G Protein–Dependent and G Protein–Independent Signaling Pathways and Their Impact on Cardiac Function

Douglas G. Tilley

Abstract: G protein-coupled receptors signal through a variety of mechanisms that impact cardiac function, including contractility and hypertrophy. G protein–dependent and G protein–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 G protein-coupled receptor signaling. (Circ Res. 2011;109:217-230.)

Key Words: β-arrestin ■ cardiac contractility ■ G protein-coupled receptor ■ hypertrophy
relay such an effect is possible because of the variety of G protein–dependent and G protein–independent pathways that can be initiated on GPCR stimulation. G protein–dependent signaling pathways have been explored in the heart for decades, revealing significant roles for the Gq11, Gt11, Gq12/13, and Gβγ families in mediating contractile or hypertrophic responses in the heart. Newer to the field of cardiac research, G protein–independent signaling has been studied for only the past 15 years, with specific roles for β-arrestin–mediated signaling in the regulation of cardiac contractility and hypertrophy reported only in the past 5 years. The multitude of cardiac signaling pathways regulated by G proteins and β-arrestins downstream of GPCR activation provide 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–dependent and β-arrestin–dependent signaling.

**G Protein–Dependent Signaling**

The heterotrimeric G protein complex comprises a Gα subunit, of which there are 4 main families (Gαs, Gαq/11, and Gαq/12/13), coupled to a combination of Gβ and Gγ subunits, of which there exist 5 and 12 members, respectively. The specific classifications, isoforms, and various subunit compositions of the numerous G proteins have been described elsewhere. The Gα proteins primarily expressed and studied in the heart include Gαs, Gαq12/23, and Gαq11, and Gαq12/13 (Table). GPCR stimulation leads to a change in conformational of the receptor such that it promotes nucleotide exchange at Gα of guanosine diphosphate (GDP) for guanosine triphosphate (GTP). The active GTP-bound form of Gα dissociates from the receptor and Gβγ subunits and subsequently activates/inhibits downstream effector proteins, although Gα subtype-selective molecular rearrangement with Gβγ subunits in the absence of dissociation has also been reported. In recent years, an expansive array of accessory proteins that modulate G protein activity has been described, including activators of G protein signaling and regulators of G protein-signaling (RGS) proteins. Members of these families may contain GTPase-activating protein, guanine nucleotide exchange factor inhibitor 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 guanine nucleotide dissociation inhibitor-containing proteins act to inhibit the dissociation of GDP from Gα subunits, thereby inhibiting Gα-protein–mediated signaling. RGS proteins containing GTPase-activating protein activity accelerate the GTPase activity of Gα subunits, thereby decreasing the amplitude and duration of Gα-protein–mediated signaling, although these effects appear to be limited to mainly Gq11 and Gαi1/2/3. The impact of G protein–dependent signaling via various GPCRs and downstream effector proteins on the regulation of cardiac function are discussed with regard to the molecular mechanisms by which they impact cardiac contractility and hypertrophy.

