (Wang *et al*., 2008; Liu *et al*., 2009) have proposed that GPCR kinase (GRK)-mediated receptor phosphorylation also enhances β_2-adrenoceptor-G_i coupling. However, our recent studies suggest that phosphorylation of the receptor alone is insufficient to trigger β_2-adrenoceptor-G_i coupling (Woo *et al*., 2009; 2014). In these scenarios, stimulation with the G_s-biased β_2-adrenoceptor agonists markedly increased β_2-adrenoceptor phosphorylation at both the PKA and GRK sites without activating the G_i signalling, suggesting that ligand-specific receptor conformation may be a previously unrecognized determinant for the coupling of the β_2-adrenoceptor to different G_s and G_i proteins (for details, see companion article). Further studies are needed to elucidate the mechanism of β_2-adrenoceptor-G_i coupling.

Receptor phosphorylation is essentially involved in the process of GPCR desensitization (uncoupling of the G protein from the cognate receptor). Homologous desensitization is initiated by stimulation of the receptor with high concentrations of its agonist resulting in a change in the receptor conformation to its active state. GRKs can then phosphorylate the threonine and the serine residues at the C-terminus of the activated receptor. Such phosphorylation increases the affinity of the multifunctional adaptor protein β-arrestin for the receptor, resulting in the uncoupling of the α subunit of the heterotrimeric G protein ($G_{\alpha s}$ in the case of the β_2-adrenoceptor) from the receptor. The β-arrestin, by interacting with components of the endocytic machinery such as clathrin and the adaptor protein 2 (AP2) adaptor complex, targets the GPCR for clathrin-mediated endocytosis and internalization (Lefkowitz, 1998) (Figure 2A). Heterologous desensitization is the desensitization of a GPCR induced by the activation of another GPCR, without the need for phosphorylation of the former GPCR by GRKs. As will be discussed below, both homologous and heterologous desensitization of the β_1-adrenoceptor occur in the failing heart and these processes participate in the pathogenesis of HF.

Subcellular localization of β-adrenoceptor subtypes

Early studies on cardiomyocytes suggest that cellular cAMP level does not correlate with the extent of Ca^{2+} mobilization across cellular membranes and phosphorylation of phospholamban (Kuschel *et al*., 1999a,b). In particular, the β_2-adrenoceptor-mediated cAMP signalling is local while the β_1-adrenoceptor-mediated cAMP signalling is global (Kuschel *et al*., 1999a,b). Studies have demonstrated that caveolin 3 plays a crucial role in the localization of β_2-adrenoceptors and the β_2-adrenoceptor-mediated cAMP signalling to the transverse tubules (T-tubules) (Nikolaev *et al*., 2010) and caveolae in adult cardiomyocytes (Rybin *et al*., 2000; Calaghan and White, 2006) (Figure 1). On the other hand, β_1-adrenoceptors appear to be distributed evenly on the caveolin 3-enriched and other plasma membrane fractions in adult cardiomyocytes (Rybin *et al*., 2000) (Figure 1). Restricting the β_2-adrenoceptor-mediated cAMP signalling to cellular subdomains allows the common second messenger, cAMP, to perform selective functions without causing a global effect.

β-Adrenoceptor subtype signalling in the aetiology of HF

A dysregulation of the β-adrenoceptor signalling plays a crucial role in the aetiology and progression of HF. As summarized in Figure 2, the sustained activation of SNS leads to a series of molecular changes in the heart including the down-regulation of β_1-adrenoceptors (Bristow *et al*., 1982; 1993) and the up-regulation of G_i (Feldman *et al*., 1988; Bohm *et al*.,

