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G protein-dependent and –independent signaling pathways and their impact on cardiac function

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Abstract

G protein-coupled receptors (GPCRs) signal through a variety of mechanisms that impact cardiac function, including contractility and hypertrophy. G protein-dependent and -independent pathways each have the capacity to initiate numerous intracellular signaling cascades to mediate these effects. G protein-dependent signaling has been studied for decades and great strides continue to be made in defining the intricate pathways and effectors regulated by G proteins and their impact on cardiac function. G protein-independent signaling is a relatively newer concept that is being explored more frequently in the cardiovascular system. Recent studies have begun to reveal how cardiac function may be regulated via G protein-independent signaling, especially with respect to the ever-expanding cohort of β-arrestin-mediated processes. This review primarily focuses on the impact of both G protein-dependent and β-arrestin-dependent signaling pathways on cardiac function, highlighting the most recent data that illustrate the comprehensive nature of these mechanisms of GPCR signaling.

Keywords

GPCR; G protein; β-arrestin; cardiac contractility; hypertrophy

G protein-coupled receptors (GPCRs) mediate numerous acute regulatory mechanisms involved in the control of cardiovascular function, such as contractility, and chronic processes, such as hypertrophy, that contribute to the development of cardiovascular diseases including heart failure[1, 2]. Several current drug therapies, such as β-adrenergic receptor (βAR) blockers and angiotensin II receptor (AT_1R) blockers, target their GPCRs to prevent hypertrophic signaling and improve clinical outcomes of heart failure patients[3]. Recent research has shown that some GPCR ligands have the capacity to block hypertrophic signaling pathways while simultaneously promoting cardiac contractility or survival[4-7], a property that could improve overall cardiac function relative to conventional GPCR blockers. The ability of a ligand to relay such an effect is possible due to the variety of G protein-dependent and -independent pathways that can be initiated upon GPCR stimulation. G protein-dependent signaling pathways have been explored in the heart for decades,

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revealing significant roles for the $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, $G\alpha_{12/13}$, and $G\beta\gamma$ families in mediating contractile and/or hypertrophic responses in the heart. Newer to the field of cardiac research, G protein-independent signaling has only been studied for the last 15 years, with specific roles for β-arrestin-mediated signaling in the regulation of cardiac contractility and hypertrophy reported only in the last 5 years. The multitude of cardiac signaling pathways regulated by G proteins and β-arrestins downstream of GPCR activation provides a number of potential targets for pharmacotherapy of heart failure. This review highlights recent molecular studies that provide novel insight into the regulation of cardiac function via G protein- and β-arrestin-dependent signaling.

1. G protein-dependent signaling

The heterotrimeric G protein complex is comprised of a Gα subunit, of which there are four main families ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$) coupled to a combination of Gβ and Gγ subunits, of which there exists 5 and 12 members, respectively. The specific classifications, isoforms and various subunit compositions of the numerous G proteins have been described elsewhere[8, 9]. The Gα proteins primarily expressed and studied in the heart include $G\alpha_s$, $G\alpha_{i1/2/3}$, $G\alpha_{q/11}$ and $G\alpha_{12/13}$ (Table 1). GPCR stimulation leads to a change in conformational of the receptor such that it promotes nucleotide exchange at Gα of GDP for GTP[9-11]. The active GTP-bound form of Gα dissociates from the receptor and Gβγ subunits, and subsequently activates/inhibits downstream effector proteins[9], though Gα subtype-selective molecular rearrangement with Gβγ subunits in the absence of dissociation has also been reported[12, 13]. In recent years, an expansive array of accessory proteins that modulate G protein activity has been described, including activators of G protein signaling (AGS) and regulators of G protein signaling (RGS) proteins[14, 15]. Members of these families may contain GTPase-activating protein (GAP), guanine nucleotide exchange factor (GEF) or guanine nucleotide dissociation inhibitor (GDI) activities, each of which contributes to the regulation of G protein activity. For instance, GEFs act to increase the rate of GTP association with Gα subunits, thereby promoting Gα protein-mediated effects, whereas GDI-containing proteins act to inhibit the dissociation of GDP from Gα subunits, thereby inhibiting Gα protein-mediated signaling[14]. RGS proteins containing GAP activity accelerate the GTPase activity of Gα subunits, thereby decreasing the amplitude and duration of Gα protein-mediated signaling, though these effects appear to be limited to mainly $G\alpha_{q/11}$ and $G\alpha_i$ proteins[15]. The impact of G protein-dependent signaling via various GPCRs and downstream effector proteins on the regulation of cardiac function will be discussed with regard to the molecular mechanisms by which they impact cardiac contractility and hypertrophy.

a) G protein-dependent effects on cardiac contractility

cAMP-mediated regulation of cardiac contractility

The mechanisms by which $G\alpha_s$ protein activity enhance heart rate and contractility are best exemplified by β_1AR signaling (Fig. 1). β_1AR stimulation results in adenylyl cyclase (AC)-mediated generation of cAMP, subsequent protein kinase A (PKA). Via phosphorylation of numerous substrates involved in the contractile response, including the ryanodine receptor (RyR), phospholamban (PLB), the L-type calcium channel (LTCC), cardiac troponin I (cTnI) and cardiac myosin-binding protein C (cMyBP-C), PKA signaling enhances contractile function, as eloquently reviewed elsewhere[16]. Briefly, PKA-mediated phosphorylation of RyR and LTCC, to increase Ca^{2+} uptake and sarcoplasmic reticulum (SR) release, and PLB, to release its inhibitory effects on the sarcoplasmic reticulum calcium ATPase (SERCA) and promote Ca^{2+} SR storage, and TnI and cMyBP-C, to decrease Ca^{2+} affinity for the myofilaments and alter crossbridge kinetics, each contribute to the inotropic and lusitopic effects of β-adrenergic stimulation. While few $G\alpha_s$ protein-

coupled receptors have been shown to augment inotropy to the same physiologic extent of βAR stimulation, modulation of βAR-dependent effects by other $G\alpha_s$ protein-coupled receptors, as recently demonstrated by type 2 adenosine receptor (A_2AR) subtype-specific effects on βAR-mediated contractility[17], may be of importance in vivo. Additionally, in the pacemaker cells PKA-mediated phosphorylation of membrane ion channels, as well as Ca^{2+} handling proteins such as RyR and PLB, tightly controls Ca^{2+} cycling and heart rate[18].

cAMP generation also leads to activation of exchange protein activated by cAMP (EPAC), and although the effects of EPAC signaling on contractile function have not been as extensively studied as PKA-mediated effects, EPAC has also been demonstrated to regulate cardiomyocyte Ca^{2+} handling and myofilament protein phosphorylation[19]. Through mechanisms involving phospholipase Cε (PLCε), protein kinase Cε (PKCε) and calmodulin-dependent protein kinase II (CAMKII), EPAC has been shown to increase cTnI, RyR and PLB phosphorylation, Ca^{2+} release from SR stores and sarcomeric shortening in response to either βAR stimulation or direct EPAC activation[20-23]. Additionally, an interaction between CAMKII, EPAC1 and the scaffolding proteins β-arrestins 1 and 2 that was enhanced upon β1AR stimulation was demonstrated in the heart (Fig. 1)[24]. By providing a scaffold for both CAMKII and EPAC1, β-arrestins facilitate β1AR-EPAC-Rap1-PLC-PKC-mediated CAMKII activation and downstream PLB phosphorylation[24].

