

Review

Cardiac GPCRs: GPCR signaling in healthy and failing hearts

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Abstract

G protein-coupled receptors (GPCRs) are widely implicated in human heart disease, making them an important target for cardiac drug therapy. The most commonly studied and clinically targeted cardiac GPCRs include the adrenergic, angiotensin, endothelin, and adenosine receptors. Treatment options focusing on the complex and integrated signaling pathways of these GPCRs are critical for the understanding and amelioration of heart disease. The focus of this review is to highlight the most commonly studied and clinically targeted cardiac GPCRs, placing emphasis on their common signaling components implicated in cardiac disease.

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1. GPCR signaling overview

G-protein-coupled receptors (GPCRs) are a conserved family of seven transmembrane receptors that is one of the largest classes of receptors to be targeted for drug therapy [1–2]. Among an estimated 200 cardiac GPCRs, drugs targeting adrenergic and angiotensin GPCR signaling pathways alone account for the majority of prescriptions for cardiovascular diseases [3–4]. However, heart failure remains a leading cause of global morbidity and mortality [4], and a better understanding of the components that make up GPCR signaling pathways in healthy and failing hearts may provide a mechanistic basis for improving heart failure treatment. GPCR signaling is activated by ligand binding to an extracellular active site of the receptor. Depending on the ligand and type of GPCR, the active site is located on either the N-terminal tail, extracellular loops, or exofacial transmembrane helices [5,6]. Ligand binding induces a conformational change in the GPCR, which disrupts the ionic interactions between the third cytoplasmic loop and the sixth transmembrane segment and allows for coupling with heterotrimeric guanine-nucleotide regulatory proteins (G-proteins) [6–11].

G-proteins consist of α , β , and γ subunits and, upon GPCR coupling, the G-protein converts GTP to GDP on its α subunit resulting in dissociation of the $G\alpha$ from the $G\beta\gamma$ subunits to mediate downstream signaling [12,13]. The four primary families of $G\alpha$ -proteins ($G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{11/12}$) [12] diverge at this point with respect to downstream signaling molecules and consequential physiological responses. Dissociated $G\alpha$ subunits couple with an effector, an enzyme such as adenylyl cyclase (AC) and phospholipase C β (PLC β), or an ion channel [12]. Dissociated $G\beta\gamma$ subunits target a range of signaling pathways involved in desensitization, downregulation, apoptosis, and ion channel activation ($I_{K_{ACh}}$) [12,14,15] (see Fig. 1).

This review will first discuss each G-protein family in terms of its signaling pathway in the healthy heart and follow with a description of individual signaling components and receptors in heart disease. Among the most commonly studied receptors are $G\alpha_s$ and $G\alpha_i$ -coupled β -adrenergic receptors; $G\alpha_q$ -coupled angiotensin, α -1-adrenergic, and endothelin receptors; $G\alpha_i$ -coupled adenosine-1, α 2-adrenergic, and muscarinic-2 receptors; and common to all of these receptors is the corresponding $G\beta\gamma$ signaling, G-protein independent signaling, and the consequences of receptor downregulation and desensitization in the heart.

2. G_s signaling and G_s -coupled receptors

2.1. Overview of G_s signaling

Following ligand stimulation, the dissociated $G\alpha_s$ subunit activates signaling with the stimulation of AC (giving G_s or ‘ $G_{stimulatory}$ ’ its name). AC exists in the human myocardium primarily as AC Types V and VI, though types IV and VII are also present at lower levels. AC V is primarily localized to the atria of the heart while AC VI is found in both atria and ventricles and is colocalized with β 1 adrenergic receptors (β 1ARs) [16]. AC is primarily responsible for increasing intracellular production of the second messenger 3'-5'-cyclic adenosine monophosphate, (cAMP) [12,17]. cAMP modulates cardiac contractility via its binding to cAMP-dependent protein kinase (PKA). The catalytic subunit of PKA phosphorylates a range of substrates within the myocyte. In the heart, PKA functions to modulate contractility via its phosphorylation of myocyte proteins including the voltage-gated L-type Ca^{2+} channel, the cardiac ryanodine receptor (RyR2), phospholamban, and troponin I.

L-type Ca^{2+} channels activate RyR to mediate Ca^{2+} -induced Ca^{2+} release (CICR); the primary source of intracellular Ca^{2+} to activate myofilament contraction [18]. Activated L-type Ca^{2+} voltage-dependent channels initiate the inward calcium entry required for CICR [19]. L-type Ca^{2+} channels are located primarily in transverse T-tubules, and in response to an action potential generate a small release of calcium into a cytosolic space known as the diadic cleft that occurs between the opposition of T-tubules with the junctional sarcoplasmic reticulum. This trigger Ca^{2+} activates the large release of Ca^{2+} by the RyR2 to generate what is known as the Ca^{2+} spark [20].

RyR2 is a macromolecular signaling complex located on the junctional sarcoplasmic reticulum (SR). It responds to trigger Ca^{2+} from the L-type Ca^{2+} channel by releasing larger Ca^{2+} stores into the cytoplasm from within the SR, which then activates myofilament contraction. RyR2 can be modulated through phosphorylation by PKA, cGMP-dependent protein kinase (PKG), and Ca^{2+} /calmodulin kinase II (CaMKII) in addition to poly-S-nitrosylation [20–22].

Myocyte relaxation occurs through Ca^{2+} reuptake in the SR via the SR Ca^{2+} -ATPase (SERCA) pump, which is modulated by phospholamban [23]. Phospholamban modulates Ca^{2+} affinity to SERCA2 which regulates the sequestration of cytosolic Ca^{2+} back into the SR [23]. Phospholamban phosphorylation is directly associated with increases in Ca^{2+} affinity for SERCA2