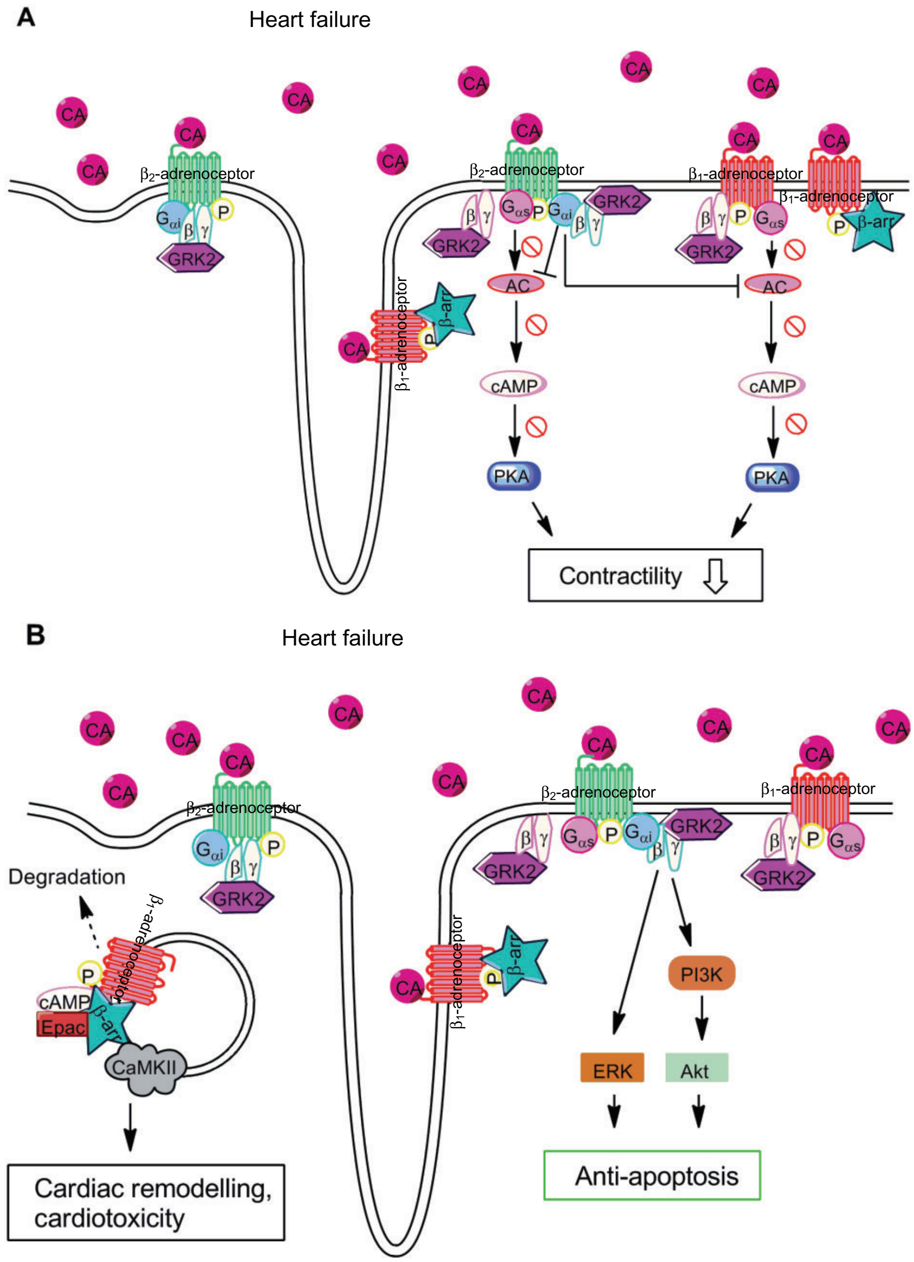

Figure 2

Cardiac β-adrenoceptor signalling during heart failure. During heart failure, the level of circulating catecholamine (CA) increases. The β_1-adrenoceptor is hyperstimulated and down-regulated. The expression level of β_2-adrenoceptors remains unchanged. The β_1- : β_2-adrenoceptor ratio drops to 3:2. The structural derangement in the failing cardiomyocytes causes the β_2-adrenoceptors to translocate from the T-tubules and the caveolae to the crests of the plasma membranes. GPCR kinase 2 (GRK2) and G_i proteins are up-regulated. The hyperstimulation of β-adrenoceptors increases the availability of activated $G_{\beta\gamma}$ dimers for binding with GRK2. The translocation of GRK2 to the plasma membranes is increased. GRK2 phosphorylates β_1-adrenoceptor (P-linked) and subsequently leads to the recruitment of β-arrestins (β-arr) to the receptor. The binding of β-arr causes the β_1-adrenoceptor to uncouple from G_s proteins and terminates β_2-adrenoceptor-G_s signalling. Moreover, GRK2 also phosphorylates β_2-adrenoceptors and leads to G_i-biased signalling. In effect, the enhanced β_2- adrenoceptor-G_i signalling causes the desensitization and uncoupling of G_s proteins to both β_1- and β_2-adrenoceptors. Therefore, the cardiac contractile reserve is markedly reduced (A). The binding of β-arr to β_1-adrenoceptors facilitates receptor internalization and interaction with exchange protein directly activated by cAMP (Epac) and CaMKII. The internalized β_1-adrenoceptor can be sorted to degradation. cAMP activates Epac which in turn induces the activation of CaMKII. The activated CaMKII induces cardiotoxic and cardiac remodelling effects. The β_2-adrenoceptor-G_i signalling is associated with an anti-apoptotic effect through activation of the ERK and the PI3K-Akt signalling cascades (some intermediate effectors are not shown). This anti-apoptotic effect partially counteracts the CaMKII-mediated cardiotoxic effect (B).

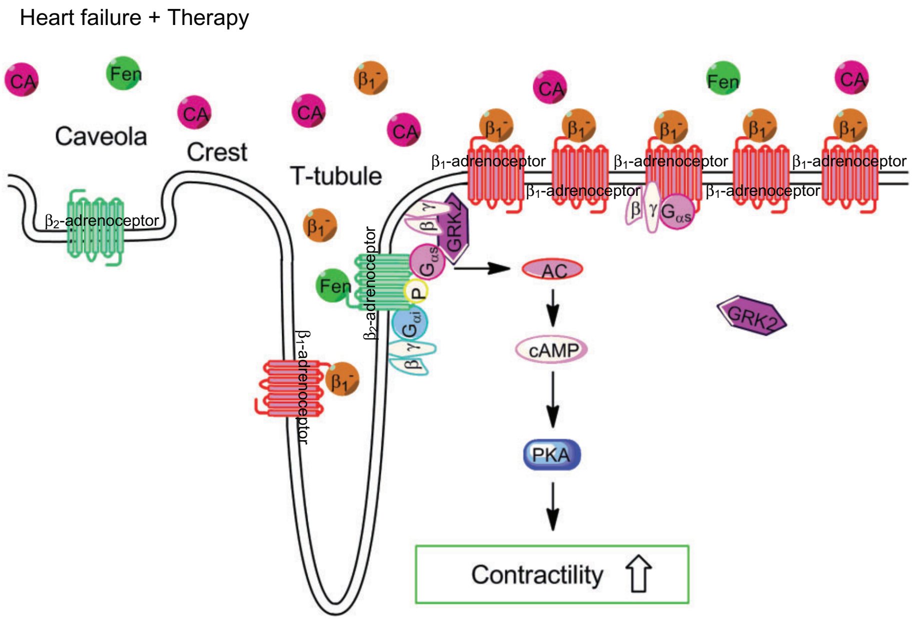

Figure 3

Cardiac β-adrenoceptor signalling in the failing heart treated with a β_1-adrenoceptor antagonist and a G_s-biased β_2-adrenoceptor agonist. Treatment of heart failure with a β_1-adrenoceptor antagonist (β_1^-) and a G_s-biased β_2-adrenoceptor agonist (fenoterol or Fen) reverses the cardiomyopathic changes. β_1^- blocks further catecholamine (CA) stimulation of the β_1-adrenoceptor, resulting in cessation of the cardiotoxic CaMKII signalling. The expression level of β_1-adrenoceptors and the location of β_2-adrenoceptors become normalized. Meanwhile, translocation of GRK2 to the plasma membrane is reduced. Fen stimulates β_2-adrenoceptors to couple to G_s irrespective of the phosphorylation status of the receptor. The activation of the G_s-biased β_2-adrenoceptor signalling provides the needed contractile support to the failing heart without activating CaMKII.

1994) and GRK2 (Ungerer *et al.*, 1993; 1994), the predominant GRK isoform expressed in the heart (Inglese *et al.*, 1993). At the same time, the expression levels of β_2-adrenoceptors (Bristow *et al.*, 1986) and G_s (Eschenhagen *et al.*, 1992) remain unchanged. This can be rationalized as the heart switches to a protected mode of operation by reducing the cardiotoxic β_1-adrenoceptor signalling and increasing the cardioprotective β_2-adrenoceptor signalling. The outcome is a change in the β_1-adrenoceptor : β_2-adrenoceptor ratio from 80:20 in the normal heart to 60:40 in the failing heart (Bristow *et al.*, 1982; 1986; 1989). However, the efficiency of the SNS-catecholamine-β-adrenoceptor axis is decreased under this mode of operation. The signalling efficiency of β_1-adrenoceptors is markedly reduced in the failing heart as a result of their desensitization and down-regulation (Bristow *et al.*, 1982). In addition, as we will explain shortly, the enhanced β_2-adrenoceptor-G_i signalling also contributes to the uncoupling of the G_s proteins to both β_1- and β_2-adrenoceptors (Bristow *et al.*, 1989; Zhu *et al.*, 2005a). If the CNS responds by further increasing the activity of SNS, a vicious cycle will ensue. Conversely, this explains why β-blockers can break this cycle. β-blockers have been used to treat HF for 25 years with success, bringing down total mortality by one-third (McMurray and Pfeffer, 2005). Subtype non-specific β-adrenoceptor blockers used in early years have subsequently been replaced with β_1-adrenoceptor subtype selective blockers with reduced side effects and increased tolerability (Waagstein *et al.*, 1993). The treated hearts improve both structurally and functionally and the β_1-: β_2-adrenoceptor ratio is normalized (Australia/New Zealand Heart Failure Research Collaborative Group, 1997; CIBIS-II Investigators and Committees, 1999; MERIT-HF Study Group, 1999; Packer *et al.*, 2001), as shown in Figure 3.

cAMP imaging in adult cardiac myocytes reveals far-reaching β_1-adrenoceptor but locally confined β_2-adrenoceptor-mediated signalling (Nikolaev *et al.*, 2006). In cardiomyocytes from healthy adult rats and mice, spatially confined β_2-adrenoceptor-induced cAMP signals are thought to concentrate at the deep T-tubules, whereas functional β_1-adrenoceptors are distributed across the entire cell surface (Nikolaev *et al.*, 2010) (Figure 1). However, recent evidence has demonstrated that functional β_2-adrenoceptor-G_s-cAMP signalling occurs almost exclusively on cell surface sarcolemma of rat ventricular myocytes (Cros and Brett, 2013). In cardiomyocytes derived from a rat model of chronic HF, β_2-adrenoceptors were redistributed from the T-tubules to the cell crest (Figure 2), which led to the diffusion of receptor-mediated cAMP signalling (Nikolaev *et al.*, 2010). Thus, the authors proposed that the redistribution of β_2-adrenoceptors in HF changes the compartmentation of cAMP and might contribute to the failing myocardial phenotype.

