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STATE-OF-THE-ART REVIEW

Designer Approaches for G Protein–Coupled Receptor Modulation for Cardiovascular Disease

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SUMMARY

The new horizon for cardiac therapy may lie beneath the surface, with the downstream mediators of G protein–coupled receptor (GPCR) activity. Targeted approaches have shown that receptor activation may be biased toward signaling through G proteins or through GPCR kinases (GRKs) and β-arrestins, with divergent functional outcomes. In addition to these canonical roles, numerous noncanonical activities of GRKs and β-arrestins have been demonstrated to modulate GPCR signaling at all levels of receptor activation and regulation. Further, research continues to identify novel GRK/effector and β-arrestin/effector complexes with distinct impacts on cardiac function in the normal heart and the diseased heart. Coupled with the identification of once orphan receptors and endogenous ligands with beneficial cardiovascular effects, this expands the repertoire of GPCR targets. Together, this research highlights the potential for focused therapeutic activation of beneficial pathways, with simultaneous exclusion or inhibition of detrimental signaling, and represents a new wave of therapeutic development. (J Am Coll Cardiol Basic Trans Science 2018;3:550–62) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

G protein-coupled receptors (GPCRs) have been mainstays of therapeutic drug targeting in the heart for decades, in particular angiotensin II type 1A receptor (AT_1R) blockers and β-adrenergic receptor (βAR) blockers (β-blockers), which improve symptoms, hemodynamics, and clinical outcomes of heart failure (HF) patients. However, improvements on these classic GPCR blockers have been slow to develop over the last couple of decades and many promising new drugs have failed to reduce morbidity and mortality highlighting the need for novel therapeutics. GPCR signaling systems are comprised of numerous molecular components and multiple pathways are emerging as potential therapeutic targets, which have the potential to shift the paradigm of standard HF treatment. Indeed, a rapidly expanding and exciting area of GPCR research is focused on the differential engagement of proximal signaling pathways in a biased manner to promote beneficial functional or survival effects in the heart while preventing activation of potentially cardiotoxic pathways.

It has been appreciated for some time now that there are at least 2 primary mechanistic signaling

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pathways engaged following ligand binding to its GPCR, represented by classical G protein–dependent signaling and noncanonical GRK/β-arrestin-mediated signaling (1-3). For both AT_1R and βARs, prolonged G protein–dependent signaling is associated with detrimental cardiac outcomes over time, while GRK/β-arrestin–dependent signaling has been demonstrated in preclinical models to promote beneficial effects in various HF models. Additionally, global gene expression analyses have identified more than 200 GPCRs present in the heart, several at equal or higher expression than AT_1R and βARs (4), which may represent novel and untapped therapeutic targets by which to improve HF outcomes in either a G protein- or GRK/-β-arrestin–dependent manner. Thus, discovery of compounds, or biased ligands (5,6), that can selectively engage GRK/β-arrestin–dependent signaling is an important and novel area of current GPCR research. Herein, we compare the impact of GRKs and β-arrestins on cardiac function, survival, and remodeling in HF; highlight the latest findings related to biased ligand-mediated engagement of both AT_1R and βARs; and discuss newly discovered GPCR systems that provide promise for the development of novel HF therapeutics.

GPCR KINASES

GPCR signaling is tightly controlled by cytosolic GRKs. Canonically, GRKs translocate to or target agonist-bound GPCRs where they phosphorylate the receptor, facilitating β-arrestin recruitment for desensitization and internalization of receptors. A thorough review of GRK structure, localization, GPCR activity, cardiac function, and regulation has recently been published (7), highlighting how GRKs control GCPR signal duration and impact. Continued research into the regulation, distribution, and noncanonical signaling of cardiac GRKs has demonstrated expanding roles in both normal cardiac function and cardiovascular disease (**Central Illustration**) (8-11). A more thorough understanding of the functional consequences of GRK activities in the heart will allow for targeted approaches for GRK modulation in human therapy.

Despite a high level of shared sequence identity and the same tissue distribution as GRK2, GRK3 has been observed to participate in the regulation of thrombin, endothelin, and α_1-adrenergic receptor (α_1AR) activity in the heart. Although cardiac GRK3 levels are not altered during human HF, these data suggest that it may play a role in cardiac growth and hypertrophy (12,13). Transgenic mice overexpressing the C-terminal pleckstrin homology domain of GRK3 exhibit a phenotype of increased systolic function (14) similar to the hypercontractile phenotype in mice with α_1AR overexpression (15). Further, both lines demonstrate improved function and reduced left ventricular remodeling in models of disease (16-18), suggesting that phosphorylation of cardiac α_1ARs by GRK3 may contribute to the detrimental cardiac signaling during disease. The full functional significance for GRK3 in cardiomyocyte signaling in the heathy heart and during pathophysiological conditions remains to be elucidated and is as yet not a target for HF therapy.