**G Protein-Dependent Effects on Cardiac Contractility**

**cAMP-Mediated Regulation of Cardiac Contractility**

The mechanisms by which Gαs protein activity enhance heart rate and contractility are best exemplified by β1AR signaling (Figure 1). β1AR stimulation results in adenyl cyclase (AC)-mediated generation of cAMP and subsequent activation of protein kinase A (PKA). Via phosphorylation of numerous substrates involved in the contractile response, including the ryanodine receptor, phospholamban (PLB), the L-type calcium channel (LTCC), cardiac troponin I (cTnl), and cardiac myosin-binding protein C, PKA signaling enhances contractile function, as eloquently reviewed elsewhere. Briefly, PKA-mediated phosphorylation of ryanodine receptor and LTCC (to increase Ca2+ uptake and sarcoplasmic reticulum (SR) release), PLB (to release its inhibitory effects on the SR calcium ATPase and promote Ca2+ SR storage), and TnI and cardiac myosin-binding protein C (to decrease Ca2+ affinity for the myofilaments and alter cross-bridge kinetics) each contribute to the inotropic and lusitropic effects of β-adrenergic stimulation. Whereas
few $G_{\alpha}$ protein-coupled receptors have been shown to augment inotropy to the same physiological extent of $\beta$AR stimulation, modulation of $\beta$AR-dependent effects by other $G_{\alpha}$ protein-coupled receptors, as recently demonstrated by type 2 adenosine receptor ($A_2\text{A}R$) subtype-specific effects on $\beta$AR-mediated contractility,\textsuperscript{27} may be of importance in vivo. Additionally, in the pacemaker cells, PKA-mediated phosphorylation of membrane ion channels and $Ca^{2+}$ handling proteins, such as ryanodine receptor and PLB, tightly control $Ca^{2+}$ cycling and heart rate.\textsuperscript{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 also has been demonstrated to regulate cardiomyocyte $Ca^{2+}$ handling and myofilament protein phosphorylation.\textsuperscript{19} Through mechanisms involving phospholipase C$\gamma$ (PLC$\gamma$), protein kinase C$\varepsilon$ (PKC$\varepsilon$), and calmodulin-dependent protein kinase II (CAMKII), EPAC has been shown to increase cTnI, ryanodine receptor, and PLB phosphorylation, $Ca^{2+}$ release from SR stores and sarcomeric shortening in response to either $\beta$AR stimulation or direct EPAC activation.\textsuperscript{20,21} Additionally, an interaction between CAMKII, EPAC1, and the scaffolding proteins $\beta$-arrestin 1 and $\beta$-arrestin 2 that was enhanced upon $\beta$AR stimulation was demonstrated in the heart (Figure 1).\textsuperscript{24} By providing a scaffold for both CAMKII and EPAC1, $\beta$-arrestins facilitate $\beta$AR-EPAC1–PKC$\gamma$–CAMKII activation and downstream PLB phosphorylation.\textsuperscript{24}

Cardiac electrophysiological processes\textsuperscript{25,26} also have been shown to be regulated by cAMP-dependent processes, because both PKA and EPAC have been shown to regulate ion channel activity. Whereas PKA-mediated phosphorylation of the ATP-sensitive $K^+$ channel increases its activity leading to hyperpolarization,\textsuperscript{27} EPAC activation leads to a $Ca^{2+}$–calciurein–dependent dephosphorylation/inactivation of vascular ATP-sensitive $K^+$ channel, potentially providing a negative feedback mechanism to inactivate the channel when cAMP levels become very high.\textsuperscript{28} Determination of EPAC-mediated effects on cardiac $K^+$ channel activity and the comparative effects of EPAC vs PKA signaling on the contractile machinery and ion flux specifically in cardiomyocytes require further exploration.

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

\textbf{A Kinase-Anchoring Protein-Mediated Regulation of Cardiac Contractility}

Via interactions with A kinase-anchoring proteins (AKAPs), PKA activity can be tethered to different substrates in subcellular environments, providing precise spatiotemporal regulation of cardiac function.\textsuperscript{32} Studies over that past decade have shown that intracellular targeting of other components of cAMP-mediated signaling immediately downstream of $\beta$ARs, including AC and cAMP phosphodiesterases by AKAPs, tightly controls $\beta$AR signaling,\textsuperscript{33} as is discussed in another review in this series. Aside from $\beta$AR–AKAP complexes, other GPCR–AKAP signaling complexes are beginning to be reported. The relaxin receptor was recently shown to be precisely regulated by constitutive association with AKAP79–AC2 and $\beta$-arrestin 2–PKA–PDE4D3 complexes, which coordinately control local generation and hydrolysis of cAMP in response to low concentrations of relaxin.\textsuperscript{34} Whereas relaxin has been shown to exert positive inotropic and chronotropic responses in the heart,\textsuperscript{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 also have been shown to regulate contractility at the level of the sarcomere as cardiac troponin T has been reported to act as an AKAP, targeting PKA activity to the sarcomere.\textsuperscript{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, which tightly controls cAMP-induced channel activity and current, thereby modulating cardiac hyperpolarization. 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.