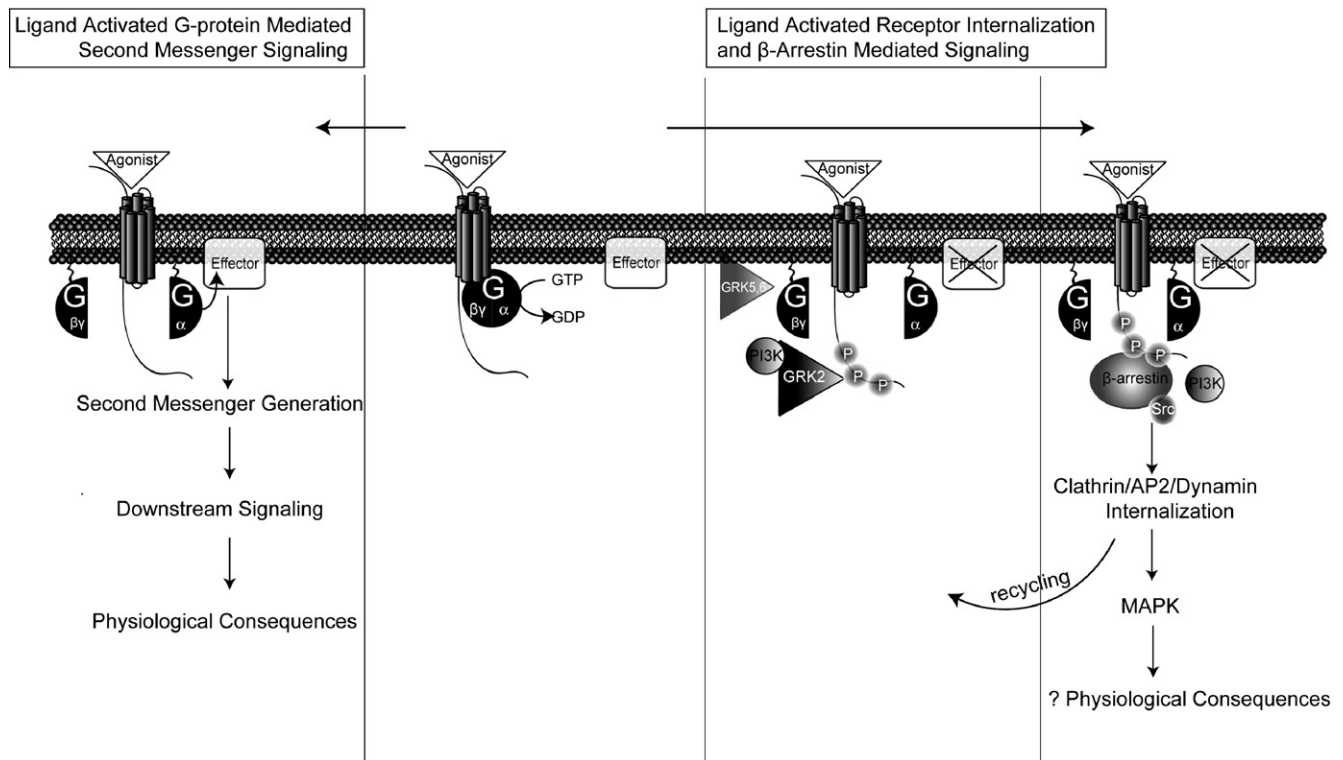


Fig. 1. Overview of GPCR Signaling. Agonist binding to receptor results in the coupling to a G-protein and the conversion of GTP to GDP to cause the dissociation of $G\alpha$ and $G\beta\gamma$ subunits. $G\alpha$ signaling is initiated with the activation of a membrane-bound effector molecule, such as adenylyl cyclase (AC) or phospholipase C β (PLC β) to produce second messengers such as cyclic adenosine 3',5' monophosphate, (cAMP), diacylglycerol (DAG), or inositol 1, 4, 5-triphosphate (IP $_3$), which activate a variety of downstream signals with a range of physiological consequences in the heart such as the regulation of contractility, hypertrophy, and apoptosis. A simultaneous process involves the ligand-activated translocation of G protein-coupled receptor kinases (GRKs) and bound phosphoinositide-3-kinase (PI3K), to the membrane by dissociated $G\beta\gamma$ subunits leading to receptor phosphorylation on the C-terminal tail. Following GRK mediated receptor phosphorylation, β -arrestin is bound and acts to (1) desensitize G-protein signaling by uncoupling the receptor from G-protein, (2) scaffold for the recruitment of proteins involved in receptor trafficking, such as AP2, clathrin, and dynamin, and (3) form a signaling complex that initiates G protein-independent downstream signaling such as the activation of mitogen-activated protein (MAP) kinases.

and enhanced rates of cardiac relaxation in intact beating hearts [24]. Phospholamban is also phosphorylated by CaMKII and can regulate excitation–contraction coupling in the heart [23]. Troponin I phosphorylation by PKA reduces myofilament sensitivity to Ca^{2+} , hence inhibiting contractile signaling and accelerating cardiac relaxation [25] (see Fig. 2).

2.2. G_s -coupled receptors in the heart

2.2.1. Adrenergic receptors

Adrenergic receptors are an important class of GPCRs responsible for translating chemical messages from the sympathetic nervous system into cardiovascular responses. Three β -adrenergic receptor (β AR) subtypes are found in mammalian hearts. β 1ARs account for approximately 80% of the β ARs in the healthy heart and couple primarily to G_s -proteins to regulate heart rate and contractility [12,26]. β 2ARs couple to G_s -proteins, but can also switch to G_i -coupling after receptor phosphorylation by PKA [27]. β 2ARs are also important for mediating smooth muscle relaxation and glycogenesis [17]. β 3ARs couple to both G_s - and G_i -proteins and are the least abundant subtype in the heart. While β 3ARs play a role in lipolysis and thermogenesis in adipose tissue, they have been shown to have both a positive inotropic effect in transgenic mice

overexpressing human β 3ARs [28] and a negative inotropic effect in the human heart [29].

2.2.2. β 1 adrenergic receptors

β 1ARs regulate the inotropic and chronotropic functions of the healthy heart primarily mediated by the effects of PKA. β 1AR knockout mice have high embryonic lethality, and those that do survive exhibit no inotropic or chronotropic response to exercise or agonist stimulation despite having a normal basal heart rate and blood pressure [30]. In contrast, overexpression of human β 1ARs in transgenic mice initially augments cardiac function but eventually leads to deleterious effects on the heart presumably due to chronic β 1AR signaling [31,32].