Cardiac electrophysiological processes (for extensive reviews of cardiac electrophysiology refer to [25, 26]) have also been shown to be regulated by cAMP-dependent processes, as both PKA and EPAC have been shown to regulate ion channel activity. Whereas PKA-mediated phosphorylation of the ATP-sensitive K^+ channel (K_{ATP}) increases its activity leading to hyperpolarization[27], EPAC activation leads to a Ca^{2+}-calcineurin-dependent dephosphorylation/inactivation of vascular K_{ATP}, potentially providing a negative feedback mechanism to inactivate the channel when cAMP levels become very high[28]. Determination of EPAC-mediated effects on cardiac K^+ channel activity and the comparative effects of EPAC versus PKA signaling on the contractile machinery and ion flux specifically in cardiomyocytes requires further exploration.

In opposition to $G\alpha_s$-mediated signaling, stimulation of cardiac $G\alpha_i$ protein-coupled receptors typically results in negative inotropy and chronotropy via $G\alpha_i$-dependent inhibition of AC activity, cAMP synthesis and PKA activation. Of the $G\alpha_i$-linked GPCRs, the muscarinic acetylcholine receptor 2 (M_2R) is the primary example of $G\alpha_i$-mediated parasympathetic antagonism of sympathetic βAR signaling, and has been shown to dampen, or block entirely, βAR-mediated inotropic and chronotropic responses (Fig. 1)[29]. The ability of $G\alpha_i$ protein-coupled receptors to mediate inhibition of AC activity may depend on membrane localization as the ability of sphingosine-1-phosphate receptor 1 ($S1P_1R$) to decrease AC activity and inotropy in adult mouse ventricular myocytes was dependent on compartmentation of $S1P_1R$ in caveolae-rich regions of the sarcolemma[30, 31].

A kinase-anchoring protein (AKAP)-mediated regulation of cardiac contractility

Via interactions with AKAPs, PKA activity can be tethered to different substrates in subcellular environments, providing precise spatiotemporal regulation of cardiac function[32]. Studies over that last decade have shown that intracellular targeting of other components of cAMP-mediated signaling immediately downstream of βARs, including AC and cAMP phosphodiesterases (PDE) by AKAPs tightly controls βAR signaling[33], as will be discussed in another review in this series. Aside from βAR-AKAP complexes, other GPCRAKAP signaling complexes are beginning to be reported. The relaxin receptor (RXFP1) was recently shown to be precisely regulated by constitutive association with AKAP79-AC2 and β-arrestin 2-PKA-PDE4D3 complexes, which coordinately control local generation and hydrolysis of cAMP in response to low concentrations of relaxin[34]. While relaxin has been

shown to exert positive inotropic and chronotropic responses in the heart[35], it is not known whether such an intricate scaffolding system mediates these responses in vivo.

Beyond regulation of local pools of cAMP at the receptor level, AKAPs have also been shown to regulate contractility at the level of the sarcomere as cardiac troponin T (cTnT) has been reported to act as an AKAP, targeting PKA activity to the sarcomere[36]. In addition, it has been shown that AKAP-9 recruits a macromolecular complex to the cardiac I_{Ks} channel consisting of PDE4D3, PKA and protein phosphatase 1 (PP-1), which tightly controls cAMP-induced channel activity and current, thereby modulating cardiac hyperpolarization[37]. In agreement with the variety of AKAP-mediated effects on cAMP signaling in cardiomyocytes, peptide-mediated disruption of PKA-AKAP interaction in the mouse heart has been shown to act as a negative inotropic, chronotropic and lusitropic stimulus[38].

Gβγ-mediated regulation of cardiac contractility

Similar to the function of AKAPs, Gβγ subunits can serve as a protein scaffold[8]. It has been shown that β_2AR-$G\alpha_i$ protein coupling leads to Gβγ-mediated confinement of $G\alpha_s$-cAMP-PKA signaling via increased phosphoinositide-3-kinase (PI3K) activity[39]. In particular, $G\alpha_i$-PI3Kγ-dependent regulation of PDE4 activity was shown to control local cAMP signaling in response to β_2AR stimulation and dampen the βAR-mediated inotropic response in cardiomyocytes[40, 41]. Further, $G\beta_1$ interaction with nucleoside diphosphate kinase B (NDPK B) was shown to regulate basal contractility in several cardiac cell models[42-44]. The NDPK B-induced transfer of a phosphate to $G\beta_1$ allows the local generation of GTP bound to $G\alpha_s$ and subsequent $G\alpha_s$ activation, AC-mediated cAMP synthesis and cardiomyocyte contractility[42, 43, 45]. Interestingly this process only impacts receptor-independent cAMP synthesis and cardiomyocyte contractility[42], since activated GPCRs, such as βARs, act as GEFs themselves to induce $G\alpha_s$ protein exchange of GDP for GTP[11].

Gβγ subunits can also promote negative inotropy via effects on ion channels, as Gβy-mediated inhibition of LTCC current following βAR stimulation has been reported[46]. Also, via a Gβγ-dependent mechanism, both M_2R and $S1P_1$R have been demonstrated in atrial and ventricular myocytes to increase the open probability of the K^+ channel $I_{K_{Ach}}$, promoting membrane hyperpolarization to decrease the action potential duration, thereby decreasing chronotropy and inotropy (Fig. 1)[31, 47]. Via a Gγ-like domain, RGS6 has been shown to interact specifically with $G\beta_5$[48] and a recent study reported that this complex binds to and promotes the deactivation of $I_{K_{Ach}}$, thereby modulating M_2R-mediated effects on myocyte current kinetics[49]. Since RGS proteins are involved in promoting the reassembly of Gα subunits and Gβγ subunits into the heterotrimeric G protein complex, this study suggests that RGS6 provides a negative feedback mechanism to turn off $G\alpha_i$-Gβγ-mediated hyperpolarization. Indeed, genetic ablation of RGS6 resulted in prolonged $I_{K_{Ach}}$ activity in both atrial myocytes and sinoatrial node cells, leading to bradycardia[49].