**Gβγ-Mediated Regulation of Cardiac Contractility**
Similar to the function of AKAPs, Gβγ subunits can serve as a protein scaffold. It has been shown that β2AR–Gαi coupling leads to Gβγ-mediated confinement of Gαi–cAMP–PKA signaling via increased phosphoinositide-3-kinase activity. In particular, Gγ, phosphoinositide-3-kinase–dependent regulation of phosphodiesterase-4 activity was shown to control local cAMP signaling in response to β2AR stimulation and to dampen the βAR-mediated inotropic response in cardiomyocytes. Further, Gβγ interaction with nucleoside diphosphate kinase B was shown to regulate basal contractility in several cardiac cell models. The nucleoside diphosphate kinase B-induced transfer of a phosphate to Gβγ allows the local generation of GTP bound to Gαi and subsequent Gαi activation, AC-mediated cAMP synthesis, and cardiomyocyte contractility. Interestingly this process only impacts receptor-independent cAMP synthesis and cardiomyocyte contractility, because activated GPCRs, such as βARs, act as GEFs themselves to induce Gαi protein exchange of GDP for GTP.

Gβγ subunits can also promote negative inotropy via effects on ion channels, because Gβγ-mediated inhibition of LTCC current after βAR stimulation has been reported. Also, via a Gβγ-dependent mechanism, both muscarinic acetylcholine receptor 2 and sphingosine-1-phosphate receptor-1 have been demonstrated in atrial and ventricular myocytes to increase the open probability of the K+ channel IKAch, promoting membrane hyperpolarization to decrease the action potential duration, thereby decreasing chronotropy and inotropy (Figure 1).

**Figure 1. Proposed G protein–dependent and β-arrestin–dependent mechanisms of contractility in ventricular myocytes.** Stimulation of the Gαs–coupled β2AR leads to AC-mediated generation of cAMP and increased PKA activity, which can be regulated in subcellular domains by AKAPs and phosphodiesterases. PKA signaling enhances contractility via phosphorylation of cTnI, RyR, LTCC, and PLB. Modulation of the contractile machinery as well as Ca2+ entry and release of SR-stored Ca2+, 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 β2AR on stimulation, allowing cAMP-EPAC–mediated activation of CAMKII and regulation of contractility. Stimulation of the Gαi–coupled muscarinic acetylcholine receptor 2 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 Gαq/11–coupled AT1R leads to PLC–mediated generation of diacylglycerol, which subsequently leads to activation of PKC and PKD, and IP3, which induces the IP3 receptor-mediated release of Ca2+ 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 AT1R stimulation, which leads to RhoA activation and effects on cytoskeletal structure, potentially influence cardiac contractility. (Illustration credit: Cosmocyte/Ben Smith).
promoting the reassembly of Goα subunits and Gβγ subunits into the heterotrimeric G protein complex, this study suggests that RGS6 provides a negative feedback mechanism to turn-off Goα-Gβγ-mediated hyperpolarization. Genetic ablation of RGS6 resulted in prolonged Ik,activity in both atrial myocytes and sinoatrial node cells, leading to bradycardia.59

**Goq11-Mediated Regulation of Cardiac Contractility**

Cardiac Goq11-protein-coupled receptors increase cardiac inotropy by modulating intracellular Ca2+ levels and contractile protein phosphorylation via PLCβ-mediated conversion of membrane inositol phospholipids into the second messenger inositol trisphosphate and diacylglycerol.60 Enhanced second messenger signaling downstream of Goq11 increases SR-dependent Ca2+ mobilization and activates a number of cardiac PKC isoforms, protein kinase D, and CAMKII.51–53 Collectively, these kinases have been shown to modulate many of the same proteins involved in cardiomyocyte contractility as PKA (Figure 1).54–61 Although acute stimulation of Goq11-protein-coupled receptors increases cardiomyocyte inotropy and chronotropy,62–64 the physiological importance of such stimulation compared to βAR-Goq-mediated inotropy is not well-established. However, a number of important Goq11-activated signaling pathways can contribute to the regulation of contractility, which may be significant because cross-talk between cardiac Goq11-protein-coupled receptors and βAR-Goq signaling has been established.65–68