Unlike GRK3, GRK2 and GRK5 expression levels are elevated in human patients during a myriad of cardiovascular diseases (19-22), where both GRKs participate in the desensitization of several important GPCRs (23). The activity of GRK5 at GPCRs is highly sensitive to phosphorylation by PKC or at other autoregulatory sites within the enzyme (24). Cardiomyocyte-restricted overexpression of GRK5 predisposes mice to a significant increase in hypertrophy and rapid transition to HF (25), in large part due to the translocation of this GRK to the nucleus where it directly and indirectly alters the transcriptional regulation of hypertrophic genes with the results dependent on the activating GPCR (25-33). Conversely, GRK5 has been shown to promote cardiomyocyte survival in response to either stretch activation of AT_1R or catecholamine-induced β_1AR stimulation via engagement of β-arrestin-dependent signaling (34,35). Together, these data present a dichotomy, wherein the noncanonical nuclear translocation of GRK5 produces pathological signaling, whereas GRK5 activity at membrane-bound GPCRs may be protective. This is an important consideration regarding the therapeutic potential of GRK5, and suggests that targeting the cellular localization of GRK5 may be more effective than a kinase inhibitor. In fact, a small molecular inhibitor of GRK5 has already been developed and was used to gain a high-resolution crystal structure for bovine GRK5 (36), which will allow for the investigation of allosteric modulation of GRK5 and may provide a means to differentially target nuclear translocation versus kinase activity. The role of GRK5 in the regulation cardiac signaling in the healthy and diseased myocardium and the pursuit for therapeutic inhibition of GRK5 has recently been reviewed in detail (37).

Despite the wealth of knowledge regarding the role of GRK2 in cardiovascular function, the full scope and impact of GRK2 regulation in cardiac physiology and disease is still being defined. Ongoing research continues to identify new interacting partners and