$G\alpha_{q/11}$-mediated regulation of cardiac contractility

Cardiac $G\alpha_{q/11}$ protein-coupled receptors increase cardiac inotropy by modulating intracellular Ca^{2+} levels and contractile protein phosphorylation via PLCβ-mediated conversion of membrane inositol phospholipids into the 2nd messenger products inositol trisphosphate (IP_3) and diacylglycerol (DAG)[50]. Enhanced 2nd messenger signaling downstream of $G\alpha_{q/11}$ increases SR-dependent Ca^{2+} mobilization and activates a number of cardiac PKC isoforms, protein kinase D (PKD) and CAMKII[51-53]. Collectively, these kinases have been shown to modulate many of the same proteins involved in cardiomyocyte contractility as PKA (Fig. 1)[54-61]. Although acute stimulation of $G\alpha_{q/11}$ protein-coupled receptors increases cardiomyocyte inotropy and chronotropy[62-64], the physiological

importance of such stimulation compared to βAR-$G\alpha_s$-mediated inotropy is not well established. However, a number of important $G\alpha_{q/11}$-activated signaling pathways can contribute to the regulation of contractility, which may be significant since crosstalk between cardiac $G\alpha_{q/11}$ protein-coupled receptors and βAR-$G\alpha_s$ signaling has been established[65-68].

LTCC, PLB and RyR each contribute to Ca^{2+} homeostasis and undergo phosphorylation by PKC and CAMKII signaling[2, 59]. For instance, phosphorylation of PLB can be increased in a PKCε-dependent manner involving activation of CAMKII[22], or can be decreased in a PKCα-dependent manner involving PP-1-mediated dephosphorylation[56]. Recently, the δ_C isoform of CAMKII was shown in transgenic mice to mediate an alteration in myocyte Ca^{2+} handling at the SR involving PLB and that inhibition of its activity specifically at the SR helps to restore diastolic Ca^{2+} handling[69]. Several putative PKC phosphorylation sites on LTCC have been reported[70], phosphorylation of which augments Ca^{2+} influx in the cardiomyocyte to promote Ca^{2+}-mediated Ca^{2+} release from the SR. Different PKC isoforms can mediate LTCC phosphorylation, including PKCα, but excluding PKCε, although it has also been shown that PKCα can transiently decrease LTCC activity via a PI3Kα-dependent mechanism following AT_1R stimulation[57].

Beyond the control of ion flux, PKC and PKD have also been reported to associate with or phosphorylate components of the cardiac contractile machinery, resulting in differential effects on Ca^{2+} sensitivity and crossbridge kinetics[54, 60, 61]. For instance, cTnI has been demonstrated to interact with PKCα following increased Ca^{2+} signaling, which may result in the maintenance of contractile force[58]. In addition, cTnI has been shown to undergo phosphorylation by PKCβII to increase Ca^{2+} sensitivity[55], and by PKCα and PKCε to decrease Ca^{2+} sensitivity in failing human myocardium[54]. In the latter study, PKCα- and PKCε-dependent phosphorylation of cMyBP-C, which is known to accelerate crossbridge cycling, was also shown to be increased in failing human myocardium. Activated PKD has been demonstrated to phosphorylate cTnI to actually decrease Ca^{2+} sensitivity[71], and may accelerate crossbride cycle kinetics via phosphorylation of cMyBP-C[72]. Through these combined mechanisms, $G\alpha_{q/11}$-mediated signaling has the capacity to regulate precise events involved in cardiac Ca^{2+} transport and contractility.

b) G protein-dependent effects on cardiac hypertrophy

$G\alpha_{q/11}$ protein-mediated effects on cardiac hypertrophy

While the physiological relevance of $G\alpha_{q/11}$ protein signaling on cardiac contractility may not be as well-established as $G\alpha_s$ protein-mediated effects, $G\alpha_{q/11}$ signaling has been shown to play an important role in the development of cardiac hypertrophy[51, 73, 74]. Cardiac hypertrophy involves enhanced transcriptional activity and cell size, which can be a normal physiologic adaptive response to increased cardiovascular workload, or can contribute to the pathologic development of heart failure[75]. Increased expression of various isoforms of sarcomeric and metabolic proteins considered to be representative of a developmental phenotype, or “fetal” gene expression, is associated with decreased cardiac function during the progression of hypertrophy and transition to heart failure (Fig. 2)[76]. The hypertrophic role of $G\alpha_{q/11}$ in the heart has been studied using genetic approaches to knockdown or inhibit $G\alpha_{q/11}$ in various mouse models of cardiomyopathy, demonstrating that hypertrophic responses to chronic agonist stimulation or pressure overload are reduced or prevented in the absence of $G\alpha_{q/11}$ activity, as reviewed by others[75, 77]. The regulation of $G\alpha_q$ activity by RGS2, which normally dampens $G\alpha_q$ signaling in the heart, has also been shown to influence cardiac hypertrophy. RGS2 knockout mice exhibit enhanced hypertrophic responses to pressure overload compared with RGS2-expressing mice, which include increased calcineurin expression, CAMKII activity and MAPK activity[78], suggesting that

RGS2-mediated inhibition of $G\alpha_q$ signaling could be an effective means by which to prevent cardiac hypertrophy. Similar results were shown in a recent study exploring the regulation of AT_1R-induced MAPK signaling via RGS5 in neonatal cardiomyocytes[79].

Studies have also begun to comprehensively characterize the transcriptional response to $G\alpha_{q/11}$ signaling, revealing hundreds of genes whose expression is altered following $G\alpha_{q/11}$ activation. These studies have reported an increase in $G\alpha_{q/11}$-dependent transcript detection following stimulation with angiotensin II (Ang II) in HEK 293 cells, or endothelin in rat neonatal cardiomyocytes[66, 80, 81]. In particular, investigators studying the effects of Ang II on transcription have shown that the Ang II-mediated increases in gene expression is primarily dependent upon $G\alpha_{q/11}$ signaling[66]. By blocking $G\alpha_{q/11}$ protein-dependent signaling, antagonists such as AT_1R blockers can diminish the hypertrophic transcription response to stimulation by endogenous factors, and reduce the rate of progression of heart failure[3].

PLC-PKC-MAPK-mediated effects on cardiac hypertrophy

Aside from the antagonism of $G\alpha_{q/11}$ protein-coupled receptors or $G\alpha_{q/11}$ itself, inhibition of several downstream regulatory proteins has been shown to interfere with $G\alpha_{q/11}$-mediated hypertrophic responses. As discussed above, $G\alpha_{q/11}$ activation initiates PLCβ-mediated phospholipid hydrolysis and 2nd messenger generation. A 32-amino acid C-terminal peptide of PLCβ1b was shown to be sufficient to prevent sarcolemmal targeting of PLCβ1b in rat neonatal cardiomyocytes[82]. By blocking PLC1β1b targeting to the membrane, PLC-mediated 2nd messenger generation and subsequent hypertrophic responses were abrogated in response to α_1AR stimulation[82]. Downstream of PLC, PKC activation leads to phosphorylation of numerous substrates, and in the context of hypertrophy initiation of MAPK signaling is a major route by which $G\alpha_{q/11}$-coupled receptors mediate cell growth responses[83]. In particular, activated PKCs are known to increase ERK1/2 activity in the heart to increase cell growth, effects that can be prevented with PKC inhibition[51]. Via both cytosolic and nuclear actions, ERK1/2 signaling has been shown in different cell types to increase DNA transcription and mRNA translation[84, 85]. Such subcellular effects of ERK1/2 have also been demonstrated in neonatal rat cardiomyocytes to contribute to cardiac growth responses, including modulation of proteins involved in gene expression and protein synthesis, such as the nuclear transcription factor family NFAT (nuclear factor of activated T cells) and the ribosomal S6 kinase p70S6K[86, 87]. Interestingly, it was recently demonstrated that ERK1/2 in particular contributes to concentric cardiomyocyte hypertrophy, or increased cardiomyocyte width, associated with the addition of new sarcomeres, likely mediated via cytosolic pools of activated ERK1/2[88]. Inhibition of ERK1/2 signaling however, led to increased cardiomyocyte length, or eccentric hypertrophy. Inhibition of downstream phosphorylation targets of ERK1/2, including mitogen and stress activated kinase 1 (MSK1) and MAP kinase-interacting kinase 1 (Mnk1), have also been shown to reduce the hypertrophic response to $G\alpha_{q/11}$-protein coupled receptors, such as the α_1AR, in cardiomyocytes[89, 90].