LTCC, PLB, and ryanodine receptor each contribute to Ca2+ homeostasis and under phosphorylation by PKC and CAMKII signaling.2,58 For instance, phosphorylation of PLB can be increased in a PKCα-dependent manner involving activation of CAMKII,22 or it can be decreased in a PKCα-dependent manner involving protein phosphatase-1-mediated dephosphorylation.56 Recently, the δs isoform of CAMKII was shown in transgenic mice to mediate an alteration in myocyte Ca2+ handling at the SR involving PLB, and that inhibition of its activity specifically at the SR helps to restore diastolic Ca2+ handling.69 Several putative PKC phosphorylation sites on LTCC have been reported,70 which augments Ca2+ influx in the cardiomyocyte to promote Ca2+-mediated Ca2+ 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 phosphoinositide-3-kinase–dependent mechanism after AT1R stimulation.57

Beyond the control of ion flux, PKC and protein kinase D also have been reported to associate with or phosphorylate components of the cardiac contractile machinery, resulting in differential effects on Ca2+ sensitivity and cross-bridge kinetics.54,60,61 For instance, cTnI has been demonstrated to interact with PKCα after increased Ca2+ signaling, which may result in the maintenance of contractile force.58 In addition, cTnI has been shown to undergo phosphorylation by PKCβ1 to increase Ca2+ sensitivity,55 and by PKCα and PKCε to decrease Ca2+ sensitivity in failing human myocardium.54 In the latter study, PKCα-dependent and PKCε-dependent phosphorylation of cardiac myosin-binding protein C, which is known to accelerate cross-bridge cycling, was also shown to be increased in failing human myocardium. Activated protein kinase D has been demonstrated to phosphorylate cTnI to actually decrease Ca2+ sensitivity,71 and may accelerate cross-bridge cycle kinetics via phosphorylation of cardiac myosin-binding protein C.72 Through these combined mechanisms, Goq11-mediated signaling has the capacity to regulate precise events involved in cardiac Ca2+ transport and contractility.

**G Protein-Dependent Effects on Cardiac Hypertrophy**

Goq11 Protein–Mediated Effects on Cardiac Hypertrophy

Whereas the physiological relevance of Goq11-protein signaling on cardiac contractility may not be as well-established as Goq-protein–mediated effects, Goq11 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 physiological adaptive response to increased cardiovascular workload or can contribute to the pathological 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 (Figure 2).76 The hypertrophic role of Goq11 in the heart has been studied using genetic approaches to knockdown or inhibit Goq11 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 Goq11 activity, as reviewed by others.75,77 The regulation of Goq activity by RGS2, which normally dampens Goq signaling in the heart, also has 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 mitogen-activated protein kinase (MAPK) activity,78 suggesting that RGS2-mediated inhibition of Goq signaling could be an effective means by which to prevent cardiac hypertrophy. Similar results were shown in a recent study exploring the regulation of AT1R-induced MAPK signaling via RGS5 in neonatal cardiomyocytes.79

Studies also have begun to comprehensively characterize the transcriptional response to Goq11 signaling, revealing hundreds of genes whose expressions are altered after Goq11 activation. These studies have reported an increase in Goq11-dependent transcript detection after stimulation with 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 are primarily dependent on Goq11 signaling.66 By blocking Goq11-protein–dependent signaling, antagonists such as AT1R blockers can diminish the hypertrophic transcription response to stimulation by endogenous factors and reduce the rate of progression of heart failure.3
Aside from the antagonism of Gαq/11-protein-coupled receptors or Gαq/11 itself, inhibition of several downstream regulatory proteins has been shown to interfere with Gαq/11-mediated hypertrophic responses. As discussed, Gαq/11 activation initiates PLCβ-mediated phospholipid hydrolysis and second 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. 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 Gαq/11-coupled receptors mediate cell growth responses. 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. Via both cytosolic and nuclear actions, ERK1/2 signaling has been shown in different cell types to increase DNA transcription and mRNA translation. Such subcellular effects of ERK1/2 also have 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 nuclear factor of activated T cells and the ribosomal S6 kinase p70S6K. 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. 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 and MAP kinase-interacting kinase 1, also has been shown to reduce the production of pro-apoptotic factors and increase cell survival.