ABBREVIATIONS AND ACRONYMS

AR = adrenergic receptor

AT_1R = angiotensin II type 1A receptor

CRF = corticotropin-releasing factor

EGFR = epidermal growth factor receptor

ERK1/2 = extracellular signal-regulated kinase

GPCR = G protein–coupled receptor

GRK = G protein–coupled receptor kinase

HF = heart failure

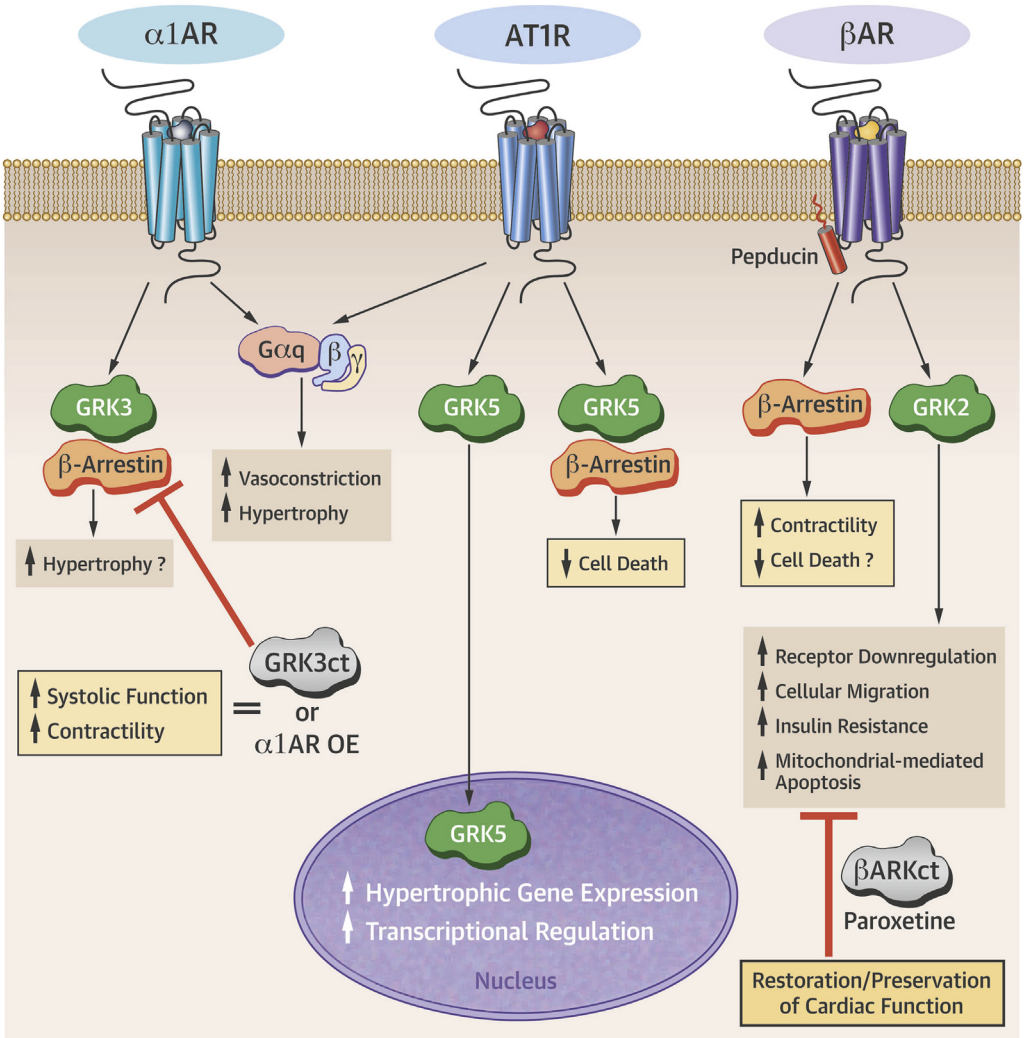
ICL = intracellular loop

PI3K = phosphoinositide 3-kinase

SERCA2a = sarco(endo) plasmic reticulum Ca^{2+}-ATPase

SII = [Sar(1), Ile (4), Ile(8)]-angiotensin II

CENTRAL ILLUSTRATION **GRKs Demonstrate Isoform-Specific Functions in the Regulation of Cardiac Signaling and Function**



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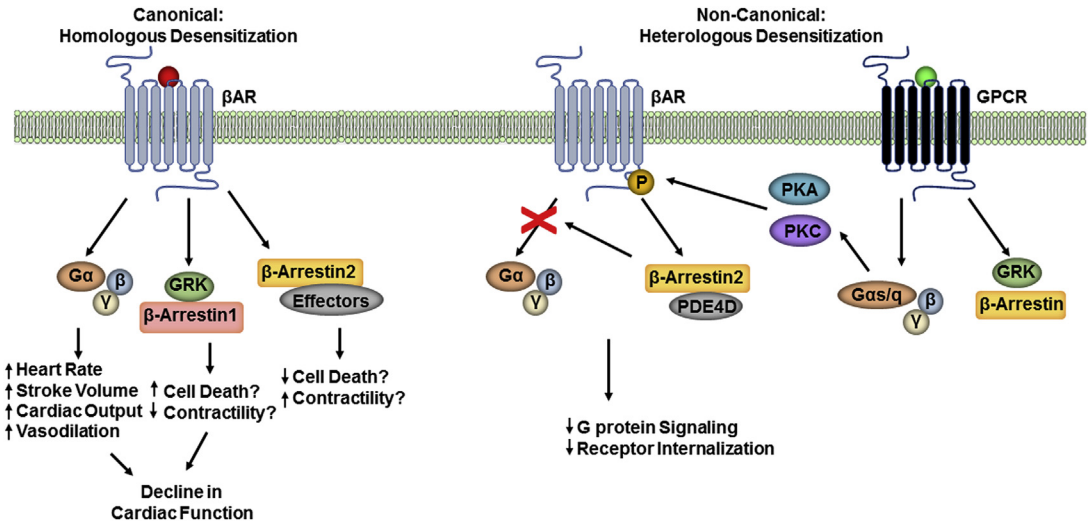
AR = adrenergic receptor; ARKct = adrenergic receptor kinase carboxy-terminal peptide; AT1R = angiotensin II type 1A receptor; beta = beta subunit; G alpha q = G alpha q subunit; gamma = gamma subunit; GRK = G protein-coupled receptor kinase; GRK3ct = G protein-coupled receptor kinase 3 carboxy-terminal peptide; OE = over-expression.

protein targets that facilitate GRK2 signaling activities in a presumably cell type- and state-dependent manner (38-45). The canonical role of GRK2 in regulating βAR signaling in the healthy heart and the diseased heart, as well as the basic and preclinical pursuit of therapeutic GRK2 inhibition, has recently been reviewed in detail (46). The expanding role of GRK2 in regulating cytoskeletal components to modulate cellular migration in physiology and pathophysiology has also been reviewed in detail (47), with implications for inflammatory responses during cardiac disease. GRK2 also plays a critical role in insulin signaling and is a mediator of insulin resistance (48-52), with particular implications for cardiovascular diseases compounded by a metabolic syndrome. Further, GRK2 is a documented regulation of mitochondrial-mediated apoptosis and cell survival (53-61), with significant consequences for cardiac disease progression regardless of etiology. Based on these diverse canonical and noncanonical