CAMKII-mediated effects on cardiac hypertrophy

In addition to PKC-MAPK signaling, activation of $G\alpha_{q/11}$ mediates hypertrophy via other mechanisms. PLCβ-generated IP_3 binds to IP_3 receptors (IP_3R) on the SR to increase the release of stored Ca^{2+} into the cytosol where it binds calmodulin (CAM). The resulting Ca^{2+}/CAM complex interacts with and activates numerous proteins, including CAMKII and calcineurin (Fig. 2), a protein phosphatase that regulates NFAT and has been shown to play a role in this development of hypertrophy in various models[83, 91]. The role of CAMKII in hypertrophy and development of heart failure has been extensively studied by the Brown group using various genetic mouse models[53]. From these studies, the notion that select

isoforms of CAMKII can play distinct, but overlapping roles in the promotion of hypertrophic signaling in the heart has emerged. In particular, it was shown that despite differential localization of the δ_B, and δ_C cardiac isoforms of CAMKII in the nucleus and cytosol, respectively, transgenic expression of each isoform enhanced cardiac hypertrophic gene expression by promoting histone deacetylase (HDAC) 4 extrusion from the nucleus[92]. Interestingly, genetic deletion of CAMKIIδ in mice did not prevent the development of hypertrophy, ostensibly due to a compensatory increase in CAMKIIγ activity, but did attenuate heart failure progression following pressure overload due to a loss of altered expression of Ca^{2+} regulatory proteins[93]. This reveals a CAMKII isoform-specific transcriptional control of subsets of cardiac proteins. Most recently, CAMKIIδ deletion has been demonstrated to improve cardiac function and reduce remodeling in various mouse models of heart failure[53], including myocardial ischemia, ischemia/reperfusion and transgenic overexpression of $G\alpha_q$. Thus, inhibition of CAMKII signaling appears to be a viable mechanism to attenuate hypertrophy and progression to heart failure, though isoform-specific targeting and compensatory effects may need further exploration.

$G\alpha_{12/13}$-mediated effects on cardiac hypertrophy

Contrary to $G\alpha_{q/11}$ signaling, $G\alpha_{12/13}$ activation does not lead to the generation of 2nd messengers, but to the activation of a small family of RhoGEFs[94]. RhoGEFs induce the activation of the small GTPase RhoA, which in turn mediates numerous cellular processes through effects on several downstream protein targets[95]. Although $G\alpha_{12/13}$ signaling in the heart is still relatively unexplored, several studies from the Kurose laboratory have shown a role for $G\alpha_{12/13}$ in mediating cardiac hypertrophy and fibrosis. α_1AR, AT_1R and ET-1 stimulation were each shown in neonatal cardiomyocytes or cardiac fibroblasts to be capable of inducing hypertrophic or fibrotic responses, mainly via $G\alpha_{12/13}$-p115RhoGEF-dependent activation of the MAPK c-Jun NH(2)-terminal kinase (JNK) (Fig. 2)[96-99]. Another group has also reported that AKAP-Lbc acts both as a scaffold to induce α_1AR-mediated p38 MAPK activation and as a RhoGEF to activate RhoA following α_1AR-$G\alpha_{12/13}$ stimulation in neonatal cardiomyocytes[100, 101]. In addition, it was shown that either mechanical stretch or direct stimulation of the purinergic $P2Y_6$ receptor increases cardiomyocyte fibrosis via $G\alpha_{12/13}$ and that $P2Y_6$ inhibition in vivo prevented fibrosis, but not hypertrophy, in response to pressure overload[102]. Thus, while $G\alpha_{12/13}$ effects in the heart have not been as extensively studied at $G\alpha_{q/11}$-mediated effects, they may be important mediators of cardiac hypertrophy and fibrosis.

cAMP-dependent effects on cardiac hypertrophy

Although a role for $G\alpha_s$ in the development of hypertrophy has been recognized for many years[103], as illustrated via transgenic overexpression of cardiac $G\alpha_s$ [104, 105], the mechanisms controlling hypertrophy downstream of $G\alpha_s$ and cAMP generation remain controversial. At the level of $G\alpha_s$, a recent study highlighted the ability of RGS2 to influence the hypertrophic response to βAR stimulation, as RGS overexpression in neonatal rat ventricular myocytes diminished βAR-mediated cAMP synthesis, ERK1/2 and Akt phosphorylation and hypertrophy[106]. Additionally, using selective activators of PKA and EPAC, the authors demonstrated reliance on PKA signaling for the induction of cardiomyocyte hypertrophy with no EPAC-mediated effects on cell growth. Conversely, another group demonstrated a role for EPAC in the hypertrophic responses to both pressure overload and βAR stimulation. In a rat model of aortic constriction, both EPAC1 expression and myocardial hypertrophy increased and it was shown in isolated adult rat ventricular myocytes that the effects of EPAC on cell growth involve Ras, calcineurin and CAMKII signaling[107]. It was subsequently shown in neonatal rat cardiomyocytes that Ras activation in response to EPAC stimulation was dependent on PLC- and IP_3R-mediated increased Ca^{2+} signaling and that both calcineurin-dependent NFAT transcription and CAMKII-dependent myocyte enhancer

factor-2 (MEF-2) activation contributed to the hypertrophic response[108]. While the mechanisms by which EPAC regulates cardiac hypertrophy are still being explored, there is evidence to support a role for EPAC in this process (Fig. 2) and could provide a novel therapeutic target.

$G\alpha_i$- and Gβγ- mediated effects on hypertrophy

Both $G\alpha_i$ and Gβγ have been implicated in the development of hypertrophy as well as the progression of heart failure. An increase in cardiac $G\alpha_{i1}$ expression was detected in an inducible genetic model of Ras-MAPK mediated hypertrophy, correlating with alterations in the regulation of intracellular Ca^{2+}-handling and leading to ventricular hypertrophy and arrhythmia, both of which were normalized with the inhibition of $G\alpha_i$ via pertussis toxin[109]. Also, genetic inhibition of $G\alpha_i$ with a cardiac-expressed inhibitory peptide (GiCT) was shown to increase apoptosis following ischemia/reperfusion injury, identifying a cardioprotective role for $G\alpha_i$ during cardiac stress[110]. More recently, it was shown that small molecule inhibition of Gβγ was able to halt the progression of heart failure in both a neurohormonal and a genetic mouse model of heart failure[111]. In each model, contractile function was improved with Gβγ inhibition, and the hypertrophic response reduced, as assessed by cardiomyocyte morphology and changes in fetal gene expression. Since small molecule inhibitors of Gβγ have been shown to differentially modulate different Gβγ-dependent signaling pathways[8], the potential to selectively inhibit distinct cardiac Gβγ-mediated hypertrophic effects while preserving contractile function could be advantageous.