**Figure 2. Proposed G protein- and β-arrestin-dependent regulation of ventricular myocyte hypertrophy and apoptosis.** Stimulation of the AT1R leads to Gαq/11-mediated signaling that can be antagonized by RGS2 and β-arrestin recruitment. PLCβ activity leads to diacylglycerol and IP3 generation and downstream activation of PKC, ERK1/2, CAMKII, and calcineurin, each of which can increase the transcriptional response in the nucleus. AT1R-Gα12/13-mediated signaling through p115RhoGEF leads to c-Jun NH2-terminal kinase activation that can also regulate transcription. βAR-Gαq stimulation leads to AG-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 Ca2+ mobilization. Increased cardiomyocyte transcription in response to hypertrophic stimuli can lead to an increase in fetal gene expression. The β-arrestin-mediated βAR signaling also can regulate hypertrophy via an unknown mechanism. Also, β-arrestin–dependent βAR-mediated EGFR transactivation decreases cardiac apoptosis, possibly via internalization of a βAR–EGFR–ERK1/2 complex that directs an unknown cytosolic cell survival response. Internalization of an AT1R–β-arrestin–ERK1/2 complex has been shown to increase MAP kinase–interacting kinase 1 activation to enhance eukaryotic translation initiation factor-4E–mediated mRNA translation, which could contribute to an increase in cell size and protein content, and thus hypertrophy and decreased cardiac function in response to hypertrophic stimuli. AT1R–β-arrestin–ERK1/2–mediated activation of 90-kDa ribosomal S6 kinase has been shown to inhibit BAD-induced apoptosis, which could contribute to cardiomyocyte cell survival. (Illustration credit: Cosmoscyte/Ben Smith).
hypertrophic response to Gq/11-protein-coupled receptors, such as the α1AR, in cardiomyocytes.89,90

CAMKII-Mediated Effects on Cardiac Hypertrophy
In addition to PKC–MAPK signaling, activation of Goq/11 mediates hypertrophy via other mechanisms. PLCβ-generated inositol trisphosphate binds to inositol trisphosphate receptors on the SR to increase the release of stored Ca2+ into the cytosol, where it binds calmodulin (CAM). The resulting Ca2+/CAM complex interacts with and activates numerous proteins, including CAMKII and calcineurin (Figure 2). A protein phosphatase that regulates nuclear factor of activated T cells 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 Brown et al73 using various genetic mouse models. 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 δn, and δc cardiac isoforms of CAMKII in the nucleus and cytosol, respectively, transgenic expression of each isoform enhanced cardiac hypertrophic gene expression by promoting histone deacetylase-4 extrusion from the nucleus.92 Interestingly, genetic deletion of CAMKIIδ in mice did not prevent the development of hypertrophy, ostensibly attributable to a compensatory increase in CAMKIIγ activity, but did attenuate heart failure progression after pressure overload attributable to a loss of altered expression of Ca2+ regulatory proteins.93 This reveals a CAMKII isomorph-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,33 including myocardial ischemia, ischemia/reperfusion, and transgenic overexpression of Goq. Thus, inhibition of CAMKII signaling appears to be a viable mechanism to attenuate hypertrophy and progression to heart failure, although isomorph-specific targeting and compensatory effects may need further exploration.

