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/).
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 AT1R 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 AT1R 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 AT1R 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 GPCR 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 α1-adrenergic receptor (α1AR) 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 α1AR overexpression (15). Further, both lines demonstrate improved function and reduced left ventricular remodeling in models of disease (16–18), suggesting that phosphorylation of cardiac α1ARs by GRK3 may contribute to the detrimental cardiac signaling during disease. The full functional significance for GRK3 in cardiomyocyte signaling in the healthy 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 observed to participate in the regulation of thrombin, endothelin, and CRF (15). Further, both lines demonstrate improved function and reduced left ventricular remodeling in models of disease (16–18), suggesting that phosphorylation of cardiac α1ARs by GRK3 may contribute to the detrimental cardiac signaling during disease. The full functional significance for GRK3 in cardiomyocyte signaling in the healthy heart and during pathophysiological conditions remains to be elucidated and is as yet not a target for HF therapy.

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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...
activities GRK2 has arisen as a significant target in diverse pathologies and tissues (62). Inhibition of GRK2 via expression of a carboxyl-terminal peptide, bARKct, that competes with GRK2 binding to Gbg, enhances cardiac function. In fact, bARKct 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
have identified that some Gs- and Gq-coupled receptors promote protein kinase A– and C–mediated phosphorylation of β2ARs in rat ventricular myocytes, leading to β-arrestin2-dependent recruitment and complex formation with phosphodiesterase 4D in a manner that impairs subsequent β2AR signaling (86). Similarly, heterologous GRK-dependent signaling initiated from the Gq-coupled vasopressin type 1A receptor was also demonstrated to be capable of reducing β1AR 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 Ca2+ 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 β1ARs 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 Ca2+-ATPase (SERCA2a) downstream of β1ARs 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 AT1R SIGNALING

The AT1R 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 Gq causes vasoconstriction (91-92) and hypertension, whereas in the heart it causes hypertrophy (6,91). Although β-arrestin signaling downstream of the AT1R 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 AT1R 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 AT1R that activate β-arrestin signaling without promoting the detrimental G protein effects are of growing interest (Figure 2).

Novel ligands for AT1R have recently been developed that can act as biased agonists for β-arrestin signaling. Work to develop and characterize biased ligands for the AT1R 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 AT1R (100-102). Comprehensive proteomic studies in human embryonic kidney 293 cells showed that stimulation of AT1R 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, AT1R 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 AT1R 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 AT1R signaling in the cardiovascular system were difficult, but in vitro and ex vivo work showed that selective engagement of β-arrestin at AT1R promotes contraction and activates mitogen-activated protein kinase pathways in a G protein- and Ca2+-independent manner, which could be beneficial in the heart (109,110).

Recently, Trevena Inc. (Chesterbrook, Pennsylvania) has developed compounds, including TRV023, TRV027, and TRVO67, using SII as a lead compound, which act as potent and selective β-arrestin-biased ligands for AT1R 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 AT1R 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 AT1R signaling with TRV023 (115,116) or TRVO67 (113) in vivo. Further, the linkage between AT1R-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 TRVO67 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 AT1R 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 AT1R-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 AT1R orthosteric ligand (118), suggesting that a β-arrestin-biased AT1R 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 AT1R agonists for ameliorating various HF etiologies led to a randomized,
double-blind, placebo-controlled, phase IIIB, 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 \( \beta \)-arrestin-biased \( \alpha \)R signaling offers a benefit in chronic forms of HF.