2. G protein-independent signaling

GPCR-mediated G protein-independent signaling is a newer concept compared to G protein-dependent signaling. The diverse nature of this signaling paradigm has become apparent over the last decade, and great strides have been made in unraveling the roles of G protein-independent signaling in the cardiovascular system. GPCR stimulation and subsequent phosphorylation of C-terminal serine/threonine residues by GPCR kinases (GRKs) relay the primary steps in the induction of G protein-independent signaling by inducing the recruitment of β-arrestins[112]. Since the role of GRKs in cardiovascular signaling and function will be reviewed elsewhere in this series, the following discussion of G protein-independent signaling will focus upon recent developments in the understanding of the signaling networks used by β-arrestins. β-arrestins 1 and 2 are ubiquitous scaffolding proteins that induce receptor desensitization, internalization as well as numerous signaling mechanisms[113]. Recently, identification of entire β-arrestin-interacting protein signalosomes via mass spectroscopy has greatly expanded the comprehension of the scope of β-arrestin signaling. In particular, the β-arrestin signalosomes that associate with AT_1R before and after Ang II stimulation have been reported in HEK 293 cells, identifying hundreds of proteins that scaffold differentially with β-arrestins 1 and 2[114]. Additionally, the identification of hundreds of proteins whose phosphorylation status is altered following stimulation of AT_1R also reveals entire AT_1R-β-arrestin-dependent phosphoproteomes involved in numerous processes including cell growth, cell survival and cytoskeletal reorganization[115, 116]. Although a majority of studies investigating β-arrestin-mediated effects have focused on the downstream responses to AT_1R or βAR stimulation, the increasing array of results and may be applicable to other cardiac GPCR systems as they relate to the control of cardiac contractility and hypertrophy.

a) β-arrestin-mediated effects on cardiac contractility

β-arrestin-dependent cardiomyocyte contractility

In the last five years, β-arrestins have been demonstrated to promote cardiomyocyte and cardiac contractility. Studies using β-arrestin-biased AT_1R ligands that do not induce $G\alpha_{q/11}$

protein activation have shown that AT_1R-β-arrestin-dependent signaling enhances contractility in isolated adult mouse cardiomyocytes[5-7]. The first study, which utilized the biased ligand [Sar^1, Ile^4, Ile^8]-angiotensin II (SII) and knockout mice to define the roles of each β-arrestin in increasing cardiomyocyte contractility, identified β-arrestin 2, but not β-arrestin 1, as the mediator of this $G\alpha_{q/11}$-independent response[5]. The reliance upon β-arrestin 2 in mediating $G\alpha_{q/11}$ protein-independent contractility in response to AT_1R stimulation was confirmed in a more recent study utilizing a distinct β-arrestin-biased AT_1R ligand[6]. In addition, unbiased activation of AT_1R with Ang II in β-arrestin 2 knockout cardiomyocytes produced a blunted contractile response[6], suggesting that β-arrestin 2-mediated effects on contractility may not be redundant with respect to $G\alpha_{q/11}$ protein-dependent signaling. Violin *et al.* have recently demonstrated in whole animals that infusion of synthetic β-arrestin-biased AT_1R peptide ligands cause increased cardiac contractility[7]. Interestingly, while these β-arrestin biased AT_1R ligands increased cardiac contractility and decreased blood pressure, they did not alter stroke volume unlike conventional AT_1R blockers[7]. The therapeutic implications for these ligands will be discussed in another review in this series, but these observations demonstrate the potential of targeting β-arrestin-mediated signaling pathways to selectively impact cardiovascular function.

β-arrestin-mediated effects on cytoskeletal reorganization

The mechanism(s) responsible for mediating β-arrestin-dependent cardiomyocyte contractility have not yet been defined, but could involve the aforementioned ability of β-arrestins to scaffold proteins involved in regulating contractility, such as EPAC and CAMKII[24]. Additionally, cytoskeletal reorganization could play a role in β-arrestin-mediated cardiac contractility. Mechanistic studies in HEK 293 cells have reported β-arrestin-mediated effects on cytoskeletal reorganization, mainly describing effects on the small GTPase RhoA downstream of AT_1R. AT_1R-β-arrestin 1-mediated signaling has been shown to increase RhoA activation and subsequent stress fiber reorganization, while β-arrestin 2 was shown to have no impact on this process[117], highlighting distinct functional roles for β-arrestins 1 and 2 in regulating this intracellular process. In addition, increased β-arrestin 1 association with a Rho GAP (ARHGAP21) following AT_1R stimulation was recently demonstrated to promote RhoA activation and stress fiber formation (Fig. 1), while disruption of this interaction diminished RhoA activity and changes in actin reorganization and cell shape[118]. Perhaps explaining the lack of effect of β-arrestin 2 in mediating RhoA activation downstream of AT_1R, it was shown that unlike β-arrestin 1, β-arrestin 2 does not interact with ARHGAP21[118]. Interestingly, another group reported a dependence on β-arrestin 2, but not β-arrestin 1, in the RhoA-RhoA kinase (ROCK)-dependent regulation of myosin light chain kinase (MLCK) activity and plasma membrane blebbing following AT_1R stimulation[119]. How AT_1R stimulation promotes one β-arrestin-mediated pathway over another to confer changes in cytoskeletal organization is not clear, but could depend on local concentrations of the mediators of these effects. While β-arrestin-mediated activation of RhoA signaling is an attractive explanation for increased cardiomyocyte contractility since RhoA activity can impact regulators of cardiac contractility such as PKC and PKD[95], the impact of RhoA signaling in β-arrestin-mediated contractility requires exploration.