Goq12/13-Mediated Effects on Cardiac Hypertrophy
Contrary to Goq/11 signaling, Goq12/13 activation does not lead to the generation of second messengers, but rather 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 Goq213 signaling in the heart is still relatively unexplored, several studies from the Kurose laboratory have shown a role for Goq12/13 in mediating cardiac hypertrophy and fibrosis. AT1R, α1AR, and ET-1 stimulation were each shown in neonatal cardiomyocytes or cardiac fibroblasts to be capable of inducing hypertrophic or fibrotic responses, mainly via Goq12/13-p115RhoGEF–dependent activation of the MAPK c-Jun NH2-terminal kinase (Figure 2).96–99 Another group also has reported that AKAP–Lbc acts both as a scaffold to induce α1AR-mediated p38 MAPK activation and as a RhoGEF to activate RhoA after α1AR–Goq12/13 stimulation in neonatal cardiomyocytes.100,101 In addition, it was shown that either mechanical stretch or direct stimulation of the purinergic P2Y1 receptor increases cardiomyocyte fibrosis via Goq12/13 and that P2Y1 inhibition in vivo prevented fibrosis, but not hypertrophy, in response to pressure overload.102 Thus, although Goq12/13 effects in the heart have not been as extensively studied as Goq/11-mediated effects, they may be important mediators of cardiac hypertrophy and fibrosis.

cAMP-Dependent Effects on Cardiac Hypertrophy
Although a role for Goq in the development of hypertrophy has been recognized for many years,103 as illustrated via transgenic overexpression of cardiac Goq,104,105 the mechanisms controlling hypertrophy downstream of Goq and cAMP generation remain controversial. At the level of Goq, a recent study highlighted the ability of RGS2 to influence the hypertrophic response to βAR stimulation, because 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 myocadrial 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-mediated and inositol trisphosphate receptor-mediated increased Ca2+ signaling, and that both calcineurin-dependent nuclear factor of activated T-cell transcription and CAMKII-dependent myocyte enhancer factor-2 activation contributed to the hypertrophic response.108 Although the mechanisms by which EPAC regulates cardiac hypertrophy are still being explored, there is evidence to support a role for EPAC in this process (Figure 2) and to suggest that EPAC could provide a novel therapeutic target.

Goq-Mediated and Gβγ-Mediated Effects on Hypertrophy
Both Goq and Gβγ have been implicated in the development of hypertrophy and the progression of heart failure. An increase in cardiac Goq1 expression was detected in an inducible genetic model of Ras-MAPK–mediated hypertrophy, correlating with alterations in the regulation of intracellular Ca2+ handling and leading to ventricular hypertrophy and arrhythmia, both of which were normalized with the inhibition of Goq via pertussis toxin.109 Also, genetic inhibition of Goq with a cardiac-expressed inhibitory peptide was shown to increase apoptosis after ischemia/reperfusion injury, identifying a cardioprotective role for Goq 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 was reduced, as assessed by cardiomyocyte morphology and changes in fetal gene expression. Because small molecule inhibitors of Gβγ have been shown to differentially modulate
different Gβγ-dependent signaling pathways, the potential to selectively inhibit distinct cardiac Gβγ-mediated hypertrophic effects while preserving contractile function could be advantageous.

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 past 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 Because the role of GRKs in cardiovascular signaling and function are reviewed elsewhere in this series, the following discussion of G protein–independent signaling focuses on 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, and 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,R 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 after stimulation of AT,R also reveals entire AT,R-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,R or βAR stimulation, the increasing array of results may be applicable to other cardiac GPCR systems as they relate to the control of cardiac contractility and hypertrophy.