The dual roles of β_2-adrenoceptor-G_i signalling in cardioprotection and cardiodepression

Activation of the β_2-adrenoceptor-G_i signalling protects the heart from the deleterious effects of excessive

β_1-adrenoceptor-G_s signalling. However, this cardioprotection comes at a price, decreased contractility. Prolonged activation of G_i through a synthetic receptor construct has been shown to lead to a depressed cardiac function and eventually the development of dilated cardiomyopathy (McCloskey *et al.*, 2008). In addition, the β_2-adrenoceptor-G_i 'switch' is a key protective mechanism underlying ischaemia/reperfusion-induced preconditioning (Tong *et al.*, 2005), although it is also implicated in the cardiac stunning associated with ischaemia (Vittone *et al.*, 2006) and in Takotsubo cardiomyopathy (Paur *et al.*, 2012; Shao *et al.*, 2013). Importantly, the enhanced β_2-adrenoceptor-G_i signalling cross-inhibits the β_1-adrenoceptor-mediated cAMP/PKA signalling as well as negating the β_2-adrenoceptor-G_s signalling and contributes to the dysfunction of both β_1- and β_2-adrenoceptors in the failing heart (Sato *et al.*, 2004; Xiao and Balke, 2004; Lokuta *et al.*, 2005; Zhu *et al.*, 2005b) (Figure 2A). Unlike in some previous *in vivo* studies where β_2-adrenoceptors were found to be cardioprotective, a recent study in two HF models has shown that β_2-adrenoceptor signalling can be harmful because of the negative regulation of the Ca^{2+} dynamics by the enhanced β_2-adrenoceptor-G_i signalling (Fajardo *et al.*, 2013). Thus, β_2-adrenoceptor-G_i signalling plays dual roles in cardioprotection and cardiodepression. This is manifested clinically, particularly in Takotsubo syndrome, also called stress-induced cardiomyopathy. During an episode of acute adrenergic challenge, the high levels of circulating catecholamines trigger cardiodepression in a β_2-adrenoceptor-G_i-dependent manner (Paur *et al.*, 2012; Shao *et al.*, 2013). The β_2-adrenoceptor-G_i signalling protects against the detrimental consequences of excessive adrenergic drive. Preventing this signalling converts the syndrome to a sudden death phenotype in rats (Paur *et al.*, 2012; Shao *et al.*, 2013).

G_i-biased β_2-adrenoceptor signalling links pathological GRK2 up-regulation to HF

In advanced HF, the greatly increased expression of G_i and GRK2 causes an exaggerated β_2-adrenoceptor-G_i signalling with important pathological consequences. Cardiac remodelling is the central process in the progression of HF from compensation to decompensation. Nevertheless, the molecular mechanism of cardiac remodelling is unclear. Multiple lines of evidence have implicated a role for GRK2 in HF (Koch *et al.*, 1995; reviewed in Rengo *et al.*, 2011; 2012). Firstly, GRK2 levels are increased in human HF (Ungerer *et al.*, 1993; 1994) and animal models of HF (Choi *et al.*, 1997; Rockman *et al.*, 1998; Anderson *et al.*, 1999). Secondly, GRK2 up-regulation is an early common event in myocardial ischaemia (Ungerer *et al.*, 1996) and hypertension (Gros *et al.*, 1997), which can lead to HF. Thirdly, Raake *et al.* (2008) have demonstrated that GRK2 is a causative factor in cardiac remodelling. Furthermore, GRK2-ct (carboxy terminus of GRK2), a peptide inhibitor of the GRK2-$G_{\beta\gamma}$ interaction, reverses the progression of HF (Koch *et al.*, 1995; Rockman *et al.*, 1998; Tachibana *et al.*, 2005; Raake *et al.*, 2008; Rengo *et al.*, 2011; 2012). These studies demonstrated that the up-regulation of GRK2 is a causative component in maladaptive cardiac remodelling and the progression of HF. Other studies have suggested that $G_{\beta\gamma}$ involves ERK in the transcriptional activation of pathological cardiac hypertrophy (Lorenz *et al.*, 2009). Interestingly, the activation of G_i protein does not lead to the dissociation of the G_i subunit from the $G_{\beta\gamma}$ dimers (Frank *et al.*, 2005) (depicted in Figure 2). Therefore, the activated $G_{\beta\gamma}$ dimers remain membrane-bound (Lorenz *et al.*, 2009). The availability of the activated $G_{\beta\gamma}$ dimers allows GRK2 to translocate to the plasma membrane to interact with the β-adrenoceptors, because GRK2 contains a $G_{\beta\gamma}$-binding domain in its C-terminus (Pitcher *et al.*, 1992). During HF, the availability of activated $G_{\beta\gamma}$ dimers is increased because of higher catecholamine levels (Figure 2A). The concerted increase in GRK2 levels (Ungerer *et al.*, 1993; 1994; Choi *et al.*, 1997) and the increase in GRK2 translocation and activity (Perrino *et al.*, 2005) finally lead to the pathological desensitization of both β-adrenoceptors (Figure 2A). GRK2-ct has been proposed to reverse cardiac remodelling at least in part by inhibiting $G_{\beta\gamma}$ (Völkers *et al.*, 2011).

Recently, we have shown that β_2-adrenoceptor G_i-biased signalling is the link between GRK2 up-regulation and the progression to decompensated HF (Zhu *et al.*, 2012). In this study, transgenic mice expressing β_2-adrenoceptors lacking all their PKA phosphorylation sites [cardiac-specific Tg-β_2-adrenoceptor(PKA-)] exhibited an accelerated HF phenotype under pressure-overload stresses as compared with transgenic mice expressing the wild-type β_2-adrenoceptor or β_2-adrenoceptors lacking all GRK phosphorylation sites. The increases in GRK2 and G_i expression levels were also highest in the hearts of the Tg-β_2-adrenoceptor(PKA-) mice. Cardiomyocytes isolated from these mice and the GRK2 transgenic mice had compromised β-adrenoceptor function typical of a failing heart. Surprisingly, inhibition of G_i by PTX fully restored the β-adrenoceptor-mediated contractile response and suppressed β-adrenoceptor desensitization in both cases. These data suggest that the GRK2-dependent β_2-adrenoceptor-G_i signalling is a harmful pathway leading to the progression to HF.