2.2.3. β 2 adrenergic receptors

β 2ARs were originally thought to signal exclusively through G_s . Like the β 1AR, G_s -signaling leads to regulation of contractility via Ca^{2+} cycling as described above. The β 2ARs differ importantly from the β 1ARs in that they can also couple to the $G_{inhibitory}$ or G_i -protein [27]. While both β AR subtypes stimulate AC through G_s , stimulation via the β 1AR activates L-type Ca^{2+} channels throughout the myocyte while β 2AR only activates L-type Ca^{2+} channels in close proximity to the receptor [33]. However, when myocytes are treated with

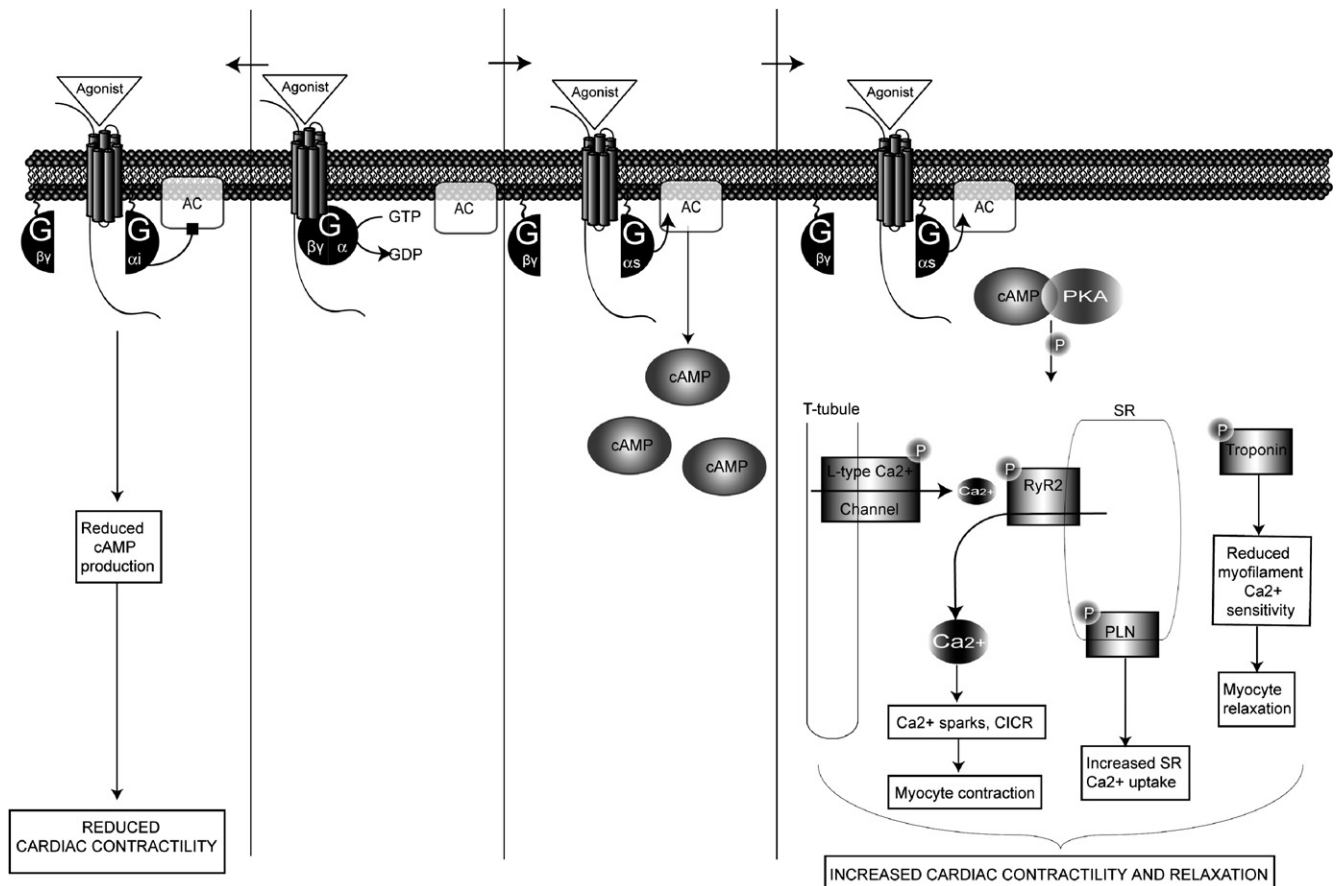


Fig. 2. G_s/G_i -coupled receptor signaling. G_s - and G_i -coupled receptors target the second messenger adenylyl cyclase (AC). While G_s stimulates AC, activation of G_i inhibits AC, reducing the accumulation of cAMP and consequently leading to reduced PKA and agonist-stimulated cardiac contractility. Downstream effectors of $G\alpha_s$ activation result from the increase in PKA whose phosphorylation substrates include: phospholamban (PLN), troponin, L-type Ca^{2+} channels, and the cardiac ryanodine receptor (RyR2).

pertussis toxin to selectively inhibit G_i signaling, activation of L-type Ca^{2+} channels by β_2 ARs becomes more widespread and induces relaxation effects similar to β_1 AR stimulation [33]. This contrast between local and diffuse mediation of contractility at the cellular level suggests that there is a competition between β_2 AR mediated G_s and G_i signaling. This relationship is illustrated by the timecourse of β_2 AR-induced contractility in neonatal cardiomyocytes: agonist binding first induces a small increase followed by a long decrease in myocyte contraction rate, representing a short G_s -coupling followed by a prolonged G_i -coupling [17,34]. Furthermore, while β_2 AR-overexpressing mice do not show an enhanced contractile response to β AR-agonists, treatment with pertussis toxin restores β AR contractile responsiveness [35]. These results suggest that β_2 AR stimulation initially activates G_s signaling pathways in a pattern analogous to β_1 AR activation. This stimulatory pathway is quickly overshadowed by the delayed activation of the G_i pathway, which negatively regulates contractility in response to prolonged β AR activation.

Transgenic mouse studies illustrate the physiological impact of these downstream β_2 AR signaling components in the heart. *In vivo*, modest cardiac-specific β_2 AR overexpression of 60-fold over basal results in enhanced contractile function without development of heart failure [36,37]. However, mice with

β_2 AR overexpression at 350 times greater than basal rapidly develop hypertrophy and heart failure [36]. Physiologically, these mice show a loss in their response to agonist, a reduction in L-type Ca^{2+} density, and a decreased systolic function [36]. In contrast, β_2 AR-deficient mice display normal development and resting cardiovascular function, and show increased exercise capacity compared to wild type mice [30]. However, they develop hypertension in response to either adrenergic system stimulation or exercise [30]. These data support the concept that β_2 ARs are important regulators of prolonged activation of the adrenergic system via stress or exercise, possibly due, in part, to their coupling to G_i .