**\( \beta \)-ARRESTIN-BIASED \( \beta \)AR SIGNALING**

\( \beta \)ARs are another well-studied and important receptor family in the cardiovascular system. \( \beta \)ARs have strong effects on increasing cardiac output via enhanced heart rate, conduction velocity, and stroke volume, mainly attributed to \( G \) protein-dependent signaling, whereas \( \beta \)ARs also have inotropic effects in the heart and also influence vascular tone (122). Both \( \beta \)AR agonists and antagonists (blockers) are used clinically for cardiovascular conditions. Similar to \( \alpha \)R, \( \beta \)-arrestin-biased signaling from \( \beta \)ARs 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 \( \beta \)AR ligands, \( \beta \)-blockers including the clinically used carvedilol, which display bias toward \( \beta \)-arrestin-dependent signaling including EGFR and ERK1/2 activation, without an increase of Gas protein activity inherent to their \( \beta \)-blocker property (124,125). A recent study showed that the naturally occurring Arg389Gly polymorphism in \( \beta \)AR confers \( \beta \)-arrestin2 tropism in response to carvedilol in cardiomyocytes (126), suggesting that gene mutations in GPCRs may promote endogenous biased signaling mechanisms. Further, \( \beta \)AR-\( \beta \)-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-522 was found to be a \( \beta \)AR- and \( \beta \)-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 \( \beta \)-blocker metoprolol in HF patients (130), suggesting that at therapeutically relevant doses carvedilol may not engage \( \beta \)-arrestin signaling with high enough efficacy to impart additional survival benefits in patients. Overall, progress on establishing whether orthosteric \( \beta \)-arrestin-biased \( \beta \)AR ligands could modulate cardiomyocyte contractility akin to \( \alpha \)R 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 \( \beta \)-arrestin-biased signaling. Pepducins, small lipitated 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 \( \beta \)AR-specific pepducins that selectively promote biased signaling via either \( G \)s or \( \beta \)-arrestin-dependent pathways (133). Characterization of the downstream signaling pathways activated in response to stimulation with \( \beta \)-arrestin-biased \( \beta \)AR pepducins designed from the first ICL of \( \beta \)AR 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 \( \beta \)2AR and either \( \beta \)-arrestin1 or \( \beta \)-arrestin2, but independently of classic \( \beta \)AR-mediated contractile processes including Ca2+ mobilization or phosphorylation of phospholamban (134). The ability of \( \beta \)2AR signaling to promote contractility in a \( \beta \)-arrestin-dependent manner is a new and exciting property with no mechanistic explanation. However, \( \beta \)2AR stimulation has been shown to activate RhoA in a \( \beta \)-arrestin-dependent manner, which regulates focal adhesion formation and migration of renal carcinoma cells (135). Thus, although the mechanistic underpinnings responsible for relaying \( \beta \)-arrestin-biased \( \beta \)2AR effects on cardiomyocyte contractility have not been elucidated as yet, they could involve engagement of pathways, similar to \( \beta \)-arrestin-biased \( \alpha \)R 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 AT₃R (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 AT₁R (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⁺-Ca²⁺ 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 (151-152). 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 Ca²⁺ transients and decay rates (163). At the highest concentration tested (1 μM), despite altering Ca²⁺ 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. 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.

**REFERENCES**

1. Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. Science 2005; 308:512-7.

2. Lettreli LM, Lefkowitz RJ. The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. J Cell Sci 2002;115:455-65.

3. Koblika BK. G protein coupled receptor structure and activation. Biochim Biophys Acta 2007; 1768:794-807.

4. Tang CM, Insel PA. GPCR expression in the heart; “new” receptors in myocytes and fibroblasts. Trends Cardiovasc Med 2004;14:94-9.

5. Violin JD, Lefkowitz RJ. Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol Sci 2007;28:416-22.

6. Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nat Rev Drug Discov 2010;9:373-86.

7. Sato PY, Chuprun JK, Schwartz M, Koch WJ. The evolving impact of g protein-coupled receptor kinases in cardiac health and disease. Physiol Rev 2015;95:377-404.

8. Inglese J, Freedman NJ, Koch WJ, Lefkowitz RJ. Structure and mechanism of the G-protein-coupled receptor kinases. J Biol Chem 1993;268:23735-8.

9. Penn RB, Prinon AN, Benovic JL. Regulation of G protein-coupled receptor kinases. Trends Cardiovasc Med 2000;10:81-9.

10. Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nature reviews. Mol Cell Biol 2002;3:639-50.

11. Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. Ann Rev Biochem 1998;67:653-92.

12. Iaccarino G, Rockman HA, Shotwell KF, Tomhave ED, Koch WJ. Myocardial overexpression of GRK3 in transgenic mice: evidence for in vivo selectivity of GRKs. Am J Physiol 1998;275: H1298-306.

13. Vinge LE, Andressen KW, Attramadal T, et al. Substrate specificities of G protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in regulation of myocardial function. Mol Pharmacol 2007;72:582-91.

14. Vinge LE, von Lueder TG, Aasm E, et al. Cardiac-restricted expression of the carboxyterminal fragment of GRK3 uncovers distinct functions of GRK3 in regulation of cardiac contractility and growth: GRK3 controls cardiac alpha1-adrenergic receptor responsiveness. J Biol Chem 2008;283:10601-10.

15. Lin F, Owens WA, Chen S, et al. Targeted alpha1(IA)-adrenergic receptor overexpression induces enhanced cardiac contractility but not hypertrophy. Circ Res 2001;89:343-50.

16. Du XJ, Fang L, Gao XM, et al. Genetic enhancement of ventricular contractility protects against pressure-overload-induced cardiac dysfunction. J Mol Cell Cardiol 2004;37:979-87.