A novel therapy for HF using the G_s-biased β_2-adrenoceptor agonist

If enhanced β_2-adrenoceptor-G_i signalling contributes to the progression to HF, will it be possible to activate β_2-adrenoceptor-G_s without activating G_i to harvest the beneficial effect of β_2-adrenoceptor-G_s signalling? Using a cardiomyocyte contractility assay, we screened different β_2-adrenoceptor agonists and found that while most β_2-adrenoceptor agonists stimulate the β_2-adrenoceptor to activate both G_s and G_i signalling, fenoterol only activates β_2-adrenoceptor-mediated G_s signalling (Xiao *et al.*, 2003). Fenoterol also produces a full contractile response in myocytes isolated from the failing hearts of spontaneous hypertensive rats (Xiao *et al.*, 2003). These results indicate that fenoterol is a potentially useful treatment for HF. Based on our understanding of the β-adrenoceptor subtype signalling, we have proposed to combine the blocking of β_1-adrenoceptors with the activation of G_s-biased β_2-adrenoceptor signalling in a novel treatment regimen for

HF (Xiao *et al.*, 2003; Zhu *et al.*, 2005a; Woo and Xiao, 2012). Subsequent studies in a rodent model of HF confirmed the effectiveness of this approach (Ahmet *et al.*, 2004; 2005; 2008). In a study comparing the long-term therapeutic effects of combined β_2-adrenoceptor stimulation with fenoterol and β_1-adrenoceptor blockade with metoprolol, we found that either fenoterol alone or metoprolol alone were somewhat effective at ameliorating the cardiomyopathic changes but the combination therapy produced the best treatment outcome (Ahmet *et al.*, 2008). Results from a recent study in a canine model of HF independently corroborated the therapeutic usefulness of selective activation of the G_s-biased β_2-adrenoceptor signalling in HF (Chakir *et al.*, 2011).

At present, there is no clinical evidence as to whether the activation of β_2-adrenoceptor-G_s-cAMP is beneficial in human HF. Preliminary studies have revealed no beneficial effect of a modest cAMP increase produced through β-adrenoceptor stimulation (Ikram and Crozier, 1990) and several reports have also revealed adverse effects associated with high dosages of β_2-adrenoceptor agonists (Pearce *et al.*, 1989; Lindmark and Ottosson, 1998; Martin *et al.*, 1998).

The obvious efficacy of β-blockers in the management of HF has halted any further efforts to explore the potential therapeutic usefulness of β_2-adrenoceptor agonism. However, further studies are needed to determine the clinical efficacy of G_s-biased β_2-adrenoceptor agonism in HF.

Diversity of β_2-adrenoceptor-G_s signalling and β_1-adrenoceptor-G_s signalling in HF

The opposite effects of β_2-adrenoceptor-G_s signalling and β_1-adrenoceptor-G_s signalling in cell survival suggest that although stimulation of both β-adrenoceptors similarly activates G_s proteins, the G_s signalling pathways mediated by the two receptors differ in major ways. Firstly, β_2-adrenoceptor-G_s signalling does not activate the harmful CaMKII; this is because the association of the β-arrestin-CaMKII-Epac1 (or exchange protein directly activated by cAMP 1) with the C-terminus of β1-AR is very specific for the activation of CaMKII to occur (Mangmool *et al.*, 2010) (Figure 2B).

As discussed earlier, cellular cAMP level does not change upon β_2-adrenoceptor stimulation, unlike the effect with β_1-adrenoceptor stimulation, suggesting that the β_2-adrenoceptor-mediated cAMP signalling is compartmentalized in adult cardiomyocytes (Kuschel *et al.*, 1999a,b). HF is manifested by substantial structural changes in ventricular myocytes and this causes the redistribution of the β_2-adrenoceptors from the caveolin 3-enriched T-tubules and caveolae to other non-caveolin 3-containing membrane fractions (He *et al.*, 2001; Louch *et al.*, 2004; Lyon *et al.*, 2009; Nikolaev *et al.*, 2010) (Figure 2). The β_2-adrenoceptor-G_s-cAMP signalling could be converted into a β_1-adrenoceptor-like global signalling in the failing heart (Nikolaev *et al.*, 2010). Stimulation of β_2-adrenoceptors in this situation might increase the incidence of arrhythmias possibly via an Epac2-dependent mechanism (Desantiago *et al.*, 2008; Pereira *et al.*, 2013). A recent study has suggested that overexpression of caveolin 3 in failing myocytes partially restores the disrupted localization of β_2-adrenoceptors and normalizes the compartmentalized β_2-adrenoceptor-G_s-cAMP signalling, implicating the important role of caveolin 3 in cardiac β-adrenoceptor signalling (Wright *et al.*, 2014).

One hypothesis is that β_2-adrenoceptor activation will produce a desirable signalling in the failing heart only if applied before the anatomical structure of the cardiomyocytes goes awry (Gorelik *et al.*, 2013). However, this interpretation does not necessarily exclude any benefits G_s-biased β_2-adrenoceptor agonism may bring to advanced HF, as β-blockers may be used in combination to reverse the structural changes in the failing cardiomyocytes (Chen *et al.*, 2012). A combination of β_1-blockade and G_s-biased β_2-adrenoceptor agonism could, therefore, restore both the structure and normalize the compartmentalized β_2-adrenoceptor-G_s-cAMP signalling in the failing cardiomyocytes (Figure 3), which is a significant improvement as compared with the standard treatment using a β_1-blocker alone. Importantly, our data have shown that failing rat hearts treated with this combination regimen have a reduced incidence of arrhythmias (Ahmet *et al.*, 2008). In another recent study on the rat cardiomyopathy model, the combined (fenoterol + metoprolol) therapy is at least as good as the clinical combination (metoprolol + ACEI) treatment with respect to mortality and exceeds the latter with respect to cardiac remodelling and infarct area expansion (Ahmet *et al.*, 2009).

Biased agonism beyond β-blockers in cardioprotection

In the present review, we have focused on the potential clinical application of G_s-biased β_2-adrenoceptor agonism in HF management. The β-adrenoceptors are also known to transduce the G protein-independent β-arrestin-dependent signalling, also called biased agonism (Violin and Lefkowitz, 2007). In particular, the subtype non-selective β-blocker carvedilol has been shown to activate ERK via β-arrestin-biased signalling at β_2-adrenoceptors (Wisler *et al.*, 2007). Carvedilol has also been found to induce the transactivation of the epidermal growth factor receptor (EGFR) via β-arrestin-biased signalling at β_1-adrenoceptor (Kim *et al.*, 2008). Recent clinical trials have indicated that carvedilol is superior to other β-blockers for treating HF (Poole-Wilson *et al.*, 2003). Recent studies have also shown that β-arrestin-dependent, G protein-independent activation of EGFR via β_1-adrenoceptors confers cardioprotection in mice chronically stimulated with catecholamines (Noma *et al.*, 2007). Therefore, it has been hypothesized that the special therapeutic effect of carvedilol could be attributed to β-arrestin-biased agonism (Wisler *et al.*, 2007; Kim *et al.*, 2008). Whether this signalling plays a role in the cardioprotection associated with carvedilol remains to be determined.

In addition, the possibility of β-blockers as G_i agonists has been advanced (Gong *et al.*, 2002) and the combination of β_1-adrenoceptor blockade plus β_2-adrenoceptor-G_i activation has also been advanced as a protective drug design strategy in the setting of mechanical left ventricular assistance for end-stage HF (Rose *et al*., 2001; Hall *et al*., 2006). In these studies, clenbuterol, a G_i-biased β_2-adrenoceptor agonist (Siedlecka *et al*., 2008), is added on top of β-blockers (and sometimes together with ACEI, angiotensin II blockers, digoxin and aldosterone receptor blockers) at a later stage under a mechanical unloading treatment protocol. Therefore, the overall therapeutic effect is likely to be the result of several different factors.