2.3. G_s -coupled receptors in heart disease

2.3.1. G_s signaling in heart disease

G_s -coupled receptors signal via AC primarily to regulate contractility. Chronic activation of G_s signaling pathways leads to cardiac hypertrophy, fibrosis, and heart failure. In fact, transgenic mice with cardiac-specific overexpression of $G\alpha_s$ display increased heart rate and contractility in response to catecholamine stimulation, but eventually develop histological evidence of myocardial damage such as cellular hypertrophy, fibrosis, and necrosis [38,39].

Recent data suggest that human heart failure may be mediated, in part, by defective regulation of RyR2, the SR channel responsible for gating Ca^{2+} efflux into the cytosol. Several human RyR2 mutations, which result in altered myocardial Ca^{2+} handling, have been linked to ventricular arrhythmias and sudden cardiac death [40–44]. Moreover, heart failure is associated with RyR2 hyperphosphorylation by PKA, leading to poor Ca^{2+} gating and uncoupling from neighboring RyR2 channels [45]. Such hyperphosphorylation causes a reduced ability to trigger Ca^{2+} release from the SR and a consequent loss of excitation–contraction coupling gain [45], which is a characteristic of hypertrophic and failing myocytes [46]. β -blockers such as metoprolol [47,48], carvedilol [47], atenolol [47], and propranolol [49] suppress the hyperphosphorylation of RyR2 and restore channel function [50]. Recent data suggest that selective inhibition of RyR2 hyperphosphorylation stabilizes the RyR2, prevents SR Ca^{2+} leak and prevents development of heart failure in experimental models [51].

Phospholamban appears to decline during heart failure, possibly contributing to decreased myocyte contractility in the failing human heart [23]. Interestingly, transgenic mice lacking in phospholamban show an increase in the total level of Ca^{2+} in the SR, an increase in the number of spontaneous Ca^{2+} sparks, and a more rapid inactivation of L-type Ca^{2+} currents [52]. Mutations of phospholamban in humans result in a disturbance in Ca^{2+} reuptake to the SR, which is implicated in the development of dilated cardiomyopathy and heart failure [53]. Humans that lack phospholamban in their hearts also develop heart failure and early mortality [54,55]. Transgenic overexpression of phospholamban *in vivo* is associated with altered systolic Ca^{2+} parameters and depressed ventricular systolic function [56,57]. However, gene-targeted ablation of phospholamban enhances cardiac contractility [58], and can rescue experimental models of heart failure [59]. Thus altering phospholamban levels in heart failure may provide a way to relieve the impairment of cardiac relaxation by enhancing Ca^{2+} reuptake to the SR [23].

2.3.2. β ARs in heart disease

During heart failure, heightened catecholamine stimulation of the adrenergic receptor system induces selective downregulation of β 1ARs. Consequently, the β 1AR to β 2AR ratio changes from an 80:20 distribution in the healthy heart to a ratio of 60:40 in the failing heart [60,61]. Chronic catecholamine stimulation also promotes cardiac hypertrophy and myocyte apoptosis while leading to a reduction in contractility [17].

Diminished contractile responsiveness to β AR stimulation and an increase in G_i signaling is a hallmark of heart failure [32]. In failing human ventricles, β 2ARs are uncoupled from AC and show a marked loss of their ability to produce cAMP, which accounts for the reduced efficiency in producing a contractile response [61]. Thus, although β 2ARs do not downregulate, they are considerably desensitized in the failing heart.

β AR signaling is a central component of heart failure, and its correlation with myocyte apoptosis has recently been clarified.

Apoptotic myocytes are more prevalent in failing human hearts, showing 200-fold increases over basal compared to healthy human hearts [62]. Within individual failing hearts, there are more apoptotic myocytes in peri-infarcted regions than in cardiac regions distal to myocardial infarction [62]. Chronic β AR agonism via norepinephrine or isoproterenol induces apoptosis in cultured cardiomyocytes [63] and *in vivo* through chronic isoproterenol administration [64].

A recently recognized detrimental pathway is the β 1AR mediated pro-apoptotic signaling of CaMKII. Both direct activation or cardiac overexpression of CaMKII induces myocyte apoptosis *in vitro* that can be blocked by CaMKII inhibition [33]. CaMKII levels are significantly elevated and show increased activation immediately following the induction of heart failure, and cardiac CaMKII overexpression induces dilated cardiomyopathy [65,66]. Importantly, direct inhibition of CaMKII *in vivo* reduces adverse LV remodeling after β 1AR agonism [67], and significantly diminishes the development of cardiac apoptosis in heart failure models [68]. Furthermore, β 1AR-mediated development of apoptosis can be prevented with transgenic overexpression of a CaMKII inhibitor [68]. When these mice are crossed with phospholamban deficient mice to normalize Ca^{2+} stores in the heart and allow activation of CaMKII, the resistance to apoptosis is abolished [68]. These data support the current search for selective CaMKII inhibitors to directly test whether CaMKII inhibition can alter the development of heart failure.

2.4. Other G_s -protein-coupled receptors in the heart

Other G_s -coupled receptors in the heart include adenosine [69], glucagon [70], prostanoid [71], and histamine [72] receptors [3].

Adenosine receptors are thought to be cardioprotective in myocardial ischemia by inducing coronary vasodilation, which increases oxygen supply by inhibiting oxygen radical-mediated cellular damage [73]. Overexpression of adenosine receptors 1 or 3 offers an overall increase in the response to adenosine agonism and consequently reduces the level of injury caused by myocardial ischemia [69,74]. Moreover, direct stimulation of adenosine receptors via adenosine administration during reperfusion has been shown to improve left ventricular (LV) function and reduce infarct size [75,76] implicating adenosine agonism as a potential therapy during myocardial ischemia.

Prostanoid receptors respond to prostaglandins in neonatal myocytes to play a role in cardiac cell growth [71]. Prostanoids regulate blood pressure by mediating vascular tone and renal sodium handling [77]. Reduction of prostacyclin *in vivo* results in an increase in blood pressure and consequent development of hypertension while reduction of the prostanoid receptor leads to the development of hypertension accompanied by robust cardiac hypertrophy and fibrosis [77].