17. Du XJ, Gao XM, Kiriazis H, et al. Transgenic alpha1A-adrenergic activation limits post-infarct ventricular remodeling and dysfunction and improves survival. Cardiovasc Res 2006;71:735-43.

18. von Lueder TG, Gravning J, How OJ, et al. Cardiomycocyte-restricted inhibition of G protein-coupled receptor kinase-3 attenuates cardiac dysfunction after chronic pressure overload. Am J Physiol Heart Circ Physiol 2012;303:H368.

19. Yi XP, Zhou J, Baker J, Wang X, Gerdes AM, Li F. Myocyte redistribution of GRK2 and GRK5 in hypertensive, heart-failure-prone rats. Hypertension 2002;39:1058-63.

20. Johnson LR, Robinson JD, Lester KN, Pitcher JA. Distinct structural features of G protein-coupled receptor kinase 5 (GRK5) regulate its nuclear localization and DNA-binding ability. PLoS One 2013;8:e62508.

21. Johnson LR, Scott MG, Pitcher JA. G protein-coupled receptor kinase 5 contains a DNA-binding nuclear localization sequence. Mol Cell Biol 2004; 24:10169-79.

22. Gold JI, Martini JS, Hullmann J, et al. Nuclear translocation of cardiac G protein-coupled receptor kinase 5 downstream of select Gq-activating hypertrophic ligands is a calmodulin-dependent process. PLoS One 2013;8:e65734.

23. Zhang Y, MatKimovich SJ, Duan X, Gold JI, Koch WJ, Dorn GW 2nd. Nuclear effects of G-protein receptor kinase 5 on histone deacetylation 5-regulated gene transcription in heart failure. Circ Heart Fail 2011;4:659-68.

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31. Pronin AN, Morris AJ, Surugachov A, Benovic JL. Syncolin are a novel class of substrates for G protein-coupled receptor kinases. J Biol Chem 2000;275:26015-22.

32. Hullmann JE, Grisanti LA, Makarewich CA, et al. GRK5-mediated exacerbation of pathological cardiac hypertrophy involves facilitation of nuclear Nfat activity. Circ Res 2014;115:976-85.

33. Liu F, Wang X, Gao N, et al. G protein-coupled receptor kinase 5, overexpressed in the alpha- NFAT activity. Circ Res 2014;115:976-85.

34. Noma T, Lemaire A, Naga Prasad SV, et al. Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. J Clin Invest 2007;117:2445-58.

35. Rakesh K, Yoo B, Kim IM, Zalaran N, Kim KS, Rockman HA. beta-Arrestin-biased agonism of the G protein-coupled receptor kinase 2 in complex with a rationally designed inhibitor. J Biol Chem 2015;290:20649-59.

36. Homan KT, Waldschmidt HV, Glukhova A, et al. Crystal structure of G protein-coupled receptor kinase 5 in complex with a rationally designed inhibitor. J Biol Chem 2015;290:20649-59.

37. Traynham CJ, Hullmann J, Koch WJ. Canonical and non-canonical actions of GRK5 in the heart. J Mol Cell Cardiol 2016;92:196-202.

38. DeWiere SM, Ahn S, Leftowizk RJ, Shenoy SK. Beta-arrestins and cell signaling. Ann Rev Physiol 2007;69:483-510.

39. Penela P, Munga C, Ribas C, Lafarga V, Mayor F Jr. The complex G protein-coupled receptor kinase 2 (GRK2) interactome unveils new physiopathological targets. Br J Pharmacol 2010;160:821-32.

40. Sterne-Marr R, Dhami GK, Tesmer J, Ferguson SS. Characterization of GRK2 RH domain-dependent regulation of GPCR coupling to heterotrimeric G proteins. Methods Enzymol 2004;390:310-36.

41. Pao CS, Benovic JL. Phosphorylation-independent desensitization of G protein-coupled receptors? Sci STKE 2002;2002:pe42.

42. Willets JM, Nahorski SR, Challiss RA. Roles of phosphorylation-dependent and -independent mechanisms in the regulation of M1 muscarinic acetylcholine receptors by G protein-coupled receptor kinase 2 in hippocampal neurons. J Biol Chem 2005;280:18950-8.

43. Dhami GK, Anborgh PH, Dale LB, Sterne-Marr R, Ferguson SS. Phosphorylation-independent regulation of metabotropic glutamate receptor signaling by G protein-coupled receptor kinase 2. J Biol Chem 2002;277:25666-72.

44. Penela P, Munga C, Ribas C, Tutor AS, Peregrin S, Mayor F Jr. Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. Cardiovasc Res 2006;69:46-56.