Existing evidence also indicates that β_2-adrenoceptor-G_i activation is not only beneficial but also life-saving in the acute heart failure associated with Takotsubo syndrome (Paur *et al*., 2012; Shao *et al*., 2013). Although Takotsubo syndrome and congestive HF share some common features such as high circulating catecholamines and reduced cardiac function, a major distinction between these diseases is in the course of disease progression. Takotsubo syndrome is an acute episode of cardiodepression, whereas congestive HF is a chronic deterioration of both the structure and function of the heart.

Thus, non-discriminately targeting a specific signalling as a general strategy in HF management should be discouraged. Therapeutic signal modulation should aim at rectifying the deregulated signalling based on a sound knowledge of the molecular mechanism of the disease. For example, combination therapy with a β_1-adrenoceptor antagonist and a G_s-biased β_2-adrenoceptor agonist may be a treatment option for HF with exaggerated β_2-adrenoceptor-G_i signalling accompanied by a high level of GRK2. Hence, the development of biomarkers to differentiate HF subtypes that could yield most benefits from biased β_2-adrenoceptor agonist treatment is as important as the development of therapeutic agents or the treatment regimen itself.

Concluding remark

The development and gradual gain in acceptance of the concept of functional selectivity in recent years have revolutionized our understanding of GPCR signal transduction and introduced new opportunities in drug discovery. In the heart, the β_2-adrenoceptor mediates an inotropic effect with much less efficiency than the β_1-adrenoceptor (Figure 1). Nevertheless, the fact that the β_2-adrenoceptor couples to G_i in addition to G_s allows it to be a key regulator in cardiac function and a potential drug target in cardiac conditions. The β_2-adrenoceptor not only mediates myocyte contractile responses without increasing the cellular cAMP level, but it also counteracts the pro-apoptotic effect of excessive β_1-adrenoceptor stimulation. However, during heart insufficiency, enhanced expression and activity of GRK2 and G_i proteins promote an exaggerated G_i-biased β_2-adrenoceptor signalling, thus blunting the cardiac reserve function mediated by both β_1- and β_2-adrenoceptors, resulting in maladaptive cardiac remodelling and failure (Figure 2). In addition, G_i-biased β_2-adrenoceptor signalling links the pathological up-regulation of GRK to maladaptive cardiac remodelling and thus defines itself as a pathogenic factor in HF. Conversely, G_s-biased β_2-adrenoceptor agonism is an attractive therapeutic strategy for the treatment of HF. When combined with β_1-adrenoceptor blockade, it may provide contractile support and protection to the failing heart (Figure 3). The therapeutic potential of fenoterol and its derivatives (Jozwiak *et al*., 2007; 2010) in HF warrants further investigation.

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Conflict of interest

The authors declare no conflicts of interests.

References

Ahmet I, Krawczyk M, Heller P, Moon C, Lakatta EG, Talan MI (2004). Beneficial effects of chronic pharmacological manipulation of beta-adrenoreceptor subtype signaling in rodent dilated ischemic cardiomyopathy. Circulation 110: 1083–1090.

Ahmet I, Lakatta EG, Talan MI (2005). Pharmacological stimulation of beta2-adrenergic receptors (beta2AR) enhances therapeutic effectiveness of beta1AR blockade in rodent dilated ischemic cardiomyopathy. Heart Fail Rev 10: 289–296.

Ahmet I, Krawczyk M, Zhu W, Woo AY, Morrell C, Poosala S *et al*. (2008). Cardioprotective and survival benefits of long-term combined therapy with beta2 adrenoreceptor (AR) agonist and beta1 AR blocker in dilated cardiomyopathy postmyocardial infarction. J Pharmacol Exp Ther 325: 491–499.

Ahmet I, Morrell C, Lakatta EG, Talan MI (2009). Therapeutic efficacy of a combination of a beta1-adrenoreceptor (AR) blocker and beta2-AR agonist in a rat model of postmyocardial infarction dilated heart failure exceeds that of a beta1-AR blocker plus angiotensin-converting enzyme inhibitor. J Pharmacol Exp Ther 331: 178–185.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al*. (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al*. (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Nuclear hormone receptors. Br J Pharmacol 170: 1652–1675.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al*. (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. Br J Pharmacol 170: 1676–1705.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al*. (2013d). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. Br J Pharmacol 170: 1797–1867.

Anderson KM, Eckhart AD, Willette RN, Koch WJ (1999). The myocardial beta-adrenergic system in spontaneously hypertensive heart failure (SHHF) rats. Hypertension 33: 402–407.

Anderson ME, Brown JH, Bers DM (2011). CaMKII in myocardial hypertrophy and heart failure. J Mol Cell Cardiol 51: 468–473.

Australia/New Zealand Heart Failure Research Collaborative Group (1997). Randomised, placebo-controlled trial of carvedilol in patients with congestive heart failure due to ischaemic heart disease. Lancet 349: 375–380.

Backs J, Song K, Bezprozvannaya S, Chang S, Olson EN (2006). CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. J Clin Invest 116: 1853–1864.

Bernstein D, Fajardo G, Zhao M, Urashima T, Powers J, Berry G *et al*. (2005). Differential cardioprotective/cardiotoxic effects mediated by beta-adrenergic receptor subtypes. Am J Physiol Heart Circ Physiol 289: H2441–H2449.

Bisognano JD, Weinberger HD, Bohlmeyer TJ, Pende A, Raynolds MV, Sastravaha A *et al*. (2000). Myocardial-directed overexpression of the human beta(1)-adrenergic receptor in transgenic mice. J Mol Cell Cardiol 32: 817–830.

Bohm M, Eschenhagen T, Gierschik P, Larisch K, Lensche H, Mende U *et al*. (1994). Radioimmunochemical quantification of Gi alpha in right and left ventricles from patients with ischaemic and dilated cardiomyopathy and predominant left ventricular failure. J Mol Cell Cardiol 26: 133–149.

Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K *et al*. (1982). Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. N Engl J Med 307: 205–211.

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R *et al*. (1986). Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res 59: 297–309.

Bristow MR, Hershberger RE, Port JD, Minobe W, Rasmussen R (1989). Beta 1- and beta 2-adrenergic receptor-mediated adenylate cyclase stimulation in nonfailing and failing human ventricular myocardium. Mol Pharmacol 35: 295–303.

Bristow MR, Minobe WA, Raynolds MV, Port JD, Rasmussen R, Ray PE *et al*. (1993). Reduced beta 1 receptor messenger RNA abundance in the failing human heart. J Clin Invest 92: 2737–2745.

Brown LA, Harding SE (1992). The effect of pertussis toxin on beta-adrenoceptor responses in isolated cardiac myocytes from noradrenaline-treated guinea-pigs and patients with cardiac failure. Br J Pharmacol 106: 115–122.

Calaghan S, White E (2006). Caveolae modulate excitation-contraction coupling and beta2-adrenergic signalling in adult rat ventricular myocytes. Cardiovasc Res 69: 816–824.

Caron MG, Lefkowitz RJ (1976). Solubilization and characterization of the beta-adrenergic receptor binding sites of frog erythrocytes. J Biol Chem 251: 2374–2384.

Chakir K, Depry C, Dimaano VL, Zhu WZ, Vanderheyden M, Bartunek J *et al*. (2011). Galphas-biased beta2-adrenergic receptor signaling from restoring synchronous contraction in the failing heart. Sci Transl Med 3: 100ra188.

Chen B, Li Y, Jiang S, Xie YP, Guo A, Kutschke W *et al*. (2012). β-Adrenergic receptor antagonists ameliorate myocyte T-tubule remodeling following myocardial infarction. FASEB J 26: 2531–2537.