Histamine is abundant in cardiac mast cells and, in the healthy heart, histamine infusion produces an increase in heart rate and atrial activity [78]. While histamine-2-receptors are coupled to G_s , histamine-1 receptors couple to G_q and histamine-3-

receptors couple to $G_{i/o}$. Histamine-2-receptor knockout mice show a decrease in heart rate in response to histamine stimulation while histamine-3-receptor knockout mice show a decrease in blood pressure and reduced norepinephrine release during ischemia [79]. In addition, blockade of histamine receptors by the antihistamines used in allergy treatments have been shown to produce cardiotoxicity and atypical forms of arrhythmia such as torsade de pointes [80].

3. G_q signaling and G_q -coupled receptors

3.1. Overview of G_q signaling

Receptors coupled to G_{α_q} play important roles in mediating cardiac growth responses, myocyte apoptosis, and cardiac hypertrophy. Ligand-stimulated receptor signaling via G_q is initiated by the membrane-recruitment and activation of PLC β . PLC β is responsible for hydrolyzation of phosphatidylinositol 4, 5 biphosphate (PIP $_2$) into two second messengers: diacylglycerol (DAG) and inositol 1, 4, 5-triphosphate (IP $_3$) [81].

DAG activates several serine-threonine isoforms of protein kinase C (PKC) [82,83]. PKC signaling as a whole is important for myocyte apoptosis, necrosis, and for triggering pro-hypertrophic gene transcription in the heart [84–86]. Recently, it has been shown that protein kinase D, a downstream effector of PKC, directly phosphorylates HDAC5 and stimulates its nuclear export, leading to activation of hypertrophic genes [87,88].

There are three primary families of PKC: “conventional”, “novel”, and “atypical” [89]. “Conventional” PKCs are both Ca^{2+} and DAG dependent, “novel” PKCs are DAG activated but Ca^{2+} independent, and “atypical” PKCs are both DAG and Ca^{2+} independent. The four most functionally significant PKC isoforms in the heart are of the conventional (PKC α , β) and novel (PKC δ , ϵ) groups. PKC α is the most prevalent isoform in the human heart [90], and is primarily a regulator of contractility via modulation of phospholamban and SERCA phosphorylation [91]. PKC β is important for the transduction of cardiac hypertrophy, contractility, and regulation of neonatal cardiac growth [92,93]. PKC δ is a critical mediator of postischemic necrosis and contractile dysfunction [94,95]. The role of PKC ϵ in hypertrophy is disputed, but it is generally accepted to be a regulator of ischemia–reperfusion injury [94–96]. Upon activation, PKC ϵ is translocated from the cytosol to the SR where it directly activates K_{ATP} channels to decrease ROS generation and calcium overload. These PKC ϵ -mediate signaling events are critical for the cardioprotective effects of ischemic preconditioning [94–96].

The other second messenger of PLC β , IP $_3$, binds several receptors on the SR to induce the release of calcium stores into the cytoplasm [21,40,84–86,97]. Unlike the short bursts of contraction and relaxation induced by CICR, IP $_3$ -mediated Ca^{2+} signaling results in a sustained Ca^{2+} release that allows for the activation of hypertrophic response genes [83]. This sustained release of Ca^{2+} results in calcineurin activation and consequent targeting of the nuclear factor of activated T-cells (NFAT) transcription factors [83]. CaMKII activation and

subsequent NFAT3 signaling act in concert to promote pathologic hypertrophic signaling and cardiac growth [26,83] (see Fig. 3).

3.2. G_q -coupled receptors in the heart

3.2.1. Angiotensin receptors

Angiotensin receptor blockers and angiotensin-converting enzyme (ACE) inhibitors have been shown to reverse cardiac remodeling, reduce cardiac hypertrophy, slow the progression of heart failure, and improve survival in heart failure patients [98–102]. The main effector of this pathway is angiotensin II (Ang II), a peptide that stimulates vascular smooth muscle contraction, aldosterone release, and production of extracellular matrix proteins [103]. Ang II and angiotensin-converting enzyme (ACE) are both present at high levels after myocardial infarction and during cardiac hypertrophy [104–106].

Ang II signals primarily via the G_q -coupled AT $_1$ R [107]. Experimental studies have reported upregulation of AT $_1$ R in both hypertrophic and ischemic states [108–110] and down-regulation with end-stage human heart failure [111,112]. Part of the mechanism for AT $_1$ R downregulation may be attributed to the prolonged elevation of AngII levels with cardiac hypertrophy and during heart failure [113,114]. Overexpression of AT $_1$ Rs in the heart results in hypertrophy and fibrosis in otherwise healthy mice. These mice also show enhanced hypertrophic responses to pressure overload and die prematurely in response to heart failure [115]. In contrast, mice deficient in AT $_1$ Rs have an initially attenuated hypertrophic response to Ang II, and show diminished left ventricular dilatation and fibrosis after myocardial infarction with improved LV systolic function over time [116]. However, AT $_1$ R-knockouts still develop hypertrophy in response to long-term pressure overload [117,118]. This suggests the possibility that other angiotensin receptor subtypes such as the AT $_2$ R or the angiotensin type II receptor may also play a role in the hypertrophic response to pathological stimuli.

3.2.2. α -1-Adrenergic receptors

α_{1A} , α_{1B} , α_{1D} -ARs activate G_{α_q} signaling in response to sympathetic nervous system stimulation [119]. Signaling via the PLC β /DAG/PKC pathway mobilizes Ca^{2+} entry into intracellular compartments, inducing cardiac growth [119]. α_{1A} and α_{1B} ARs crosstalk with β 1ARs to regulate cardiac inotropy and, although the mechanism is not clearly understood, will diminish the β 1AR response to isoproterenol [120]. The abundance of α ARs increases during heart failure [121], with varying consequences from each α AR subtype. Overexpression of α_{1A} AR results in increased cardiac contractility without hypertrophy [94]. Cardiac-specific overexpression of α_{1B} AR induces a blunted response to β AR stimulation mediated in part by enhanced G_i coupling [122], while α_{1B} AR deficiency results in a worsened dilated cardiomyopathy only after pressure overload [123]. Deficiency in α_{1B} AR additionally blocks increased blood pressure responses to the α_{1A} agonist phenylephrine [124]. Although animals deficient in individual α_{1A} AR subtypes do not show significant