45. Lafarga V, Mayor F Jr., Penela P. The interplay between G protein-coupled receptor kinase 2 (GRK2) and histone deacetylase 6 (HDAC6) at the crossroads of epithelial cell motility. Cell Adh Migr 2012;6:495-501.

46. Hullmann J, Traynham CJ, Coleman RC, Koch WJ. The expanding GRK interactome: Implications in cardiovascular disease and potential for therapeutic development. Pharm Res 2016;31:52-64.

47. Penela P, Fogues L, Mayor F Jr. Role of G protein-coupled receptor kinases in cell migration. Curr Opin Cell Biol 2014;27:10-7.

48. Garcia-Guerra L, Nieto-Vazquez I, Vil-Bedmar R, et al. G protein-coupled receptor kinase 2 plays a relevant role in insulin resistance and obesity. Diabetes 2010;59:2407-17.

49. Uusi I, Inamura T, Babendure JL, et al. G protein-coupled receptor kinase 2 mediates endothelin-1-induced insulin resistance via the inhibition of both Galphα/11 and insulin receptor substrate-1 pathways in 3T3-L1 adipocytes. Mol Endocrinol 2005;19:2760-8.

50. Mayor F Jr., Lucas E, Jurado-Pueyo M, et al. G Protein-coupled receptor kinase 2 (GRK2): A novel modulator of insulin resistance. Arch Physiol Biochem 2011;117:125-30.

51. Woodall MC, Ciccarelli M, Woodall BP, Koch WJ. G protein-coupled receptor kinase 2: a link between myocardial contractile function and cardiac metabolism. Circ Res 2014;114:1661-70.

52. Taguchi K, Hida M, Hasegawa M, Narimatsu H, Matsuomo T, Kobayashi T. Suppression of GRK2 expression reduces endothelial dysfunction by restoring glucose homeostasis. Sci Rep 2017;7:8436.

53. Eichmann T, Lorence K, Hoffmann M, et al. The amino-terminal domain of G-protein-coupled receptor kinase 2 is a regulatory Gβγ gamma binding site. J Biol Chem 2003;278:8052-7.

54. Fusco A, Santulli G, Sorriento D, et al. Mitochondrial localization unveils a novel role for GRK2 in organelle biogenesis. Cell Signal 2012;24:468-75.

55. Obrenovich ME, Palacios HH, Gasimov E, Leszek J, Aliev G. The GRK2 overexpression is a primary hallmark of mitochondrial lesions during early Alzheimer disease. Cardiovasc Psychiatry Neurol 2009;2009:327360.

56. Brinks H, Boucher M, Gao E, et al. Level of G protein-coupled receptor kinase-2 determines myocardial ischemia/reperfusion injury via pro- and anti-apoptotic mechanisms. Circ Res 2010;107:1140-9.

57. Fan Q, Chen M, Zuo L, et al. Myocardial ablation of G protein-coupled receptor kinase 2 (GRK2) decreases ischemia/reperfusion injury through an anti-infectious apoptotic pathway. PLoS One 2013;8:e66234.

58. Chen M, Sato PY, Chuprun JK, et al. Prodeath signaling of G protein-coupled receptor kinase 2 in cardiac myocytes after ischemic stress occurs via extracellular signal-regulated kinase-dependent heat shock protein 90-mediated mitochondrial targeting. Circ Res 2013;112:1121-34.

59. Luo J, Benovic JL. G protein-coupled receptor kinase interaction with Hop90 mediates kinase maturation. J Biol Chem 2003;278:50908-14.
**Protein-coupled receptor kinase 2 inhibitors.** Mol Pharm 2017;92:707–17.

75. Waldschmidt HV, Homan KT, Cato MC, et al. Structure-based design of highly selective and potent G protein-coupled receptor kinase 2 inhibitors based on paroxetine. J Med Chem 2017;60:3052–69.

76. Tian X, Wang G, Guo R, Xu L, Chen QM, Hou Y. Effects of paroxetine-mediated inhibition of GRK2 expression on depression and cardiovascular function in patients with myocardial infarction. Neuropsychiatr Dis Treat 2016;12:2333–41.

77. Sorrentino D, Ciccarelli M, Cipolletta E, Trimarco B, laccarino G. Freeze, don’t move: how to arrest a suspect in heart failure - a review on available GRK2 inhibitors. Front Cardiovasc Med 2016;3:48.

78. Okawa T, Aramaly M, Yamamoto M, et al. Design, synthesis, and evaluation of the highly selective and potent G-protein-coupled receptor kinase 2 (GRK2) inhibitor for the potential treatment of heart failure. J Med Chem 2017;60:6942–90.