Chesley A, Lundberg MS, Asai T, Xiao RP, Ohtani S, Lakatta EG *et al*. (2000). The beta(2)-adrenergic receptor delivers an antiapoptotic signal to cardiac myocytes through G(i)-dependent coupling to phosphatidylinositol 3′-kinase. Circ Res 87: 1172–1179.

Choi DJ, Koch WJ, Hunter JJ, Rockman HA (1997). Mechanism of beta-adrenergic receptor desensitization in cardiac hypertrophy is increased beta-adrenergic receptor kinase. J Biol Chem 272: 17223–17229.

Christensen GL, Aplin M, Hansen JL (2010). Therapeutic potential of functional selectivity in the treatment of heart failure. Trends Cardiovasc Med 20: 221–227.

CIBIS-II Investigators and Committees (1999). The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial. Lancet 353: 9–13.

Cros C, Brett F (2013). Functional subcellular distribution of β1- and β2-adrenergic receptors in rat ventricular cardiac myocytes. Physiol Rep 1: e00038.

Daaka Y, Luttrell LM, Lefkowitz RJ (1997). Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. Nature 390: 88–91.

Desantiago J, Ai X, Islam M, Acuna G, Ziolo MT, Bers DM *et al*. (2008). Arrhythmogenic effects of beta2-adrenergic stimulation in the failing heart are attributable to enhanced sarcoplasmic reticulum Ca load. Circ Res 102: 1389–1397.

Dixon RA, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T *et al*. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. Nature 321: 75–79.

El-Armouche A, Zolk O, Rau T, Eschenhagen T (2003). Inhibitory G-proteins and their role in desensitization of the adenylyl cyclase pathway in heart failure. Cardiovasc Res 60: 478–487.

Engelhardt S, Hein L, Wiesmann F, Lohse MJ (1999). Progressive hypertrophy and heart failure in beta1-adrenergic receptor transgenic mice. Proc Natl Acad Sci U S A 96: 7059–7064.

Eschenhagen T, Mende U, Nose M, Schmitz W, Scholz H, Haverich A *et al*. (1992). Increased messenger RNA level of the inhibitory G protein alpha subunit Gi alpha-2 in human end-stage heart failure. Circ Res 70: 688–696.

Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ (2010). Ligand-directed signalling at beta-adrenoceptors. Br J Pharmacol 159: 1022–1038.

Fajardo G, Zhao M, Urashima T, Farahani S, Hu DQ, Reddy S *et al*. (2013). Deletion of the β2-adrenergic receptor prevents the development of cardiomyopathy in mice. J Mol Cell Cardiol 63: 155–164.

Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL *et al*. (1988). Increase of the 40 000-mol wt pertussis toxin substrate (G protein) in the failing human heart. J Clin Invest 82: 189–197.

Frank M, Thumer L, Lohse MJ, Bunemann M (2005). G protein activation without subunit dissociation depends on $G_{\alpha i}$-specific region. J Biol Chem 280: 24584–24590.

Gong H, Sun H, Koch WJ, Rau T, Eschenhagen T, Ravens U *et al*. (2002). The specific beta(2)AR blocker, ICI 118,551, actively decreases contraction through a G(i)-coupled form of the beta(2)AR in myocytes from failing human heart. Circulation 105: 2497–2503.

Gorelik J, Wright PT, Lyon AR, Harding SE (2013). Spatial control of the βAR system in heart failure: the transverse tubule and beyond. Cardiovasc Res 98: 216–224.

Gros R, Benovic JL, Tan CM, Feldman RD (1997). G-protein-coupled receptor kinase activity is increased in hypertension. J Clin Invest 99: 2087–2093.

Hall JL, Birks EJ, Grindle S, Cullen ME, Barton PJ, Rider JE *et al.* (2006). Molecular signature of recovery following combination left ventricular assist device (LVAD) support and pharmacologic therapy. Eur Heart J 28: 613–627.

He JQ, Conklin MW, Foell JD, Wolff MR, Haworth RA, Coronado R *et al.* (2001). Reduction in density of transverse tubules and L-type Ca2+ channels in canine tachycardia-induced heart failure. Cardiovasc Res 49: 298–307.

Hussain RI, Aronsen JM, Afzal F, Sjaastad I, Osnes JB, Skomedal T *et al.* (2013). The functional activity of inhibitory G protein (G(i)) is not increased in failing heart ventricle. J Mol Cell Cardiol 56: 129–138.

Ikram H, Crozier IG (1990). Xamoterol in severe heart failure. Lancet 336: 517–518.

Inglese J, Freedman NJ, Koch WJ, Lefkowitz RJ (1993). Structure and mechanism of the G protein-coupled receptor kinases. J Biol Chem 268: 23735–23738.

Jozwiak K, Khalid C, Tanga MJ, Berzetei-Gurske I, Jimenez L, Kozocas JA *et al.* (2007). Comparative molecular field analysis of the binding of the stereoisomers of fenoterol and fenoterol derivatives to the beta2 adrenergic receptor. J Med Chem 50: 2903–2915.

Jozwiak K, Woo AY, Tanga MJ, Toll L, Jimenez L, Kozocas JA *et al.* (2010). Comparative molecular field analysis of fenoterol derivatives: a platform towards highly selective and effective beta(2)-adrenergic receptor agonists. Bioorg Med Chem 18: 728–736.

Kenakin T (2007). Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. Trends Pharmacol Sci 28: 407–415.

Kilts JD, Gerhardt MA, Richardson MD, Sreeram G, Mackensen GB, Grocott HP *et al.* (2000). Beta(2)-adrenergic and several other G protein-coupled receptors in human atrial membranes activate both G(s) and G(i). Circ Res 87: 705–709.

Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD *et al.* (2008). Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. Proc Natl Acad Sci U S A 105: 14555–14560.

Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA *et al.* (1995). Cardiac function in mice overexpressing the beta-adrenergic receptor kinase or a beta ARK inhibitor. Science 268: 1350–1353.

Kuschel M, Zhou YY, Cheng H, Zhang SJ, Chen Y, Lakatta EG *et al.* (1999a). G(i) protein-mediated functional compartmentalization of cardiac beta(2)-adrenergic signaling. J Biol Chem 274: 22048–22052.

Kuschel M, Zhou YY, Spurgeon HA, Bartel S, Karczewski P, Zhang SJ *et al.* (1999b). beta2-adrenergic cAMP signaling is uncoupled from phosphorylation of cytoplasmic proteins in canine heart. Circulation 99: 2458–2465.

Lefkowitz RJ (1998). G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. J Biol Chem 273: 18677–18680.

Liggett SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC *et al.* (1998). The Ile164 beta2-adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. J Clin Invest 102: 1534–1539.

Lindmark B, Ottosson A (1998). Beta2 agonists and heart failure. Lancet 352: 1709–1710.

Ling H, Zhang T, Pereira L, Means CK, Cheng H, Gu Y *et al.* (2009). Requirement for Ca2+/calmodulin-dependent kinase II in the transition from pressure overload-induced cardiac hypertrophy to heart failure in mice. J Clin Invest 119: 1230–1240.

Liu R, Ramani B, Soto D, De Arcangelis V, Xiang Y (2009). Agonist dose-dependent phosphorylation by protein kinase A and G protein-coupled receptor kinase regulates beta2 adrenoceptor coupling to G(i) proteins in cardiomyocytes. J Biol Chem 284: 32279–32287.