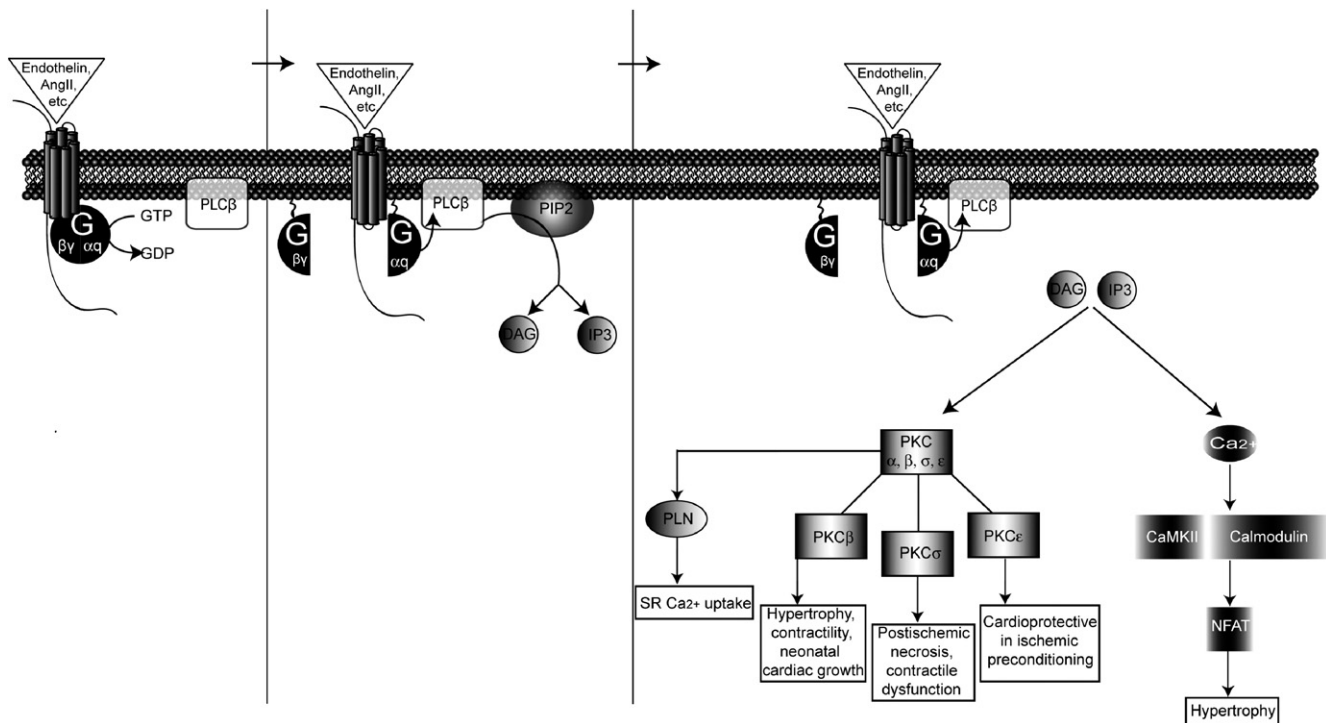


Fig. 3. G_q-coupled receptor signaling. G_q-coupled receptors such as endothelin, angiotensin, or α -1-adrenergic receptors activate the second messenger phospholipase C β resulting in the hydrolysis of phosphatidylinositol 4, 5 biphosphate (PIP₂) into two components: diacylglycerol (DAG) and inositol 1, 4, 5-triphosphate (IP₃). DAG activates several isoforms of protein kinase C (PKC). PKC β activation regulates hypertrophy, contractility, and neonatal cardiac growth. PKC σ activation regulates postischemic necrosis and contractile function. PKC ϵ activation induces a cardioprotective effect in response to ischemic preconditioning. Activation of G_q-coupled receptors stimulates the release of calcium, which activates CaMKII and calcineurin to promote the nuclear translocation of nuclear factor of activated T-cells (NFAT) whereby the hypertrophic transcriptional machinery is activated.

hypertrophy, knockout mice lacking both α 1_{A/C}AR and α 1_BAR genes show increased sympathetic innervation and the development of cardiac hypertrophy after weaning [125]. Together these studies demonstrate that α 1AR subtypes interact with each other, and with other adrenergic receptors, to actively regulate blood pressure, contractile, and hypertrophic responses.

3.2.3. Endothelin receptors

Another cardiac GPCR that promotes cardiac remodeling and hypertrophy via G α_q is the endothelin-1 receptor (ET1R). Endothelin is a powerful vasoconstrictor, and a chronotropic, inotropic, and hypertrophic promoting growth factor [126]. Of the three isoforms, endothelin-1 (ET-1) is expressed in the cardiovascular system and signals via the G_q-coupled ET1R [127].

Cardiac-specific murine overexpression of ET-1 results in myocardial inflammation, hypertrophy, and premature death from heart failure [128]. ET-1 overexpression also induces nuclear translocation of NF- κ B, implicating a potential role of ET-1 in initiating an inflammatory cascade that ultimately leads to myocardial damage and heart failure [126,128]. Various studies to date investigating changes in endothelin receptor levels in failing human hearts have shown contradicting results [126]: while first reports showed unchanged or diminished ETIR levels [129,130], subsequent reports have shown elevated ETIR levels [131]. Furthermore, it is known that ET-1 signaling is

activated in human heart failure [132,133]. While ET1R antagonists are beneficial in experimental heart failure [132–136], and the elevated levels of ET1_AR in heart failure support it as a potential target for selective antagonism in human heart failure, recent clinical trials have failed to show significant benefits [137,138].

3.3. G_q-coupled receptor signaling in heart disease

3.3.1. G_q signaling in heart disease

Modulation of G α_q itself can result in apoptotic and hypertrophic responses. While low overexpression of G α_q in mice does not significantly affect the heart; mild overexpression results in increased hypertrophic markers and minimal cardiac dysfunction [139,140], and high overexpression of G α_q results in marked hypertrophy, heart failure, and increased mortality [139]. G α_q -overexpressing hearts that are exposed to pressure overload show increased apoptosis that can be rescued with a caspase inhibitor [141], implicating apoptotic pathways as mediators in the development of heart failure [142,143]. Importantly, overexpression of an inhibitory peptide that interferes with G_q coupling prevents the development of cardiac hypertrophy and dysfunction in pressure-overloaded mice [144,145]. These data support the concept that activation of G_q-signaling pathways *in vivo* are critical mediators of the hypertrophic response and the transition to heart failure.