FIGURE 1 β-Arrestins Participate in the Regulation of Cardiac GPCR Signaling Through Homologous and Heterologous Desensitization



This is a cartoon representation of the selective roles of β-arrestin1 versus β-arrestin2 in regulating the canonical homologous desensitization of G protein–coupled receptors (GPCRs) versus heterologous GPCR desensitization and their consequences for cardiac signaling. Gα = G alpha subunit; Gαs = G alpha subunit; P = phosphorylation; PKA = protein kinase A; PKC = protein kinase C.

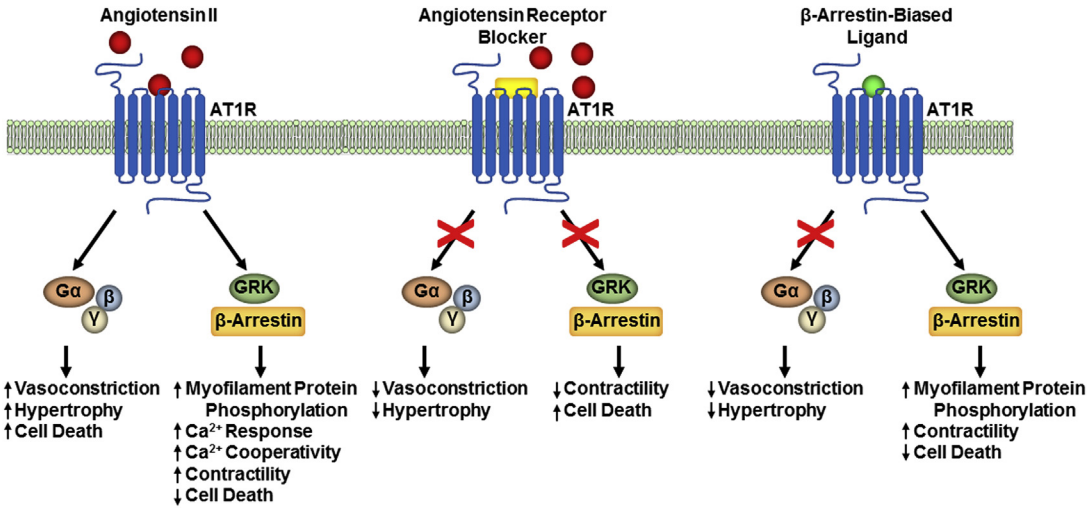
activities GRK2 has arisen as a significant target in diverse pathologies and tissues (62). Inhibition of GRK2 via expression of a carboxyl-terminal peptide, βARKct, that competes with GRK2 binding to Gβγ, enhances cardiac function. In fact, βARKct expression has been shown to prevent and reverse HF in numerous animal models of disease, including a preclinical porcine model of myocardial infarction (63–69). Further, a high-throughput screen for small molecular inhibitors of GRK2 revealed that the Food and Drug Administration–approved selective serotonin reuptake inhibitor paroxetine could selectively inhibit GRK2, and in a mouse model of myocardial infarction paroxetine was able to significantly enhance cardiac function and impair left ventricular remodeling without adverse effects in control animals (70,71). This compound served as a starting point for the rational design of compounds with increased efficacy and selectivity for GRK2 and reduced central nervous system activity (72–75). In addition, many other studies have investigated the therapeutic relevance of paroxetine as a HF therapy, and the design and synthesis of alternative GRK2 inhibitors (76–78). Whether or not such compounds will translate to relevant therapies for human HF, they will be useful research tools to investigate GRK2 kinase function in cell- and disease-dependent states to better understand the diverse activities of this enzyme.

β-ARRESTINS

The canonical role of the GPCR adapter proteins β-arrestin1 and β-arrestin2 in the heart is to participate in 2 ways in the homologous desensitization of GPCRs. Following agonist binding and phosphorylation of receptors by GRKs, β-arrestins associate with the activated receptor to sterically block reassociation of the heterotrimeric G protein subunits and simultaneously facilitate receptor internalization via clathrin-coated vesicles (79). In addition, it is now well recognized that β-arrestins can also act as scaffold proteins or effectors to initiate downstream signaling. Although the concept of β-arrestin-mediated signaling has been around for some time, ongoing research continues to uncover new signaling targets and mechanisms of β-arrestin signaling in altering cardiovascular function. Recent advances in technology have revealed that the receptor type–specific binding interaction between GPCRs and β-arrestins induces a conformational change in the β-arrestins that persists for some time even after dissociation from receptor, allowing for prolonged cell surface signaling (80).