β-Arrestin–Mediated Effects on Cardiac Contractility

β-Arrestin–Dependent Cardiomyocyte Contractility

In the past 5 years, β-arrestins have been demonstrated to promote cardiomyocyte and cardiac contractility. Studies using β-arrestin–biased AT,R ligands that do not induce Goαq11 protein activation have shown that AT,R–β-arrestin–dependent signaling enhances contractility in isolated adult mouse cardiomyocytes. The first study, which utilized the biased ligand [Sar1, Ile8]-angiotensin II 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 Goαq11–independent response.5 The reliance on β-arrestin 2 in mediating Goαq11–protein–independent contractility in response to AT,R stimulation was confirmed in a more recent study utilizing a distinct β-arrestin–biased AT,R ligand.6 In addition, unbiased activation of AT,R with Ang II in β-arrestin 2 knockout cardiomyocytes produced a blunted contractile response, suggesting that β-arrestin 2–mediated effects on contractility may not be redundant with respect to Goαq11 protein–dependent signaling. Violin et al7 have recently demonstrated in whole animals that infusion of synthetic β-arrestin–biased AT,R peptide ligands cause increased cardiac contractility. Interestingly, although these β-arrestin–biased AT,R ligands increased cardiac contractility and decreased blood pressure, they did not alter stroke volume, unlike conventional AT,R blockers.7 The therapeutic implications for these ligands are 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 mechanisms 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,R. AT,R–β-arrestin 1–mediated signaling has been shown to increase RhoA activation and subsequent stress fiber reorganization, whereas 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 GTPase-activating protein (ARHGAP21) after AT,R stimulation was recently demonstrated to promote RhoA activation and stress fiber formation (Figure 1), whereas 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,R, 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–Rho kinase–dependent regulation of myosin light chain kinase activity and plasma membrane blebbing after AT,R stimulation.119 How AT,R 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. Although β-arrestin-mediated activation of RhoA signaling is an attractive explanation for increased cardiomyocyte contractility because RhoA activity can impact regulators of cardiac contractility such as PKC and protein kinase D, 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,R stimulation. These include RhoA kinase, actin, coflin, myosin, and the myosin-binding subunit of myosin phosphatase.
and extend to other proteins involved in more generalized signaling processes. Further, β-arrestin–dependent regulation of Ca2+ transport via transient receptor potential channel has been reported in vascular smooth muscle cells. After Ang II stimulation, a β-arrestin 1–dependent AT1R- transient receptor potential-4 complex undergoes internalization away from the plasma membrane, reducing cation influx in response to continued AT1R stimulation. Altogether, the expanding roles for β-arrestins in the regulation of cation influx, cytoskeletal structure, and cardiomyocyte contractility suggest 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 AT1R remains to be tested.

β-Arrestin-Mediated Effects on Cardiac Hypertrophy

β-Arrestin–Mediated MAPK Activity

Some GPCRs, such as the AT1R, form stable complexes with β-arrestins after 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; however, β-arrestin–mediated scaffolding of ERKs has been shown for several receptors to restrict ERK1/2 signaling to the cytosol. 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 and protein synthesis. AT1R–β-arrestin 2–dependent cytosolic ERK1/2 signaling allows phosphorylation and activation of 90-kDa ribosomal S6 kinase, shown in neonatal cardiomyocytes to increase DNA synthesis and proliferation. In addition, MAP kinase–interacting kinase 1 has been shown to interact with β-arrestin 2 and to become activated in an AT1R–β-arrestin–ERK1/2–dependent manner in vascular smooth muscle cell, leading to phosphorylation of the cap binding complex member protein eukaryotic translation initiation factor-4E and increased protein synthesis, which could be a mechanism common to cardiomyocytes as well (Figure 2). 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 AT1R, 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. Although an initial report has revealed opposing roles for β-arrestins 1 and 2 in the regulation of neointimal hyperplasia, 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. Whereas 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, because siRNA-mediated deletion of either β-arrestin or overexpression of mutant forms of β-arrestins prevents EGFR transactivation, βAR internalization, and ERK1/2 activation. The importance of βAR-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. The mechanisms relaying survival in response to βAR-mediated EGFR transactivation have not been elucidated but may involve the interaction and cytosolic trafficking of a βAR-EGFR-ERK1/2 complex in a β-arrestin–dependent manner (Figure 2). Similar to βAR, 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. 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. Sadoshima et al. have shown that G protein–independent AT1R-mediated transactivation of EGFR after Ang II stimulation augments isolated cardiac fibroblast proliferation, as well as both cardiac hypertrophy and apoptosis in vivo, although these studies did not specifically explore β-arrestins in these processes. Conversely, a recent report from Smith et al. indicates that AT1R-mediated EGFR transactivation and subsequent hypertrophy is completely dependent on Goq/11 protein-coupling in neonatal rat ventricular cardiomyocytes, whereas β-arrestins play no role in this process. Interestingly, ligand-independent AT1R-mediated EGFR transactivation in the heart in response to mechanical stretch was shown to relay prosurvival signaling, enhancing Akt activation and maintaining lower rates of apoptosis in a β-arrestin 2–dependent manner. Therefore, EGFR transactivation can enhance both cardiac survival and hypertrophy, although the roles of β-arrestins vs G proteins in mediating these processes appear to be GPCR-specific and ligand-specific.