PKC expression and activity are markedly increased in failing hearts compared to healthy human hearts [146]. Importantly, PKC activity levels are also markedly increased in failed hearts compared to nonfailed human hearts [146]. However, this overall trend varies by subtype: PKC α and β are both upregulated, PKC ϵ is either upregulated or preferentially activated, and PKC δ levels are not changed [83]. Knocking out PKC α improves contractility while overexpression of PKC α diminishes contractile function in the heart without effecting cardiac growth [83,91]. Animals with high overexpression of G α_q and resultant increased PKC ϵ show overt heart failure and high mortality rates, implicating the critical role of PKC ϵ activation in the development of hypertrophy and heart failure [139]. Transgenic animals lacking PKC ϵ lose the ability to reduce infarct size in response to reactive oxygen species after ischemia reperfusion, suggesting that PKC ϵ is also required for cardioprotective signaling pathways in ischemic preconditioning [128].

3.4. Other G $_q$ -protein-coupled receptors in the heart

Other G $_q$ -coupled receptors in the heart include: subtype 5-HT $_{2b}$ of the serotonin [147,148], bradykinin [149], subtypes I, III, and V of the muscarinic [18], and subtype 1 of the histamine [150] receptors. Muscarinic receptor stimulation by acetylcholine mediates parasympathetic system activation of the heart to modulate heart rate, conduction velocity, and contractility [18]. More specifically, muscarinic receptors I, III, and V are involved in regulating anti-apoptotic pathways [151]. Serotonin is a known vasoconstrictor that has been shown to regulate embryonic cardiac development via its action on serotonin 5-HT $_{2b}$ receptors [148]. Bradykinin, part of the kallikrein–kinin system, is found in vascular smooth muscle cells of the heart. Reduced bradykinin signaling may contribute to the onset of hypertension, cardiac failure, ischemia, myocardial infarction and left ventricular hypertrophy. In contrast to other G $_q$ -receptors in the heart, the activation of the bradykinin system may be beneficial for heart function [152].

4. G $_i$ signaling and G $_i$ -coupled receptors

4.1. Overview of G $_i$ signaling

G $_{inhibitory}$, or G $_i$, signaling opposes G $_s$ signaling by inhibiting AC and reducing AC mediated production of cAMP and its downstream targets. Specifically, a decrease in cAMP leads to a reduction in L-type Ca $^{2+}$ currents which inhibits the force of contraction in myocytes [26]. Heart rate is slowed by the effects of activation of G $_i$ -protein-coupled receptors on pacemaker cells [1]. Sustained G $_i$ stimulation by genetic overexpression of a synthetic G $_i$ -coupled receptor results in bradycardia and cardiomyopathy [153]. Elevated levels of G $_i$ are not known to cause cardiomyopathy, though levels are increased in patients with dilated cardiomyopathy and heart failure [154,155]. G $_i$ couples with A1 adenosine receptors (A1Rs), M2 muscarinic receptors (M2Rs), and α -2-adrenergic receptors (α 2ARs) in the heart (see Fig. 2).

4.2. G $_i$ -coupled receptors in the heart

4.2.1. Adenosine-1 receptors

Adenosine receptors 1 and 3 (A1, A3) signal primarily through G $_i$ to inhibit cAMP production while adenosine 2 receptors signal primarily via G $_s$. Adenosine receptor 1 agonists have been used as anti-arrhythmic agents or in unmasking atrial tachyarrhythmias [156]. The administration of adenosine prior to ischemia reduces infarction size and improves recovery of ventricular function during reperfusion [157]. It is postulated that the protective effect is secondary to A1 receptor-mediated phosphorylation and opening of mitochondrial potassium channels [157]. These data support the concept that the activation of A1 receptors is cardioprotective and mediated by a reduction in heart rate, contractility, and an attenuation of catecholamine stimulation in the heart [158].

4.2.2. Muscarinic-2 receptors

While most muscarinic receptors couple primarily to G $_q$, the predominant muscarinic isoform in the heart, M2, couples to G $_i$. M2 receptor stimulation mediates negative inotropic effects via G α_i , while the negative chronotropic effects are mediated by G $\beta\gamma$ subunit activation of the I $_{K_{ACh}}$ channel [159]. *In vivo*, injection of the muscarinic agonist carbachol reduces resting heart rate without affecting basal contractility [160]. In humans, acetylcholine infusion does not change the force of contraction basally, but attenuates increases in contractile force induced by β AR stimulation [161,162]. M2 density in the heart has been found to decrease with age, accompanied with a reduced ability to respond to muscarinic agonists [129]. While M2 receptor density has been shown to increase in patients with congestive heart failure [163], a change in total muscarinic receptor density in patients with congestive heart failure has not been shown [26].

4.2.3. α 2-Adrenergic receptors

Presynaptic α 2ARs are responsible for suppressing norepinephrine (NE) release from synaptic nerves of healthy hearts, and reducing the excessive amounts of NE in the synaptic terminals during increased adrenergic drive [129,164,165]. As increased sympathetic stimulation activates β 1AR, β 2AR, and α 1AR signaling, the role of α 2ARs in attenuating these pathways is of critical importance. In fact, mice deficient in α ARs have elevated catecholamine levels and develop contractile dysfunction [17,129]. Furthermore, individuals with a loss of function deletion in α 2ARs show a severely attenuated ability to suppress norepinephrine release, leading to higher instances of heart failure [166]. Treatments that stimulate the α 2AR to decrease adrenergic receptor stimulation at its source may provide a way of moderately attenuating β AR and α 1AR signaling.

5. Turning off the signal: desensitization, downregulation, and G $\beta\gamma$

In synchrony with G α -protein signaling is the simultaneous activation of G $\beta\gamma$ -dependent and β -arrestin-mediated pathways

that are responsible for the desensitization, downregulation, and further signaling of GPCRs.

Desensitization describes the tendency of biological systems and receptors to lose their responsiveness to continuous or repeated stimulation [14]. Desensitization occurs via receptor downregulation, the net loss of receptors from the cell membrane, and via receptor uncoupling from its G-protein through a process of receptor phosphorylation [12].