A less well-defined function of β-arrestins is in facilitating heterologous desensitization of GPCRs, in which receptors are phosphorylated and desensitized through interactions with other kinases such as protein kinase A or C (**Figure 1**) (81–85). For example, studies

FIGURE 2 The Physiological Consequences of Agonism, Antagonism, and Biased Agonism of the AT_1R in the Cardiovascular System



This is a cartoon representation outlining the effect on cardiovascular signaling and outcomes when the AT_1R is differentially affected by direct agonism or antagonism versus the potential beneficial effect of β-arrestin–targeted biased agonism. Ca^{2+} = calcium ion; other abbreviations as in Figure 1.

have identified that some Gs- and Gq-coupled receptors promote protein kinase A- and C-mediated phosphorylation of β_2ARs in rat ventricular myocytes, leading to β-arrestin2-dependent recruitment and complex formation with phosphodiesterase 4D in a manner that impairs subsequent β_2AR signaling (86). Similarly, heterologous GRK-dependent signaling initiated from the Gq-coupled vasopressin type 1A receptor was also demonstrated to be capable of reducing β_1AR responsiveness (87), although a β-arrestin–dependent facet of this response was not reported. Altogether, these data suggest an additional means by which elevated neurohormonal stimuli acting at other GPCRs can impair βAR-mediated Ca^{2+} signaling and myocyte contractile responses during disease.

Although thought for some time to be functionally redundant, significant evidence points to distinct outcomes of β-arrestin1 versus β-arrestin2 on cardiomyocyte function and survival, wherein β-arrestin1 may be viewed as cardiotoxic in pathological conditions through its desensitization of β_1ARs and promotion of apoptotic and proinflammatory signaling, whereas β-arrestin2 may generally oppose cell death signaling (88). Recent evidence suggests that β-arrestin2 may also increase contractility through a direct interaction with sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA2a) downstream of β_1ARs in vivo and in vitro, inducing SUMOylation of SERCA2a to enhance its activity (88). Further, in a mouse model of cardiac dysfunction after myocardial infarction, mice with cardiac overexpression of β-arrestin2 demonstrated significantly improved function and increased SERCA2a SUMOylation and activity, with a corresponding decrease in adverse remodeling through apoptosis and fibrosis (88). Although these data may suggest cardiac gene transfer of β-arrestin2 as a viable therapy for HF, opposing data suggest that during acute injury, β-arrestin2 overexpression may be detrimental. In this study, β-arrestin2 expression was selectively upregulated in cultured myocytes and rat models of ischemia-reperfusion injury, promoting cardiomyocyte death and enhanced ischemia-reperfusion-induced injury, whereas β-arrestin2 knockdown or functional deficiency conferred resistance to ischemia-reperfusion injury (89). The worsening phenotype occurred via an interaction between β-arrestin2 and the p85 subunit of phosphoinositide 3-kinase (PI3K) that negatively regulated p85-PI3K/Cav3 complex formation and subsequently blocked PI3K-mediated activation of Akt and glycogen synthase kinase 3β survival signaling (89). These conflicting reports will need to be resolved to confirm the relevance of β-arrestin2 as a therapeutic target for the treatment of human HF. Some of these differential roles for β-arrestin1 and β-arrestin2 and their therapeutic implications have been recently reviewed (90).

β-ARRESTIN–BIASED AT_1R SIGNALING

The AT_1R is of interest for the treatment of HF due to its role in the development and progression of cardiac dysfunction. G protein–mediated signaling through Gαq causes vasoconstriction (91,92) and hypertension, whereas in the heart it causes hypertrophy (6,91). Although β-arrestin signaling downstream of the AT_1R is important for receptor desensitization (93) and internalization (94), it can also activate growth and prosurvival signaling mechanisms independent of G protein signaling (95–97). Angiotensin-converting enzyme inhibitors and AT_1R blockers are used in certain clinical settings but these block the maladaptive effects of G protein signaling as well as the cardioprotective effects of β-arrestin signaling (98,99). Thus, interest in the development of biased agonists for AT_1R that activate β-arrestin signaling without promoting the detrimental G protein effects are of growing interest (**Figure 2**).