Lokuta AJ, Maertz NA, Meethal SV, Potter KT, Kamp TJ, Valdivia HH *et al.* (2005). Increased nitration of sarcoplasmic reticulum Ca2+-ATPase in human heart failure. Circulation 111: 988–995.

Lorenz K, Schmitt JP, Schmitteckert EM, Lohse MJ (2009). A new type of ERK1/2 autophosphorylation causes cardiac hypertrophy. Nat Med 15: 75–83.

Louch WE, Bito V, Heinzel FR, Macianskiene R, Vanhaecke J, Flameng W *et al.* (2004). Reduced synchrony of Ca2+ release with loss of T-tubules comparison to Ca2+ release in human failing cardiomyocytes. Cardiovasc Res 62: 63–73.

Lyon AR, MacLeod KT, Zhang Y, Garcia E, Kanda GK, Lab MJ *et al.* (2009). Loss of T-tubules and other changes to surface topography in ventricular myocytes from failing human and rat heart. Proc Natl Acad Sci U S A 106: 6854–6859.

Mailman RB (2007). GPCR functional selectivity has therapeutic impact. Trends Pharmacol Sci 28: 390–396.

Mangmool S, Shukla AK, Rockman HA (2010). beta-Arrestin-dependent activation of Ca(2+)/calmodulin kinase II after beta(1)-adrenergic receptor stimulation. J Cell Biol 189: 573–587.

Martin RM, Dunn NR, Freemantle SN, Mann RD (1998). Risk of non-fatal cardiac failure and ischaemic heart disease with long acting beta 2 agonists. Thorax 53: 558–562.

McCloskey DT, Turcato S, Wang GY, Turnbull L, Zhu BQ, Bambino T *et al.* (2008). Expression of a Gi-coupled receptor in the heart causes impaired Ca2+ handling, myofilament injury, and dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 294: H205–H212.

McMurray JJ, Pfeffer MA (2005). Heart failure. Lancet 365: 1877–1889.

MERIT-HF Study Group (1999). Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 353: 2001–2007.

Mialet Perez J, Rathz DA, Petrashevskaya NN, Hahn HS, Wagoner LE, Schwartz A *et al.* (2003). Beta 1-adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. Nat Med 9: 1300–1305.

Molenaar P, Savarimuthu SM, Sarsero D, Chen L, Semmler AB, Carle A *et al.* (2007). (–-)-Adrenaline elicits positive inotropic, lusitropic, and biochemical effects through beta2-adrenoceptors in human atrial myocardium from nonfailing and failing hearts, consistent with Gs coupling but not with Gi coupling. Naunyn Schmiedebergs Arch Pharmacol 375: 11–28.

Nikolaev VO, Bünemann M, Schmitteckert E, Lohse MJ, Engelhardt S (2006). Cyclic AMP imaging in adult cardiac myocytes reveals far-reaching beta1-adrenergic but locally confined beta2-adrenergic receptor-mediated signaling. Circ Res 99: 1084–1091.

Nikolaev VO, Moshkov A, Lyon AR, Miragoli M, Novak P, Paur H *et al.* (2010). Beta2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation. Science 327: 1653–1657.

Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J *et al*. (2007). β-arrestin-mediated β_1-adrenergic receptor transactivation of the EGFR confers cardioprotection. J Clin Invest 117: 2445–2458.

van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL *et al*. (2010). Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 122: 2669–2679.

Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM (2006). Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 355: 251–259.

Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P *et al*. (2001). Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med 344: 1651–1658.

Patterson AJ, Zhu W, Chow A, Agrawal R, Kosek J, Xiao RP *et al*. (2004). Protecting the myocardium: a role for the beta2 adrenergic receptor in the heart. Crit Care Med 32: 1041–1048.

Paur H, Wright PT, Sikkel MB, Tranter MH, Mansfield C, O'Gara P *et al*. (2012). High levels of circulating epinephrine trigger apical cardiodepression in a β2-adrenergic receptor/Gi-dependent manner: a new model of Takotsubo cardiomyopathy. Circulation 126: 697–706.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al*.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–D1106.

Pearce N, Crane J, Burgess C, Beasley R, Jackson R (1989). Fenoterol and asthma mortality. Lancet 1: 1196–1197.

Pereira L, Cheng H, Lao DH, Na L, van Oort RJ, Brown JH *et al*. (2013). Epac2 mediates cardiac beta1-adrenergic-dependent sarcoplasmic reticulum Ca2+ leak and arrhythmia. Circulation 127: 913–922.

Perrino C, Naga Prasad SV, Schroder JN, Hata JA, Milano C, Rockman HA (2005). Restoration of beta-adrenergic receptor signaling and contractile function in heart failure by disruption of the betaARK1/phosphoinositide 3-kinase complex. Circulation 111: 2579–2587.

Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C *et al*. (1992). Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. Science 257: 1264–1267.

Poole-Wilson PA, Swedberg K, Cleland JG, Di Lenarda A, Hanrath P, Komajda M *et al*. (2003). Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. Lancet 362: 7–13.

Raake PW, Vinge LE, Gao E, Boucher M, Rengo G, Chen X *et al*. (2008). G protein-coupled receptor kinase 2 ablation in cardiac myocytes before or after myocardial infarction prevents heart failure. Circ Res 103: 413–422.

Rengo G, Lymperopoulos A, Leosco D, Koch WJ (2011). GRK2 as a novel gene therapy target in heart failure. J Mol Cell Cardiol 50: 785–792.

Rengo G, Perrone-Filardi P, Femminella GD, Liccardo D, Zincarelli C, de Lucia C *et al*. (2012). Targeting the beta-adrenergic receptor system through G-protein-coupled receptor kinase 2: a new paradigm for therapy and prognostic evaluation in heart failure: from bench to bedside. Circ Heart Fail 5: 385–391.

Rich MW (1997). Epidemiology, pathophysiology, and etiology of congestive heart failure in older adults. J Am Geriatr Soc 45: 968–974.

Rockman HA, Chien KR, Choi DJ, Iaccarino G, Hunter JJ, Ross J Jr *et al*. (1998). Expression of a beta-adrenergic receptor kinase 1 inhibitor prevents the development of myocardial failure in gene-targeted mice. Proc Natl Acad Sci U S A 95: 7000–7005.

Rodefeld MD, Beau SL, Schuessler RB, Boineau JP, Saffitz JE (1996). Beta-adrenergic and muscarinic cholinergic receptor densities in the human sinoatrial node: identification of a high beta 2-adrenergic receptor density. J Cardiovasc Electrophysiol 7: 1039–1049.

Rose EA, Gelijns AC, Moskowitz AJ, Heitjan DF, Stevenson LW, Dembitsky W *et al*. (2001). Long-term mechanical left ventricular assistance for end-stage heart failure. N Engl J Med 345: 1435–1443.

Rybin VO, Xu X, Lisanti MP, Steinberg SF (2000). Differential targeting of beta -adrenergic receptor subtypes and adenylyl cyclase to cardiomyocyte caveolae. A mechanism to functionally regulate the cAMP signaling pathway. J Biol Chem 275: 41447–41457.

Sato M, Gong H, Terracciano CM, Ranu H, Harding SE (2004). Loss of beta-adrenoceptor response in myocytes overexpressing the Na+/Ca(2+)-exchanger. J Mol Cell Cardiol 36: 43–48.