Additional proteins known to be involved in the regulation of contractility have been demonstrated to interact with β-arrestins or have their phosphorylation status altered in a β-arrestin-dependent manner downstream of AT_1R stimulation. These include ROCK, actin, cofilin, myosin and the myosin-binding subunit of myosin phosphatase (MYPT1)[114-116], but extend to other proteins involved in more generalized signaling processes. Further, β-arrestin-dependent regulation of Ca^{2+} transport via transient receptor potential channel (TRP4) has been reported in vascular smooth muscle cells (VSMC)[120]. Following Ang II stimulation, a β-arrestin 1-dependent AT_1R-TRP4 complex undergoes internalization away

from the plasma membrane, reducing cation influx in response to continued AT_1R stimulation. Altogether, the expanding roles for β-arrestins in the regulation of cation influx, cytoskeletal structure and cardiomyocyte contractility suggests that they provide a previously unrecognized mechanism to regulate cardiac contractile function. Whether the mechanistic observations reported thus far extend from cell culture models to the heart and apply to cardiac GPCRs other than AT_1R remains to be tested.

b) β-arrestin-mediated effects on cardiac hypertrophy

β-arrestin-mediated MAPK activity

Some GPCRs, such as the AT_1R, form stable complexes with β-arrestins following ligand stimulation and internalization, which promotes prolonged MAPK signaling compared to G protein-initiated signaling, as exemplified by β-arrestin-ERK1/2 signaling[113]. Often, G protein-dependent ERK1/2 signaling results in increased nuclear ERK1/2 activity[85, 121], however β-arrestin-mediated scaffolding of ERKs has been shown for several receptors to restrict ERK1/2 signaling to the cytosol[122-125]. The function of this type of ERK1/2 signaling is still being explored, but the major effects of cytosolic β-arrestin-ERK1/2 signaling thus far have been shown to impact processes involved in cardiomyocyte survival and hypertrophy such as apoptosis, discussed below, and protein synthesis[84, 125, 126]. AT_1R-β-arrestin2-dependent cytosolic ERK1/2 signaling allows phosphorylation and activation of ribosomal S6 kinase (p90RSK), shown in neonatal cardiomyocytes to increase DNA synthesis and proliferation[125]. In addition, Mnk1 has been shown to interact with β-arrestin 2 and become activated in an AT_1R-β-arrestin-ERK1/2-dependent manner in VSMC, leading to phosphorylation of the cap binding complex member protein eukaryotic translation initiation factor 4E (eIF4E) and increased protein synthesis, which could be a mechanism common to cardiomyocytes as well (Fig. 2)[84]. Interestingly, ERK1/2 activity downstream of some GPCRs has been shown to be reciprocally regulated by β-arrestins 1 and 2. G protein-independent ERK2 activation downstream of the AT_1R, for instance, has been demonstrated to be mediated by β-arrestin 2, whereas β-arrestin 1 impedes β-arrestin 2-mediated ERK2 scaffolding and subsequent activation[127]. Although an initial report has revealed opposing roles for β-arrestins 1 and 2 in the regulation of neointimal hyperplasia[128], the consequence of such reciprocal regulation of ERK signaling in the heart has not been studied.

β-arrestin-dependent EGFR transactivation

An additional mechanism by which β-arrestins direct ERK1/2 signaling and may impact cardiomyocyte hypertrophy, as well as survival, is via transactivation of epidermal growth factor receptor (EGFR). Several GPCRs have been reported to induce EGFR transactivation and ERK1/2 activity, which may contribute to hypertrophy[129-131]. While the molecular pathways involved in this process vary for different GPCRs, a significant role for β-arrestins in βAR-mediated EGFR transactivation has been demonstrated, as siRNA-mediated deletion of either β-arrestin or overexpression of mutant forms of β-arrestins prevents EGFR transactivation, βAR internalization and ERK1/2 activation[132-134]. The importance of β_1AR-mediated EGFR transactivation has been demonstrated in a mouse model of heart failure in which chronic catecholamine stimulation induced dilated cardiomyopathy and increased cardiac apoptosis in mice unable to induce transactivation, compared to mice that were capable of inducing this pathway[133]. The mechanisms relaying survival in response to β_1AR-mediated EGFR transactivation have not been elucidated, but may involve the interaction and cytosolic trafficking of a β1AR-EGFR-ERK1/2 complex in a β-arrestin-dependent manner (Fig. 2)[134]. Similar to β_1AR, urotensin II-mediated EGFR transactivation was recently shown to be β-arrestin-dependent and to reduce cardiac apoptosis in a mouse model of pressure overload compared to mice in which EGFR was inhibited[129]. However,

the role of β-arrestins in GPCR-mediated EGFR transactivation and the effect of this signaling paradigm on cardiomyocyte growth and survival may be GPCR-specific. The Sadoshima group has shown that G protein-independent AT_1R-mediated transactivation of EGFR following Ang II stimulation augments isolated cardiac fibroblast proliferation, as well as both cardiac hypertrophy and apoptosis in vivo[131, 135], though these studies did not specifically explore β-arrestins in these processes. Conversely, a recent report from Smith *et al.* indicates that AT_1R-mediated EGFR transactivation and subsequent hypertrophy is completely dependent upon $G\alpha_{q/11}$ protein coupling in neonatal rat ventricular cardiomyocytes, whereas β-arrestins play no role in this process[136]. Interestingly, ligand-independent AT_1R-mediated EGFR transactivation in the heart in response to mechanical stretch was shown to relay pro-survival signaling, enhancing Akt activation and maintaining lower rates of apoptosis in a β-arrestin 2-dependent manner[137]. Therefore, EGFR transactivation can enhance both cardiac survival and hypertrophy, though the roles of β-arrestins versus G proteins in mediating these processes appear to be GPCR- and ligand-specific.

β-arrestin-mediated anti-apoptotic signaling

Aside from playing a role in EGFR transactivation-mediated anti-apoptotic signaling, the ability of β-arrestins to negatively regulate apoptosis downstream of various GPCRs has been known for some time[138]. The mechanisms relaying this effect have not been completely elucidated, though β-arrestin interaction with proteins involved in the regulation of apoptosis have been identified[114]. Thus far, β-arrestin 2-mediated stabilization of inactive glycogen synthase kinase-3β (GSK3β) has been shown to contribute to a decrease in apoptosis[139], as have interactions of β-arrestins with other proteins. Heat shock protein 27 has been identified as a β-arrestin-interacting protein that confers cytoprotective signaling following β_2AR stimulation by decreasing caspase activity[140]. Also, apoptosis signal-regulating kinase 1 (ASK1) has been shown to associate with β-arrestins, which promote the ubiquitination and proteosomal degradation of ASK1, thereby decreasing rate of apoptosis[141]. ERK1/2 signaling has been shown to mediate many β-arrestin-dependent effects, including apoptosis. AT_1R-β-arrestin 2-ERK-mediated phosphorylation of p90RSK in VSMC has been demonstrated to promote phosphorylation of BAD (Fig. 2), a regulatory protein involved in the promotion of cellular apoptosis[126]. p90RSK-mediated phosphorylation of BAD increases its association with the scaffolding protein 14-3-3 and conversely decreases its association with the pro-apoptotic Bcl-xL, thereby diminishing VSMC apoptosis[126]. This mechanism has since been confirmed by another group studying GLP-1 receptor-β-arrestin 1-mediated effects on apoptosis[142]. Identification of the mechanisms by which β-arrestins regulate apoptosis downstream of GPCRs specifically in the heart requires additional study, but will help define the impact of β-arrestins on cardiac remodeling during the progression of a pathologic state such as heart failure.