79. Perry SJ, Lefkowitz RJ. Arresting developments in heptahelical receptor signaling and regulation. Trends Cell Biol 2002;12:130–9.

80. Nuber S, Zabel U, Lorenz K, et al. β-arrestin biosensors reveal a rapid, receptor-dependent activation/deactivation cycle. Nature 2016;531:661–4.

81. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 2004;27:107–44.

82. Chung TT, Iacovelli L, Sallese M, De Blasi A. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. Trends Pharmacol Sci 1996;17:416–21.

83. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. Nature 2002;415:206–13.

84. Moulédaul L, Froment C, Dauvillier S, et al. GRK2 protein-mediated transphosphorylation contributes to loss of function of mu-opioid receptors induced by neuropeptide FF (NPF/F2) receptors. J Biol Chem 2012;287:12736–40.

85. Cheng Y, Tao YM, Sun JF, et al. Adenosine A(1) receptor agonist agonist N(6)-cyclohexyl-adenosine induced phosphorylation of delta opioid receptor and desensitization of its signaling. Acta Pharmacol Sinica 2010;31:784–90.

86. Shi Q, Li M, Mika D, et al. Heterologous desensitization of cardiac beta-adrenergic signal via hormone-induced betaAR/alpha(R)/PDE4 complex. Cardiovasc Res 2017;113:656–67.

87. Tilley DG, Zhu W, Myers VD, et al. β-adrenergic receptor-mediated cardiac contractility is inhibited via vasopressin type 1A receptor-dependent signaling. Circulation 2014;130:1800–11.

88. McCrink KA, Maning J, Vu A, et al. β-arrestin2 improves post-myocardial infarction heart failure via sarco(endo)plasmic reticulum Ca(2+)-ATPase-dependent positive inotropy in cardiomyocytes. Hypertension 2017;70:972–81.

89. Wang Y, Jin L, Song Y, et al. β-arrestin 2 mediates cardiac ischemia-reperfusion injury via inhibiting GPCR-independent cell survival signaling. Cardiovasc Res 2017;113:1615–26.

90. Zhabyeyev P, Zhang H, Oudit GY. Is β-arrestin 2 a magic bullet for heart failure treatment? Hypertension 2017;70:887–9.

91. Hoi M, Oliverio MI, Mannion PJ, et al. Regulation of blood pressure by the type 1A angiotensin II receptor gene. Proc Natl Acad Sci U S A 1995;92:3521–5.

92. Kawai T, Forrester SJ, O’Brien S, Baggett A, Rizzo V, Evuchi S. AT1 receptor signaling pathways in the cardiovascular system. Pharmacol Res 2017;125:4–13.

93. Violin JD, Dewire SM, Barnes WG, Lefkowitz RJ. G protein-coupled receptor kinase and beta-arrestin-mediated desensitization of the angiotensin II type 1A receptor elucidated by dicylglyceroil dynamics. J Biol Chem 2006;281:36411–9.

94. Kule CE, Karoor V, Day JN, et al. Agonist-dependent internalization of the angiotensin II type one receptor (AT1): role of C-terminus phosphorylation in recruitment of beta-arrestins. Regul Pept 2004;120:141–8.

95. Barnes WG, Reiter E, Violin JD, Ren XR, Milligan G, Lefkowitz RJ. beta-Arrestin 1 and Galphai/βII coordinately activate RhoA and stress fiber formation following receptor stimulation. J Biol Chem 2005;280:8041–50.

96. DeWire SM, Kim J, Whalen EJ, Ahn S, Chen M, Lefkowitz RJ. Beta-arrestin-mediated signaling regulates protein synthesis. J Biol Chem 2008;283:10611–20.

97. Ahn S, Kim J, Hara MR, Ren XR, Lefkowitz RJ. (beta)-Arrestin-2 mediates anti-apoptotic signaling through regulation of BAD phosphorylation. J Biol Chem 2009;284:8855–65.

98. Hoogwerf BJ. Renin-angiotensin system blockade and cardiovascular and renal protection. Am J Cardiol 2010;105:30–45A.

99. Mercier K, Smith H, Biederman J. Renin-angiotensin-aldosterone system inhibition: overview of the therapeutic use of angiotensin-converted enzyme inhibitors, renin inhibitor blockers, mineralocorticoid receptor antagonists, and direct renin inhibitors. Prim Care 2014;41:765–78.

100. Wei H, Ahn S, Shenoy SK, et al. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. Proc Natl Acad Sci U S A 2003;100:7872–7.