Shao Y, Redfors B, Scharin Täng M, Möllmann H, Troidl C, Szardien S *et al*. (2013). Novel rat model reveals important roles of β-adrenoreceptors in stress-induced cardiomyopathy. Int J Cardiol 168: 1943–1950.

Siedlecka U, Arora M, Kolettis T, Soppa GK, Lee J, Stagg MA *et al*. (2008). Effects of clenbuterol on contractility and Ca2+ homeostasis of isolated rat ventricular myocytes. Am J Physiol Heart Circ Physiol 295: H1917–H1926.

Swaminathan PD, Purohit A, Hund TJ, Anderson ME (2012). Calmodulin-dependent protein kinase II: linking heart failure and arrhythmias. Circ Res 110: 1661–1677.

Tachibana H, Naga Prasad SV, Lefkowitz RJ, Koch WJ, Rockman HA (2005). Level of beta-adrenergic receptor kinase 1 inhibition determines degree of cardiac dysfunction after chronic pressure overload-induced heart failure. Circulation 111: 591–597.

Tong H, Bernstein D, Murphy E, Steenbergen C (2005). The role of beta-adrenergic receptor signaling in cardioprotection. FASEB J 19: 983–985.

Torio CM, Andrews RM (2013). National Inpatient Hospital Costs: the Most Expensive Conditions by Payer, 2011. Agency for Healthcare Research and Quality: Rockville, MD. HCUP Statistical Brief #160.

Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ (1993). Altered expression of beta-adrenergic receptor kinase and beta 1-adrenergic receptors in the failing human heart. Circulation 87: 454–463.

Ungerer M, Parruti G, Bohm M, Puzicha M, DeBlasi A, Erdmann E *et al*. (1994). Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. Circ Res 74: 206–213.

Ungerer M, Kessebohm K, Kronsbein K, Lohse MJ, Richardt G (1996). Activation of beta-adrenergic receptor kinase during myocardial ischemia. Circ Res 79: 455–460.

Urban JD, Clarke WP, von Zastrow M, Nichols DE, Kobilka B, Weinstein H *et al*. (2007). Functional selectivity and classical concepts of quantitative pharmacology. J Pharmacol Exp Ther 320: 1–13.

Violin JD, Lefkowitz RJ (2007). Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol Sci 28: 416–422.

Vittone L, Said M, Mattiazzi A (2006). beta 2-Adrenergic stimulation is involved in the contractile dysfunction of the stunned heart. Naunyn Schmiedebergs Arch Pharmacol 373: 60–70.

Völkers M, Weidenhammer C, Herzog N, Qiu G, Spaich K, von Wegner F *et al*. (2011). The inotropic peptide βARKct improves βAR responsiveness in normal and failing cardiomyocytes through G(βγ)-mediated L-type calcium current disinhibition. Circ Res 108: 27–39.

Waagstein F, Bristow MR, Swedberg K, Camerini F, Fowler MB, Silver MA *et al*. (1993). Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group. Lancet 342: 1441–1446.

Wang W, Zhu W, Wang S, Yang D, Crow MT, Xiao RP *et al*. (2004). Sustained beta1-adrenergic stimulation modulates cardiac contractility by Ca2+/calmodulin kinase signaling pathway. Circ Res 95: 798–806.

Wang Y, De Arcangelis V, Gao X, Ramani B, Jung YS, Xiang Y (2008). Norepinephrine- and epinephrine-induced distinct beta2-adrenoceptor signaling is dictated by GRK2 phosphorylation in cardiomyocytes. J Biol Chem 283: 1799–1807.

Wisler JW, DeWire SM, Whalen EJ, Violin JD, Drake MT, Ahn S *et al*. (2007). A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. Proc Natl Acad Sci U S A 104: 16657–16662.

Woo AY, Xiao RP (2012). β-Adrenergic receptor subtype signaling in heart: from bench to bedside. Acta Pharmacol Sin 33: 335–341.

Woo AY, Wang TB, Zeng X, Zhu W, Abernethy DR, Wainer IW *et al*. (2009). Stereochemistry of an agonist determines coupling preference of beta2-adrenoceptor to different G proteins in cardiomyocytes. Mol Pharmacol 75: 158–165.

Woo AY, Jozwiak K, Toll L, Tanga MJ, Kozocas JA, Jimenez L *et al*. (2014). Tyrosine 308 is necessary for ligand-directed Gs-biased signaling of β2-adrenoceptor. J Biol Chem 289: 19351–19363.

World Health Organization (2011). Global Status Report on Noncommunicable Diseases 2010. WHO Press: Geneva, Switzerland.

Wright PT, Nikolaev VO, O'Hara T, Diakonov I, Bhargava A, Tokar S *et al*. (2014). Caveolin-3 regulates compartmentation of cardiomyocyte beta2-adrenergic receptor-mediated cAMP signaling. J Mol Cell Cardiol 67: 38–48.

Wu Y, Temple J, Zhang R, Dzhura I, Zhang W, Trimble R *et al*. (2002). Calmodulin kinase II and arrhythmias in a mouse model of cardiac hypertrophy. Circulation 106: 1288–1293.

Xiao RP, Balke CW (2004). Na+/Ca2+ exchange linking beta2-adrenergic G(i) signaling to heart failure: associated defect of adrenergic contractile support. J Mol Cell Cardiol 36: 7–11.

Xiao RP, Ji X, Lakatta EG (1995). Functional coupling of the beta 2-adrenoceptor to a pertussis toxin-sensitive G protein in cardiac myocytes. Mol Pharmacol 47: 322–329.

Xiao RP, Avdonin P, Zhou YY, Cheng H, Akhter SA, Eschenhagen T *et al*. (1999). Coupling of beta2-adrenoceptor to Gi proteins and its physiological relevance in murine cardiac myocytes. Circ Res 84: 43–52.

Xiao RP, Zhang SJ, Chakir K, Avdonin P, Zhu W, Bond RA *et al*. (2003). Enhanced G(i) signaling selectively negates beta2-adrenergic receptor (AR) – but not beta1-AR-mediated positive inotropic effect in myocytes from failing rat hearts. Circulation 108: 1633–1639.

Zhu W, Zeng X, Zheng M, Xiao RP (2005a). The enigma of beta2-adrenergic receptor Gi signaling in the heart: the good, the bad, and the ugly. Circ Res 97: 507–509.

Zhu W, Petrashevskaya N, Ren S, Zhao A, Chakir K, Gao E *et al*. (2012). Gi-biased beta2AR signaling links GRK2 upregulation to heart failure. Circ Res 110: 265–274.

Zhu WZ, Zheng M, Koch WJ, Lefkowitz RJ, Kobilka BK, Xiao RP (2001). Dual modulation of cell survival and cell death by beta(2)-adrenergic signaling in adult mouse cardiac myocytes. Proc Natl Acad Sci U S A 98: 1607–1612.

Zhu WZ, Wang SQ, Chakir K, Yang D, Zhang T, Brown JH *et al*. (2003). Linkage of beta1-adrenergic stimulation to apoptotic heart cell death through protein kinase A-independent activation of Ca2+/calmodulin kinase II. J Clin Invest 111: 617–625.

Zhu WZ, Chakir K, Zhang S, Yang D, Lavoie C, Bouvier M *et al*. (2005b). Heterodimerization of beta1- and beta2-adrenergic receptor subtypes optimizes beta-adrenergic modulation of cardiac contractility. Circ Res 97: 244–251.