β-arrestin-dependent regulation of gene expression

Recent studies have begun to describe the effect of β-arrestin signaling on gene expression as well as on specific transcriptional regulators. Stimulation of various GPCRs can increase the association of β-arrestins with proteins including the NFκB inhibitor protein IκBα and the histone acetyltransferase p300, which can enhance or diminish transcriptional activity[143-145]. In addition, β-arrestins have been demonstrated to play a complex role in ET_AR-mediated control of β-catenin phosphorylation and nuclear translocation, promoting a transcriptional response in ovarian cancer cells, also reviewed recently[146]. While many studies exploring the impact of β-arrestin-mediated signaling on transcription have focused on cancer progression, immune responses and CNS signaling[145-147], the role of β-arrestins in regulating gene expression in response to cardiac-expressed GPCRs has begun to be characterized. β-arrestin 1 was demonstrated to be essential for the β_1AR-mediated increase

in protein content and fetal gene expression in neonatal rat cardiomyocytes in response to catecholamine stimulation[148]. While β-arrestin-1 was demonstrated to be important in this process, the intermediate signaling components between β-arrestin 1 and increased gene expression were not completely elucidated, although a role for Akt was confirmed[148], a protein kinase known to be involved in mediating cardiac hypertrophy[51].

The role of β-arrestin signaling in the induction of cell proliferation and gene expression has been explored most extensively in response to AT_1R stimulation in cell culture models. AT_1R-mediated EGFR transactivation was shown to increase VSMC DNA synthesis in a β-arrestin 2-ERK-dependent manner[130], though the regulation of gene expression was not directly measured. While Ang II was shown in increase the expression of hundreds of genes in HEK 293 cells, AT_1R-β-arrestin-dependent signaling was found to increase the expression of very few genes, indicating that β-arrestins normally act to dampen the AT_1R-$G\alpha_{q/11}$ protein-induced hypertrophic response without directly contributing to a significant alteration in gene expression[66, 81]. Although β-arrestin signaling was shown to lack a robust impact on the regulation of genes directly downstream of AT_1R, AT_1R-β-arrestin signaling was demonstrated to potentiate the gene regulation response induced by β_2AR stimulation[66]. Crosstalk between AT_1R- and βAR-induced signaling on cardiomyocyte contractility and ERK activation has been established[65, 67], thus could be an important feature of β-arrestin-mediated effects on hypertrophy. AT_1R-β-arrestin signaling has also recently been demonstrated to increase phosphorylation of transcriptional regulators commonly associated with distinct GPCR systems[116], therefore studying the coordinated effects of multiple stimulated GPCRs could provide a more comprehensive understanding of gene expression changes.

3. Concluding remarks

With the increasingly diverse array of G protein-dependent and -independent signaling pathways identified that contribute to GPCR-mediated regulation of cardiac function, there exist several challenges in trying to interpret and translate them into therapeutic strategies. A significant challenge lies in extrapolating information from the diverse array of model systems used for exploring signaling mechanisms to a clinical setting, especially with regard to β-arrestin-mediated effects on cardiac function. Since G protein-dependent signaling has been investigated for decades, studies have been performed in several neonatal and adult cardiomyocyte cell systems and in whole heart in vivo, giving credence to the importance of these pathways in humans. Still, as more detailed information describing previously unappreciated roles for known effectors or novel regulators of G protein-dependent signaling are reported, further validation of their contribution to the regulation of human cardiac function is needed. Although numerous signaling pathways have been shown to be activated in a β-arrestin-dependent manner, as demonstrated mainly in AT_1R- and βAR-focused studies in non-cardiomyocyte cell models, the use of these networks in the regulation of cardiac function under normal or pathologic conditions, and in response to other GPCRs, remains to be fully explored. Another challenge relates to determining the significance of two or more signaling pathways mediating similar processes via modulation of either the same or different targets. For instance, while AT_1R stimulation may regulate hypertrophy via $G\alpha_{q/11}$-, $G\alpha_{12/13}$- and β-arrestin-dependent pathways, is there redundancy in the activation of these pathways, or do they each serve a specific purpose during pathological development of heart failure? As well, the precise spatiotemporal targeting of signaling scaffolds by AKAPs, $G\beta\gamma$ subunits and β-arrestins introduces an extra level of consideration for how GPCR-mediated effects on cardiac function may be fine-tuned. Further complexity lies within the interaction of different receptor systems at a given time in physiologic or pathologic regulation of cardiac function. Since the simultaneous activation of numerous GPCRs has the potential to initiate myriad signaling pathways, how are these

independent and/or overlapping events integrated to regulate cardiac contractility and/or hypertrophy? Although assembling a comprehensive interpretation of GPCR-mediated regulation of cardiac function is difficult, it is also exciting as points of interaction between G protein-dependent and -independent pathways continue to be discovered.

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Non-standard Abbreviations and Acronyms

α_1AR	α_1-adrenergic receptor
AC	adenylyl cyclase
AKAP	a kinase-anchoring protein
Ang II	angiotensin II
ASK1	apoptosis signal-regulating kinase 1
AT_1R	angiotensin II type 1 receptor
βAR	β-adrenergic receptor
CAMKII	calmodulin-dependent protein kinase II
cMyBP-C	cardiac myosin-binding protein C
CREB	cAMP response element binding protein
cTnI	cardiac troponin I
cTnT	cardiac troponin T
DAG	diacylglycerol
DKG	diacylglycerol kinase
EGFR	epidermal growth factor receptor
eIF4E	eukaryotic translation initiation factor 4E
EPAC	exchange protein activated by cAMP
ERK	extracellular signal-regulated kinase
ET_AR	endothelin type A receptor
GAP	GTPase-activating protein (GAP)
GDI	guanine nucleotide dissociation inhibitor
GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
GSK3β	glycogen synthase kinase-3β
IP_3	inositol trisphosphate
JNK	c-Jun NH(2)-terminal kinase
K_{ATP}	ATP-sensitive K^+ channel

LTCC	L-type calcium channel
M_2R	muscarinic acetylcholine receptor 2
MAPK	mitogen-activated protein kinase
MEF	myocyte enhancer factor
MLCK	myosin light chain kinase
MMP/ADAM	matrix metalloproteinase/a disintegrin and metalloproteinase
Mnk	MAP kinase-interacting kinase
MSK	mitogen and stress activated kinase
MYPT1	myosin-binding subunit of myosin phosphatase
NDPK B	nucleoside diphosphate kinase B
NFAT	nuclear factor of activated T cells
p70S6K	70 kDa ribosomal S6 kinase
p90RSK	90 kDa ribosomal S6 kinase
$P2Y_6$	purinergic $P2Y_6$ receptor
PDE	phosphodiesterase
PI3K	phosphoinositide-3-kinase
PKA	protein kinase A
PKC	protein kinase C
PKD	protein kinase D
PLB	phospholamban
PLC	phospholipase C
PP-1	protein phosphatase-1
RGS	regulators of G protein signaling
ROCK	RhoA kinase
RyR	ryanodine receptor
$S1P_1R$	sphingosine-1-phosphate receptor 1
SERCA	sarcoplasmic reticulum calcium ATPase
SII	[Sar^1, Ile^4, Ile^8]-angiotensin II
SR	sarcoplasmic reticulum
VSMC	vascular smooth muscle cell