Novel ligands for AT_1R have recently been developed that can act as biased agonists for β-arrestin signaling. Work to develop and characterize biased ligands for the AT_1R began a decade ago with the production of synthetic angiotensin II analogs including [Sar(1), Ile (4), Ile(8)]-angiotensin II (SII) that acted as β-arrestin–biased agonists at AT_1R (100–102). Comprehensive proteomic studies in human embryonic kidney 293 cells showed that stimulation of AT_1R induces acute changes in the interaction of β-arrestin with hundreds of proteins and that SII alters the phosphorylation status of hundreds more, including numerous cytoskeletal and motor proteins that could ostensibly be involved in the regulation of contractile processes (103,104). Notably, AT_1R has been reported to enhance rho kinase 1 activity via β-arrestin–dependent activation of RhoA to mediate changes in stress fibers, focal adhesions, and membrane blebbing (95,105,106). Rho kinase 1 activation in cardiomyocytes is known to regulate myosin light chain phosphatase to influence contraction; however, studies concerning AT_1R were performed in noncardiomyocytes with no insight into contractile function (107,108). Furthermore, due to poor affinity and selectivity of SII, determining the effects of biased AT_1R signaling in the cardiovascular system were difficult, but in vitro and ex vivo work showed that selective engagement of β-arrestin at AT_1R promotes contractility and activates mitogen-activated protein kinase pathways in a G protein– and Ca^{2+}-independent manner, which could be beneficial in the heart (109,110).

Recently, Trevena Inc. (Chesterbrook, Pennsylvania) has developed compounds, including TRV023, TRV027, and TRV067, using SII as a lead compound, which act as potent and selective β-arrestin–biased ligands for AT_1R while competitively antagonizing G protein signaling (111). These compounds have been shown to activate prosurvival mechanisms such as extracellular signal-regulated kinase 1/2 (ERK1/2) and Akt, leading to protection from cardiomyocyte death (111,112). Additionally, these compounds can lead to phosphorylation of contractile proteins thus promoting contractility (111–113). β-arrestin–biased AT_1R stimulation has been shown to alter vascular smooth muscle cell myosin light chain phosphatase targeting subunit 1, which can modify myosin light chain phosphorylation and vascular smooth muscle cell migration (114), and phosphorylation of select contractile proteins was shown to occur in response to chronic β-arrestin–biased AT_1R signaling with TRV023 (115,116) or TRV067 (113) in vivo. Further, the linkage between AT_1R-stimulated engagement of β-arrestin and phosphorylation-dependent regulation of myofilament proteins may involve at least some of the signaling pathways described in earlier studies as TRV067 was shown to increase both the sarcomeric localization of β-arrestin and myosin light chain phosphatase targeting subunit 1/2 phosphorylation, which was sensitive to ERK1/2 and ribosomal s6 kinase inhibition in myocytes (113).

In the absence of ligand, mechanical diastolic stretch of Langendorff-perfused mouse hearts was demonstrated to activate β-arrestin2-dependent AT_1R signaling to enhance left ventricular pressure, an effect that was independent of Gq protein activation but associated with increased ERK1/2 and Akt phosphorylation and sensitive to epidermal growth factor receptor (EGFR) inhibition (35). Consistent with these findings, both β-arrestin1 and β-arrestin2 were shown to mediate AT_1R-dependent left ventricular force generation in response to volume loading in vivo, indicating that the Frank-Starling mechanism is sensitive to β-arrestin–dependent signaling (117). Further, using an in vitro model, osmotic stretch was shown to allosterically augment the affinity and potency of a β-arrestin–biased AT_1R orthosteric ligand (118), suggesting that a β-arrestin–biased AT_1R agonist could be more effective at promoting left ventricular contractility under conditions of mechanical strain, such as chronic HF, consistent with in vivo mouse models (113,115,116). Together, these positive findings for the potential use of β-arrestin–biased AT_1R agonists for ameliorating various HF etiologies led to a randomized,

double-blind, placebo-controlled, phase IIB, dose-ranging trial (BLAST-AHF [Biased Ligand of the Angiotensin Receptor Study in Acute Heart Failure]) to assess the use of TRV027 in the treatment of acute HF (119). Although TRV027 was well tolerated (120), the acute use of this biased compound (2- to 4-day intravenous infusion) did not improve clinical status through 30-day follow-up compared with placebo (121). However, interest remains in determining whether β-arrestin-biased AT_1R signaling offers a benefit in chronic forms of HF.