101. Ahn S, Shenoy SK, Wei H, Lefkowitz R. Differential kinetic and spatial patterns of beta-arrestin and G protein-mediated ERK activation by the angiotensin II receptor. J Biol Chem 2004;279:35518–25.

102. Kim J, Ahn S, Rajagopal K, Lefkowitz RJ. Independent beta-arrestin2 and Go protein kinase Czeta pathways for ERK stimulation by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor. J Biol Chem 2009;284:11953–62.

103. Xiao K, McClatchy DB, Shukla AK, et al. Functional specialization of beta-arrestin interactions revealed by proteomic analysis. Proc Natl Acad Sci U S A 2007;104:12091–6.

104. Xiao K, Sun J, Kim J, et al. Global phosphorylation analysis of beta-arrestin-mediated signaling downstream of a seven transmembrane receptor (7TMR). Proc Natl Acad Sci U S A 2010;107:299–304.

105. Anthony DF, Sin YY, Vadrevu S, et al. Beta-Arrestin 1 inhibits the GTPase-activating protein function of ARH GAP21, promoting activation of RhoA following angiotensin II type 1A receptor stimulation. Mol Cell Biol 2011;31:1066–75.

106. Godin CM, Ferguson SS. The angiotensin II type 1 receptor induces membrane blebbing by coupling to Rho A, Rho kinase, and myosin light chain kinase. Mol Pharmacol 2010;77:901–11.

107. Chang AN, Battiprolu PK, Cowley PM, et al. Constitutive phosphorylation of cardiac myosin regulatory light chain in vivo. J Biol Chem 2015;290:10703–16.

108. Okamoto R, Kato T, Mizoguchi A, et al. Characterization and function of MYPT2, a target subunit of myosin phosphatase in heart cell. Cell Signal 2006;18:1408–16.

109. Rajagopal K, Whalen EJ, Violin JD, et al. Beta-arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. Proc Natl Acad Sci U S A 2006;103:16284–9.

110. Aplin M, Christensen GL, Schneider M, et al. The angiotensin type 1 receptor activates extracellular signal-regulated kinases 1 and 2 by G protein-dependent and -independent pathways in cardiac myocytes and Langendorff-perfused hearts. Basic Clin Pharmacol Toxicol 2007;100:289–95.

111. Violin JD, DeWire SM, Yamashita D, et al. Selectively engaging beta-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. J Pharmacol Exp Ther 2010;335:572–8.

112. Kim KS, Abraham D, Williams B, Violin JD, Mao L, Rockman HA. Beta-arrestin-biased AT1R stimulation promotes cell survival during acute cardiac injury. Am J Physiol Heart Circ Physiol 2012;303:H1001–10.

113. Ryba DM, Li J, Cowan CL, Russell B, Wolska BM, Solaro RJ. Long-term biased beta-arrestin signaling improves cardiac structure and function in dilated cardiomyopathy. Circulation 2017;135:1056–70.

114. Sinard E, Kovacs JJ, Miller WE, Kim J, Grandbois M, Lefkowitz RJ. Beta-Arrestin regulation of myosin light chain phosphorylation promotes AT1R-mediated cell contraction and migration. PLoS One 2013;8:e80532.

115. Monksy MM, Taglieri DM, Henze M, et al. The beta-arrestin-biased ligand TRV12003 inhibits angiotensin II-induced cardiac hypertrophy while preserving enhanced myofilament response to calcium. Am J Physiol Heart Circ Physiol 2013;305:H565–66.

116. Tariqopula M, Davis RT 3rd, Mungai PT, et al. Cardiac myosin light chain phosphorylation and...
responsive microRNAs, miR-199a-3p and -214

Park KM, Teoh JP, Wang Y, et al. Carvedilol-cardiac myocytes. Pharmacogenomics 2016;17:226-34.

117. Abraham DM, Davis RT 3rd, Warren CM, et al. β-Arrestin mediates the Frank-Starling mechanism of cardiac contractility. Proc Natl Acad Sci U S A 2016;113:14426-31.

118. Tang W, Strachan RT, Leffkowitz RJ, Rockman HA. Allosteric modulation of beta-arrestin-biased angiotensin II type 1 receptor signaling by membrane stretch. J Biol Chem 2014;289:28271-83.

119. Felker GM, Butler J, Collins SP, et al. Heart failure therapies on the basis of a biased ligand of the angiotensin-2 type 1 receptor. Rationale and design of the BLAST-AHF study (Biased Ligand of the Angiotensin Receptor Study in Acute Heart Failure). J Am Coll Cardiol HF 2015;3:193-201.