β-Arrestin–Mediated Antiapoptotic Signaling

Aside from playing a role in EGFR transactivation-mediated antiapoptotic signaling, the ability of β-arrestins to negatively regulate apoptosis downstream of various GPCRs has been known for some time. The mechanisms relaying this effect have not been completely elucidated, although β-arrestin interaction with proteins involved in the regulation of apoptosis have been identified. Thus far, β-arrestin 2–mediated stabilization of inactive glycogen synthase kinase-3β has been shown to contribute to a decrease in apoptosis, 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 after βAR stimulation by decreasing caspase activity. Also, apoptosis...
signal-regulating kinase 1 has been shown to associate with β-arrestins, which promote the ubiquitination and proteasomal degradation of apoptosis signal-regulating kinase 1, thereby decreasing rate of apoptosis.141 ERK1/2 signaling has been shown to mediate many β-arrestin–dependent effects, including apoptosis. AT1R-β-arrestin 2-ERK–mediated phosphorylation of 90-kDa ribosomal S6 kinase in vascular smooth muscle cells has been demonstrated to promote phosphorylation of BAD (Figure 2), a regulatory protein involved in the promotion of cellular apoptosis.126 The 90-kDa ribosomal S6 kinase–mediated phosphorylation of BAD increases its association with the scaffolding protein 14-3-3 and conversely decreases its association with the proapoptotic Bcl-xL, thereby diminishing vascular smooth muscle cell 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 that will help define the impact of β-arrestins on cardiac remodeling during the progression of a pathological 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 nuclear factor-κB 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 ETαR-mediated control of β-catenin phosphorylation and nuclear translocation, promoting a transcriptional response in ovarian cancer cells (also reviewed recently).146 Although many studies exploring the impact of β-arrestin–mediated signaling on transcription have focused on cancer progression, immune responses, and central nervous system 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 β2AR-mediated increase in protein content and fetal gene expression in neonatal rat cardiomyocytes in response to catecholamine stimulation.148 Whereas 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 was confirmed for Akt,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 AT1R stimulation in cell culture models. AT1R-mediated EGFR transactivation was shown to increase vascular smooth muscle cell DNA synthesis in a β-arrestin 2-ERK–dependent manner,130 although the regulation of gene expression was not directly measured. Whereas Ang II was shown in increase the expression of hundreds of genes in HEK 293 cells, AT1R-β-arrestin–dependent signaling was found to increase the expression of very few genes, indicating that β-arrestins normally act to dampen the AT1R-Gαq/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 AT1R, AT1R–β-arrestin signaling was demonstrated to potentiate the gene regulation response induced by β2AR stimulation.66 Cross-talk between AT1R, R-induced and βAR-induced signaling on cardiomyocyte contractility and ERK activation has been established65,67 and thus could be an important feature of β-arrestin–mediated effects on hypertrophy. AT1R, β-arrestin signaling also has been recently 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.

**Conclusion**

With the increasingly diverse array of G protein–dependent and G protein–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. Because 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 AT1R-focussed and βAR-focussed studies in noncardiomyocyte cell models, the use of these networks in the regulation of cardiac function under normal or pathological 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, although AT1R stimulation may regulate hypertrophy via Gαq/11-dependent, Gα12/13-dependent, 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? Also, the precise spatiotemporal targeting of signaling scaffolds by AKAPs, Gβγ 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 physiological or pathological regulation of cardiac function. Because the simultaneous activation of numerous GPCRs has the potential to initiate myriad signaling pathways, how are these indepen-
dent or overlapping events integrated to regulate cardiac contractility 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 G protein–independent pathways continue to be discovered.

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References


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Douglas G. Tilley

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