β-ARRESTIN–BIASED βAR SIGNALING

βARs are another well-studied and important receptor family in the cardiovascular system. β_1ARs have strong effects on increasing cardiac output via enhanced heart rate, conduction velocity, and stroke volume, mainly attributed to G protein-dependent signaling, whereas β_2ARs also have inotropic effects in the heart and also influence vascular tone (122). Both βAR agonists and antagonists (blockers) are used clinically for cardiovascular conditions. Similar to AT_1R, β-arrestin-biased signaling from βARs has also been elucidated and was initially associated with the promotion of prosurvival pathways including activation of EGFR and ERK1/2 (34,123). Subsequent studies identified orthosteric βAR ligands, β-blockers including the clinically used carvedilol, which display bias toward β-arrestin-dependent signaling including EGFR and ERK1/2 activation, without an increase of Gαs protein activity inherent to their β-blocker property (124,125). A recent study showed that the naturally occurring Arg389Gly polymorphism in β_1AR confers β-arrestin2 tropism in response to carvedilol in cardiomyocytes (126), suggesting that gene mutations in GPCRs may promote endogenous biased signaling mechanisms. Further, β_1AR-β-arrestin1-biased agonism by carvedilol significantly increased miR-199a-3p and miR-214 in the heart, with a microRNA-dependent activation of P-Akt survival signaling and repression of apoptotic genes in cardiomyocytes in a model of ischemia-reperfusion (127). Through this same biased agonism, carvedilol stimulated miR-125b-5p processing in the mouse heart, again increasing the levels of P-Akt and suppressing a different profile of proapoptotic genes to enhance cardiomyocyte survival during acute myocardial infarction (128). Additionally, miR-532 was found to be a β_2AR- and β-arrestin-responsive microRNA that repressed a protease serine 23 in cardiac endothelial cells, decreasing endothelial-to-mesenchymal transition and eliciting cardioprotection in a myocardial infarction model (129). However, meta-analysis revealed a lack of clinical difference between carvedilol versus the unbiased β-blocker metoprolol in HF patients (130), suggesting that at therapeutically relevant doses carvedilol may not engage β-arrestin signaling with high enough efficacy to impart additional survival benefits in patients. Overall, progress on establishing whether orthosteric β-arrestin-biased βAR ligands could modulate cardiomyocyte contractility akin to AT_1R ligands has been hampered by a lack of identification of more potent and efficacious compounds than carvedilol.

Recent attention has focused instead on the development of allosteric modulators that could promote β-arrestin-biased signaling. Pepducins, small lipidated peptides from the intracellular loops (ICLs) of GPCRs, were first shown to be capable of allosterically modulating the activity of protease-activated receptors (131). Over the last decade, pepducins have been reported to selectively regulate an expanding cohort of GPCRs and have even begun to be tested in vivo (132). Recent work detailed the development of β_2AR-specific pepducins that selectively promote biased signaling via either Gs- or β-arrestin-dependent pathways (133). Characterization of the downstream signaling pathways activated in response to stimulation with β-arrestin-biased β_2AR pepducins designed from the first ICL of β_2AR in human embryonic kidney 293 cells confirmed that they also activate the EGFR and ERK1/2 signaling pathways, suggesting that they may be beneficial in promoting cardiomyocyte survival. Additionally, one of these pepducins, ICL1-9, was further tested in isolated cardiomyocytes to determine whether it impacted contractility (134). Compared to either its scrambled pepducin control or, notably, carvedilol, ICL1-9 increased cardiomyocyte contractility in a manner dependent on expression of β_2AR and either β-arrestin1 or β-arrestin2, but independently of classic βAR-mediated contractile processes including Ca^{2+} mobilization or phosphorylation of phospholamban (134). The ability of β_2AR signaling to promote contractility in a β-arrestin-dependent manner is a new and exciting property with no mechanistic explanation. However, β_2AR stimulation has been shown to activate RhoA in a β-arrestin-dependent manner, which regulates focal adhesion formation and migration of renal carcinoma cells (135). Thus, although the mechanistic underpinnings responsible for relaying β-arrestin-biased β_2AR effects on cardiomyocyte contractility have not been elucidated as yet, they could involve engagement of pathways, similar to β-arrestin-biased AT_1R signaling, that converge on the regulation of contractile proteins at the level of the sarcomere.

APELIN-APJ SYSTEM

A newly identified GPCR system of therapeutic interest in the cardiovascular system is the apelin-APJ system. Apelin was discovered in 1998 as an endogenous ligand for the previously orphan receptor APJ, which shows homology and similar tissue distribution as the AT_1R (136,137). Apelin is synthesized as preproapelin and cleaved by angiotensin-converting enzyme into several shorter, active fragments that appear to differ in their ability to activate, internalize, and recycle the receptor (136,138). APJ is a Gi-coupled GPCR, with potential coupling to Gq (136,139,140). In the cardiovascular system, apelin plays a role in both peripheral and central cardiovascular effects by influencing vascular tone, promoting neovascularization and acting as an inotropic agent. In the vasculature, apelin acts as a vasodilator through the Gi-dependent release of nitric oxide and is thought to counter the effects of AT_1R (141–144). In the heart, apelin is expressed at moderate levels but is a potent inotropic agent through activation of Gq, phospholipase C or protein kinase C, Na^+-H^+ sarcolemmal exchange, and Na^+-Ca^{++} exchange pathways (140,145–147). Endogenous ligands for APJ including apelin fragments appear to exert a G protein bias (139); however, these events still lead to receptor internalization through classical β-arrestin-dependent mechanisms (139). In contrast, activation of APJ through stretch leads to β-arrestin-dependent hypertrophy (147).