120. Soergel DG, Subach RA, Cowan CL, Violin JD, Lark MW. First clinical experience with TRV027: pharmacokinetics and pharmacodynamics in healthy volunteers. J Clin Pharmacol 2013;53:892-9.

121. Pang PS, Butler J, Collins SP, et al. Biased ligand of the angiotensin II type 1 receptor in patients with acute heart failure: a randomized, double-blind, placebo-controlled, phase IIb, dose ranging trial (BLAST-AHF). Eur Heart J 2017;38:2364-73.

122. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res 2003;93:896-906.

123. Maudsley S, Pierce KL, Zamah AM, et al. The β(2)-adrenergic receptor mediates extracellular signal-regulated kinase activation via assembly of a multi-receptor complex with the epididymal growth factor receptor. J Biol Chem 2000;275:9572-80.

124. Kim IM, Tilley DG, Chen J, et al. Beta-blockers alprenolol and carvedilol stimulate beta-arrestin-mediated EGFR transactivation. Proc Natl Acad Sci U S A 2008;105:14555-60.

125. Wisler JW, DeVire SM, Whalen EJ, et al. A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. Proc Natl Acad Sci U S A 2007;104:16657-62.

126. McCrink KA, Brill A, Jafferjee M, et al. β1-adrenoceptor Arg389Gly polymorphism confers differential beta-arrestin-binding tropism in cardiac myocytes. Pharmacogenomics 2016;17:1611-20.

127. Park KM, Teoh JP, Wang Y, et al. Carvedilol-responsive microRNAs, miR-199a-3p and -214 protect cardiomyocytes from simulated ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2015;313:H371-83.

128. Bayoumi AS, Park KM, Wang Y, et al. A carvedilol-responsive microRNA, miR-125b-5p protects the heart from acute myocardial infarction by repressing pro-apoptotic bax and kitl3 in cardiomyocytes. J Mol Cell Cardiol 2017;114:72-82.

129. Bayoumi AS, Teoh JP, Aonuma T, et al. MicroRNA-3532 protects the heart in acute myocardial infarction, and represses p73, a positive regulator of endothelial-to-mesenchymal transition. Cardiovasc Res 2017;113:1603-14.

130. Briasoulis A, Palla M, Alfonso L. Meta-analysis of the effects of carvedilol versus metoprolol on all-cause mortality and hospitalizations in patients with heart failure. Am J Cardiol 2015;116:1111-5.

131. Covic L, Gresser AL, Talalava J, Swift S, Kulopulos A. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. Proc Natl Acad Sci U S A 2002;99:643-8.

132. Zhang P, Covic L, Kulopulos A. Pdpeps and other lipidated peptides as mechanistic probes and therapeutics. Methods Mol Biol 2015;124:191-203.

133. Carr R 3rd, Du Y, Quoy J, et al. Development and characterization of pepducins as Gs-biased allosteric agonists. J Biol Chem 2014;289:35668-84.

134. Carr R 3rd, Schilling J, Song J, et al. β-arrestin-biased signaling through the beta(2)-adrenergic receptor promotes cardiomyocyte contraction. Proc Natl Acad Sci U S A 2016;113:E4107-16.

135. Ma X, Zhao Y, Daeku Y, Nie Z. Acute activation of beta2-adrenergic receptor regulates focal adhesion via release through betaArrestin2- and p15SrGFR protein-mediated activation of RhoA. J Biol Chem 2012;287:18925-36.

136. Tatetomo K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun 1998;251:471-6.

137. O’Dowd BF, Heiber M, Chan A, et al. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. Gene 1993;136:355-60.

138. Hosoya M, Kawamata Y, Fukusumi S, et al. Molecular and functional characteristics of APJ, a unique receptor. J Biol Chem 2000;275:21061-7.

139. Sheikh AY, Chun HJ, Glassford AJ, et al. In vivo genetic profiling and cellular localization of apelin reveals a hypothia-sensitive, endothelial-centered pathway activated in ischemic heart failure. Circ Res 2003;103:1342-9.

140. Chong KS, Gardner RS, Morton JJ, Ashley EA, McDonagh TA. Plasma concentrations of the novel peptide apelin are decreased in patients with chronic heart failure. Eur J Heart Fail 2006;8:355-60.

141. Kleinz MJ, Baxter GF. Apelin reduces myocardial reperfusion injury independently of PI3K/Akt and P70S6 kinase. Regul Pept 2008;146:271-7.

142. Attuiri P, Morine KJ, Liao GP, et al. Ischemic heart failure enhances endogenous myocardial apelin and APJ receptor expression. Cell Mol Biol Lett 2007;12:127-38.