Receptor desensitization induced by phosphorylation results in the rapid (minutes) waning of G-protein-dependent second messenger signaling. Second messengers such as cAMP or DAG activate protein kinases can phosphorylate GPCRs and induce desensitization in a ‘non-agonist-specific’ manner. This mechanism of desensitization is termed heterologous desensitization.

In contrast, ‘agonist specific’, or homologous desensitization, occurs when ligand-activated receptors recruit G-protein-coupled receptor kinases (GRKs). GRKs phosphorylate residues on the c-terminal tail of agonist-occupied GPCRs leading to the binding of the multifunctional scaffold protein, β -arrestin, which then sterically hinders further G-protein coupling [12,167]. In the case of β ARs, β -arrestin blocks the activation of AC, thus reducing β AR responsiveness to catecholamines [168]. An important new concept of GPCR desensitization and downregulation is that additional signaling pathways are activated at the same time G-protein-dependent signaling is turned off. These G-protein-independent pathways are mediated by β -arrestin (see Fig. 1).

5.1. $G\beta\gamma$ subunits

$G\beta\gamma$ subunits signal via effectors such as PI3K, AC, PLC, potassium channels, and GRKs [22,169–175]. In addition, $G\beta\gamma$ subunits promote GPCR desensitization via their action on GRK2. Upon agonist stimulation, the dissociation of $G\beta\gamma$ from the heterotrimeric G-protein promotes the translocation and targeting of GRK2 to the membrane. GRK2 is a member of a protein kinase family of seven subtypes: GRKs1–7, that are primarily responsible for the phosphorylation and subsequent downregulation/desensitization of GPCRs.

5.2. G protein-coupled receptor kinases

Of the 7 GRK isoforms, GRK 2, 3, 4, and 6 are expressed in the heart [168]. GRK2, or β ARK1 as it is commonly known, is best studied and seems to have the greatest impact on cardiac function. It has been shown that GRK2 mRNA levels are increased almost 3 fold in the failing heart, with enhanced activity and consequent decrease in β 1AR levels [60]. Moreover, mice overexpressing GRK2 have a reduced response to β AR stimulation as a result of enhanced receptor desensitization [176]. In contrast, inhibition of GRK2 via the competitive peptide inhibitor β ARKct enhances myocyte contractility and β AR responsiveness *in vivo* [144,176]. GRK2-homozygous-deleted mice are embryonically lethal but exhibit pronounced hypoplasia of the ventricular myocardium and greater than 70% decrease in cardiac ejection fraction *in utero*, implicating a role of GRK2 in cardiac development [177]. GRK2-heterozygous

deleted mice with a 50% reduction in GRK2, however, survive to adulthood with normal cardiac development but show enhanced contractile response to β AR agonists, confirming the primary role of GRK2 in mediating adrenergic receptor desensitization [144].

Mice with cardiac specific overexpression of GRK5 show increased β AR desensitization and reduced β AR responsiveness with no effect on angiotensin receptor signaling [177]. GRK3 myocardial overexpression, in contrast, presents normal β AR and angiotensin receptor signaling but attenuated cardiac signaling via the thrombin receptor [144], demonstrating the substrate specificity of GRK3.

5.3. β -arrestins

β -arrestins 1 and 2 were originally characterized as scaffolding proteins responsible for receptor desensitization. Larger roles in GPCR signaling were soon found, as β -arrestins are now also known for their functions as endocytic adaptors and mediators of β -arrestin-dependent signaling pathways [168]. β -arrestin binding to GRK-phosphorylated receptors shuts off further G-protein signaling by interdicting G-protein coupling [168]. β -arrestin allows for the assembly of multi-protein signaling complexes such as ERK and tyrosine kinases [178,179]. β -arrestin-mediated signaling downstream of the $AT_{1A}R$ requires GRK5 and 6 and is independent of G-protein signaling [10]. Transgenic mouse studies to date show that while β -arrestin-2 knockouts do not seem to show a cardiac phenotype [180], β -arrestin-1-heterozygous knockouts show increased contractile responsiveness to β 1AR stimulation [181].

6. Phosphoinositide-3-kinase and heart failure

An important modulator of β AR trafficking and function is phosphoinositide-3-kinase (PI3K) [182]. PI3Ks are a family of enzymes containing both lipid and protein kinase activities that mediate cell proliferation, cytoskeletal trafficking, and endocytosis via such downstream effectors as PKB/Akt, GSK3 β , and p70S6K [182].

One isoform, PI3K α , which is activated by receptor tyrosine kinases, is important in the regulation of cardiomyocyte size [183]. Cardiac-specific overexpression of a constitutively active form of PI3K α results in increased heart size and PKB/Akt levels, while a dominant negative overexpression of PI3K α reduces heart size [184]. The other isoform, PI3K γ , is activated by $G\beta\gamma$ subunits and phosphorylates phosphoinositides on the cell membrane to ultimately activate PKB/Akt signaling. Furthermore, PI3K γ regulates contractility by negatively regulating AC and PLB activity [185], as cardiac-deletion of PI3K γ increases cAMP levels and enhances contractility [183].

PI3K is important for β AR recycling in the healthy heart, but its role in heart failure may be detrimental. Recent studies have shown that PI3K binds to GRK2 to regulate β AR endocytosis [186,187]. When the PI3K γ / β ARK interaction is disrupted by a PI3K blocking protein, heart failure is ameliorated in a number of experimental models [182,187,188]. Moreover, inhibition of the endogenous PI3K γ interaction with GRK2, results in

normalization of β AR levels and improved β AR responsiveness to agonist stimulation [182,187,188]. This is also accompanied by marked improvement of cardiac function and increased survival. Hence, disruption of PI3K–GRK2 interactions improves β AR function and may improve cardiac function and survival in failing hearts.

7. Conclusions

With over 200 GPCRs in the heart, we have discussed the key GPCRs that have been implicated in heart failure, such as the β and α adrenergic receptors, the angiotensin receptors, endothelin receptors, and the muscarinic receptors. We have discussed their downstream signaling pathways, including those mediated by PKA, PKC, and PI3K, in both healthy and failing hearts. GPCR signaling cascades are complex, involving receptor cross-talk and convergence of a variety of pathways. Interestingly, the G-protein signaling pathways of many of the so called “orphaned” GPCRs remain to be identified [3]. Elucidating the detailed components of these pathways and their effects on cardiac function will likely provide exciting therapeutic options for treating heart failure.

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