Apelin-APJ are also thought to play a role in cardiac dysfunction. Patients show elevations in plasma apelin in patients with early (148,149), which is decreased in later stages (148,150). In murine models of ischemic HF, acute up-regulation of apelin and APJ occurs following ischemic injury and these elevations in expression persist long-term (151–153). APJ and apelin knockout mice have impairments in contractility (154,155) and impaired healing following ischemia-reperfusion (156). Furthermore, administration of apelin or stable apelin analogs protects against ischemia-reperfusion in rodent models of ischemic heart disease, supporting the therapeutic potential of targeting apelin-APJ for treatment of HF (156–160). However, the effects of biased ligands in this receptor system remains to be determined.

UROCORTIN-CRF SYSTEM

Another GPCR system that is gaining ground as a relevant therapeutic target in cardiovascular disease is the urocortin-CRF system. The urocortins and stresscopin are biologically active endogenous peptides that bind to the corticotropin-releasing factor (CRF) family of GPCRs to alter cell signaling in a wide variety of tissues and organs. Urocortin1, urocortin2, urocortin3, and stresscopin have been recognized as affecting diverse multisystem functions, including the heart, vasculature, kidneys, and adrenal glands, among others where they affect a variety of downstream signaling cascades. Urocortin2 acting at type 2 CRF receptors was found to enhance cardiomyocyte contractility and calcium handling in isolated adult mouse cardiomyocytes in an 5′ adenosine monophosphate-activated protein kinase and protein kinase A-dependent manner (161,162). Similarly, delivery of urocortin2, urocortin3, or stresscopin to adult feline left ventricular myocytes significantly increased myocyte contractility in a concentration-dependent manner, with increased peak systolic Ca^{2+} transients and decay rates (163). At the highest concentration tested (1 μM), despite altering Ca^{2+} handling and cAMP levels to a similar degree as isoproterenol, the CRF peptide effect on myocyte contraction was much less robust (163). Although the mechanism of action of these CRF peptides has not been fully elucidated, it has been hypothesized that they may differ from classic inotropes in altering post-receptor signaling pathways in the cell that warrant further investigation (164). Interestingly, circulating urocortin levels are elevated in human HF patients, and antagonism of endogenous peptide elevation worsens disease outcomes in animal models of disease (165). Urocortin2, in particular, exhibits beneficial hemodynamic, neurohormonal, and renal effects in animal models of HF and human HF patients, with increased cardiac output, and reduced systemic vascular resistance and systolic blood pressure (166–169). Based on these initial studies, the number of preclinical and clinical investigations into the effect of CRF peptides on various aspects of cardiovascular function and in numerous disease etiologies has expanded (161,170–174), with various implications for their clinical outcomes. The currently known biological actions of these peptides within the cardiovascular system, including what information has been gathered from preclinical and clinical trials regarding therapeutic potential, has been recently reviewed in detail (175). Although this system is promising for novel HF therapeutics, the impact of GRK- or arrestin-biased signaling on functional outcomes remains to be tested vigorously.

SUMMARY

The last several decades have seen an increasing focus on GPCRs as targets for therapeutic

intervention in cardiovascular disease. More recently, this research has moved beyond the classic pharmacology of agonists and antagonists to more targeted approaches. The concept of biased agonism to activate a beneficial downstream signaling pathway at the expense of undesired effects is becoming a reality with the advent of small molecules and peptide that selectively activate β-arrestin–mediated signaling to improve contractility and cardiomyocyte survival. β-arrestins have also been shown to directly interact with effector proteins to inhibit signaling via inactive GPCRs and alter contractility. Further, GRK isoforms expressed in the heart demonstrate receptor specificity and diverse protein-protein and protein-DNA interactions with significant impact on cardiovascular physiology and pathophysiology. This complex web of GPCR modulators is ever expanding with the identification and characterization of endogenous cardioprotective ligands and their receptors. Although the long-term impact of these discoveries on patient health remains to be seen, this research highlights the diversity of signaling mechanisms downstream of GPCRs and identifies new avenues for therapeutic development in the treatment of HF.

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