143. Sheikh AC, Chen HJ, Glassford AJ, et al. In vivo genetic profiling and cellular localization of apelin reveals a hypothia-sensitive, endothelial-centered pathway activated in ischemic heart failure. Am J Physiol Heart Circ Physiol 2008;294:H86-90.

144. Charo DN, Ho M, Fajardo G, et al. Endogenous regulation of cardiovascular function by apelin-APJ. Am J Physiol Heart Circ Physiol 2009;297:H1904-13.

145. Kuba K, Zhang L, Inai Y, et al. Impaired heart contractility in apelin gene-deficient mice associated with aging and pressure overload. Circ Res 2007;101:e32-42.

146. Wang W, Mckinnie SM, Patel VB, et al. Loss of Apelin exacerbates myocardial infarction adverse remodeling and ischemia-reperfusion injury: therapeutic potential of synthetic Apelin analogues. J Am Heart Assoc 2013;2:e002249.

147. Simpkin JC, Yellon DM, Davidson SM, Lim SY, Wynn AM, Smith CC. Apelin-13 and apelin-36 exhibit direct cardioprotective activity against ischemia-reperfusion injury. Basic Res Cardiol 2007;102:518-28.

148. Zeng XJ, Zhang LK, Wang HX, Lu LQ, Ma LQ, Tang CS. Apelin protects heart against ischemia-reperfusion injury in rat. Peptides 2009;30:1144-52.

149. Tao J, Zhu W, Li Y, et al. Apelin-13 protects the heart against ischemia-reperfusion injury
through inhibition of ER-dependent apoptotic pathways in a time-dependent fashion. Am J Physiol Heart Circ Physiol 2011;301:H1471-86.

160. Azizi Y, Faghihi M, Imani A, Roghani M, Nazari A. Post-infarct treatment with [Pyr1]-apelin-13 reduces myocardial damage through reduction of oxidative injury and nitric oxide enhancement in the rat model of myocardial infarction. Peptides 2013;46:76-82.

161. Chen S, Wang Z, Xu B, et al. The modulation of cardiac contractile function by the pharmacological and toxicological effects of urocortin2. Toxicol Sci 2015;149:581-93.

162. Yang LZ, Kockskämper J, Heinzl FR, et al. Urocortin II enhances contractility in rabbit ventricular myocytes via CRF(2) receptor-mediated stimulation of protein kinase A. Cardiovasc Res 2006;69:402-11.

163. Makarewich CA, Troupes CD, Schumacher SM, et al. Comparative effects of urocortins and stresscopin on cardiac myocyte contractility. J Mol Cell Cardiol 2015;86:179-86.

164. Zheng M, Han QD, Xiao RP. Distinct beta-adrenergic receptor subtype signaling in the heart and their pathophysiological relevance. Sheng Li Xue Bao 2004;56:1-15.

165. Tsuda T, et al. Corticotropin releasing hormone receptor 2 exacerbates chronic cardiac dysfunction. The Journal of experimental medicine 2013;214:1877-88.

166. Rademaker MT, Cameron VA, Charles CJ, Richards AM. Integrated hemodynamic, hormonal, and renal actions of urocortin 2 in normal and paced sheep: beneficial effects in heart failure. Circulation 2005;112:3624-32.

167. Rademaker MT, Charles CJ, Nichols G, Richards M. Urocortin 2 sustains haemodynamic and renal function during introduction of beta-blockade in experimental heart failure. J Hypertens 2011;29:1787-95.

168. Davis ME, Pemberton CJ, Yandle TG, et al. Urocortin 2 infusion in human heart failure. Eur Heart J 2007;28:2589-97.

169. Chan WY, Frampton CM, Crozier IG, et al. Cardiovascular effects of urocortin 2 and urocortin 3 in patients with chronic heart failure. Br J Clin Pharmacol 2016;82:974-82.

170. Stirrat CG, Venkatasubramanian S, Pawade T, et al. Cardiovascular effects of urocortin 2 and urocortin 3 in patients with chronic heart failure. Br J Clin Pharmacol 2016;82:974-82.

171. Williams TA, Bergstrom JC, Scott J, Bernier NJ. CRF and urocortin 3 protect the heart from hypoxia/reoxygenation-induced apoptosis in zebrafish. Am J Physiol Regul Integr Comp Physiol 2017;313:R91-100.

172. Rademaker MT, Richards AM. Urocortins: actions in health and heart failure. Clin Chim Acta 2017;474:76-87.

**KEY WORDS** biased ligands, G protein-coupled receptor kinases, G protein-coupled receptors