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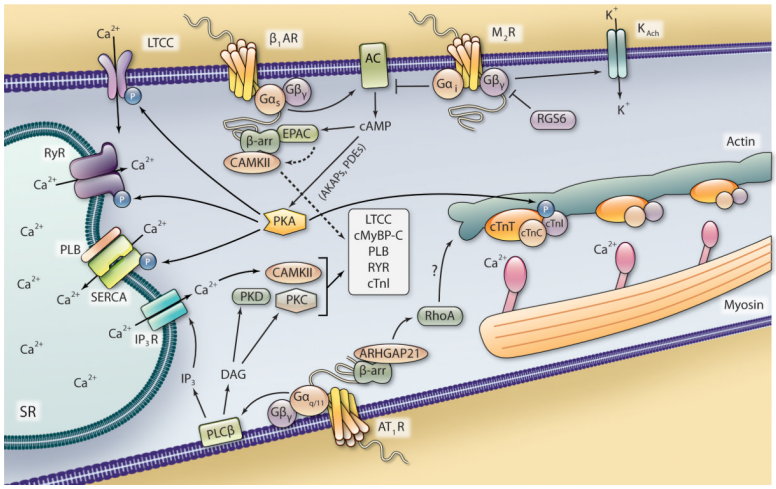

Figure 1. Proposed G protein- and β-arrestin-dependent mechanisms of contractility in ventricular myocytes

Stimulation of the $G\alpha_s$-coupled β_1AR leads to AC-mediated generation of cAMP and increased PKA activity, which can be regulated in subcellular domains by AKAPs and PDEs. PKA signaling enhances contractility via phosphorylation of cTnI, RyR, LTCC and PLB. Modulation of the contractile machinery as well as Ca^{2+} entry and release of SR-stared Ca^{2+}, which binds to the myofilaments (actin, myosin and troponin complex), act to induce contraction. A β-arrestin-dependent scaffold including EPAC and CAMKII can be recruited to β_1AR upon stimulation, allowing cAMP-EPAC-mediated activation of CAMKII and regulation of contractility. Stimulation of the $G\alpha_i$-coupled M_2R antagonizes AC activity and releases Gβγ subunits that can open K^+ channels to hyperpolarize the cardiomyocyte and dampen the contractile response, which is antagonized by RGS6. Stimulation of the $G\alpha_{q/11}$-coupled AT_1R leads to PLCβ-mediated generation of DAG, which subsequently leads to activation of PKC and PKD, and IP_3, which induces the IP_3R-mediated release of Ca^{2+} from the SR that can activate CAMKII, all of which can regulate some or all of the same myofilament and ion channel targets as PKA. β-arrestin scaffolds ARHGAP21 in response to AT_1R stimulation, which leads to RhoA activation and effects on cytoskeletal structure, potentially influence cardiac contractility.

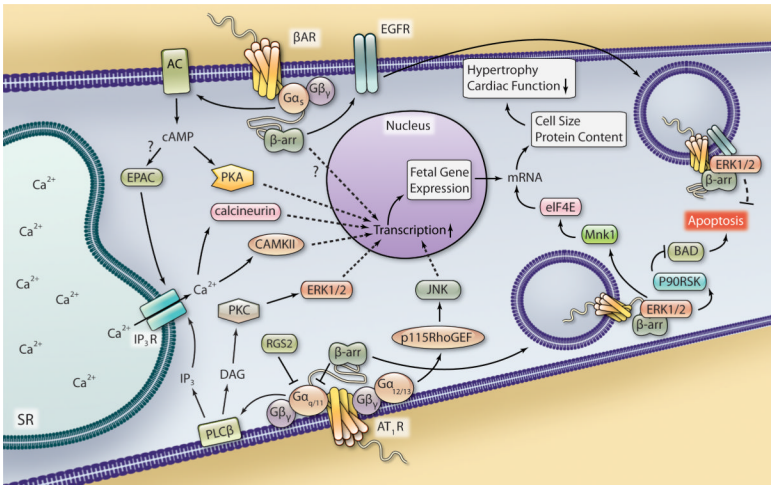

Figure 2. Proposed G protein- and β-arrestin-dependent regulation of ventricular myocyte hypertrophy and apoptosis

Stimulation of the AT_1R leads to $G\alpha_{q/11}$-mediated signaling that can be antagonized by RGS2 and β-arrestin recruitment. PLCβ activity leads to DAG and IP_3 generation and downstream activation of PKC, ERK1/2, CAMKII and calcineurin, each of which can increase the transcriptional response in the nucleus. AT_1R-$G\alpha_{12/13}$-mediated signaling through p115RhoGEF leads to JNK activation that can also regulate transcription. βAR-$G\alpha_s$ stimulation leads to AC-generated cAMP accumulation and increased PKA activity, which can also modulate gene transcription. EPAC activation, possibly downstream of βAR stimulation, also leads to CAMKII and calcineurin activation via Ca^{2+} mobilization. Increased cardiomyocyte transcription in response to hypertrophic stimuli can lead to an increase in fetal gene expression. β-arrestin-mediated βAR signaling can also regulate hypertrophy via an unknown mechanism. Also, β-arrestin-dependent β_1AR-mediated EGFR transactivation decreases cardiac apoptosis, possibly via internalization of a β_1AR-EGFR-ERK1/2 complex that directs an unknown cytosolic cell survival response. Internalization of an AT_1R-β-arrestin-ERK1/2 complex has been shown to increase Mnk1 activation to enhance eIF4E-mediated mRNA translation, which could contribute to an increase in cell size and protein content, thus hypertrophy and decreased cardiac function in response to hypertrophic stimuli. AT_1R-β-arrestin-ERK1/2-mediated activation of p90RSK has been shown to inhibit BAD-induced apoptosis, which could contribute to cardiomyocyte cell survival.

Table 1

Cardiac Gα proteins

Gα Protein	Primary Effectors (2nd messengers)	Downstream Mediators of Signaling	Functional Effects	GPCR Examples	Refs
$G\alpha_s$	AC (↑cAMP)	PKA, EPAC, MAPK, CAMKII	↑Inotropy ↑Chronotropy ↑Hypertrophy	A_2AR, β_1AR, RXFP1	16, 17, 21, 22, 24, 35, 65, 104-108
$G\alpha_{i1/2/3}$	AC (↓cAMP)	PI3K, MAPK (via Gβγ scaffolding)	↓Inotropy ↓Chronotropy ↓Hypertrophy	β_2AR, M_2R, $S1P_1R$	29-31, 40, 41, 47, 109, 110
$G\alpha_{q/11}$	PLCβ (↑DAG and IP_3)	PKC, PKD, CAMKII, MAPK	↑Inotropy ↑Chronotropy ↑Hypertrophy	α_1AR, AT_1R, ET_aR	53, 57, 61-65, 73-75, 77, 78, 80, 82, 88, 89
$G\alpha_{12/13}$	RhoGEFs (↑RhoA activity) [no 2nd messenger]	ROCK, MAPK	?Inotropy ?Chronotropy ↑Hypertrophy	α_1AR, AT_1R, $P2Y_6$	96-100, 102

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