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G protein-coupled receptor kinases in normal and failing myocardium

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1. ABSTRACT

Heart failure (HF) is the end stage of many underlying cardiovascular diseases and is among the leading causes of morbidity and mortality in industrialized countries. One of the striking characteristics of HF is the desensitization of G protein-coupled receptor (GPCR) signaling, particularly the β-adrenergic receptor (βAR) system. GPCR desensitization is initiated by phosphorylation by GPCR kinases (GRKs), followed by downregulation and functional uncoupling from their G proteins. In the heart, the major GRK isoforms, GRK2 and GRK5, undergo upregulation due to the heightened sympathetic nervous system activity that is characteristic of HF as catecholamine levels increase in an effort to drive the failing pump. This desensitization leads to the distinctive loss of inotropic reserve and functional capacity of the failing heart. Moreover, GRK2 and GRK5 have an increasing non-GPCR interactome, which may play critical roles in cardiac physiology. In the current review, the canonical GPCR kinase function of GRKs and the novel non-GPCR kinase activity of GRKs, their contribution to the pathogenesis of cardiac hypertrophy and HF, and the possibility of GRKs serving as future drug targets will be discussed.

2. INTRODUCTION

Heart failure (HF) is the end stage of many underlying cardiovascular diseases such as myocardial ischemia, hypertension, valve disease, and congenital malformation. HF is marked by the inability of the heart to adequately pump blood throughout the body. HF is among the leading causes of morbidity and mortality in industrialized countries with 1 in 8.6 death related to HF in 2006 in the United States (1). Regardless of the underlying cause, one striking characteristic of HF is the dysregulation of G protein-coupled receptor (GPCR) signaling, particularly the β-adrenergic receptor (βAR) system. In HF, β_1ARs are significantly down regulated and both β_1ARs and β_2ARs are functionally uncoupled from their G proteins due to increased desensitization by over-active GPCR kinases (GRKs). Other GPCRs in the heart are also likely affected by heightened GRK activity. Hence, signals through all G proteins are altered, including Gq, a nodal signaling switch for adaptive and maladaptive cardiac hypertrophy (2). Receptors implicated in cardiac hypertrophy that have been shown to be GRK targets include α–adrenergic receptors (αARs), angiotensin II receptors (AT_1Rs) and endothelin-1 receptors (ET_1Rs) (3, 4). The regulation of GPCRs by GRKs in the heart, the

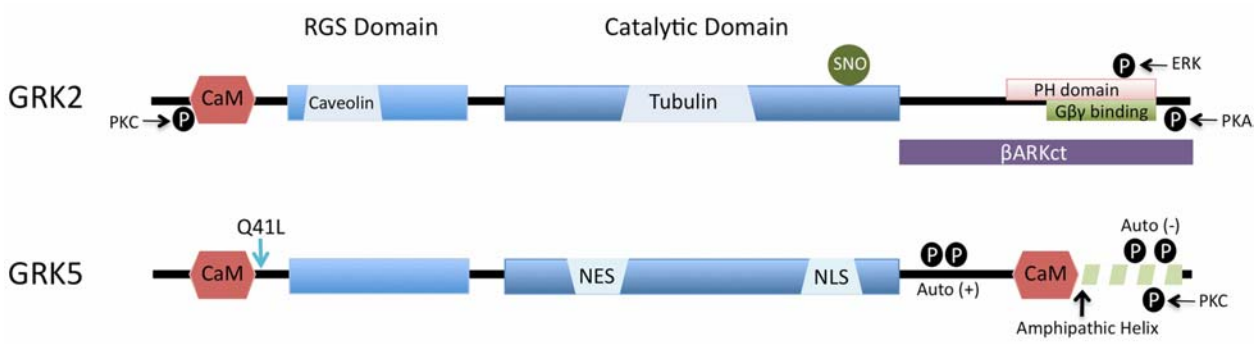

Figure 1. Linear Diagram of GRK2 and GRK5 displaying the classical tri-domain structure of the GRKs. Important interaction and regulation sites are highlighted, including the RGS domain within the amino-terminus of GRK2, the carboxyl terminal region of GRK2 that includes the βARKct and also the amino-terminal GRK5 polymorphism Q41L, which is important in human HF.

corresponding effects on cardiac diseases, and the possibility of GRKs to be future drug targets will be the focus of this review.

3. CARDIAC GPCRS AND GRKS

It is apparent that some of the most important GPCRs in the cardiovascular system are the βAR, the αAR, the AT_1R and the ET_1R. These receptors individually and collectively regulate cardiac growth and function, including heart rate, contractility, and blood pressure in response to catecholamines and other neurohormones. Since GPCRs are an important factor in the heart's response to stress and injury, they are dynamically regulated to prevent overstimulation. This dampening process, known as desensitization, is initiated through GPCR phosphorylation by second-messenger kinases (for example, protein kinase A (PKA) and protein kinase C (PKC)) or the GRKs. Phosphorylation of GPCRs decreases their affinity to bind and activate G proteins. PKA and PKC initiate heterologous desensitization while GRKs initiate homologous desensitization, phosphorylating only agonist-occupied GPCRs (5).

Seven mammalian GRKs have been characterized to date and can be segregated into three subfamilies: (1) GRK1 and GRK7 (rhodopsin kinase sub-family); (2) GRK2 and GRK3, formerly β-adrenergic receptor kinase 1 and 2, respectively (βARK sub-family); and (3) GRKs 4, 5 and 6 (GRK4-like family) (5). Division into subfamilies is due to differences in expression and receptor specificity (6). This review will focus on GRK2 and GRK5, the predominant GRKs in the heart. Both have been shown to be critical for physiological and pathological cardiac signaling and function and, most importantly, these GRKs are upregulated in the failing heart (7-10).

In general, the GRKs share a tri-domain structure -- a central, strongly conserved catalytic domain homologous to other serine/threonine kinases is flanked by amino and carboxyl termini that vary in structure. These flanking domains contain elements involved in regulation and membrane localization (5, 11). The amino-terminal domain has been proposed to be important for receptor recognition (11), as well as alteration of GRK activity and subcellular localization (12-14). Additionally, GRK2's amino terminus contains a Regulator of G-protein Signaling (RGS) homology domain that has been demonstrated to bind and inactivate $G_{(}q/11$ (14). Determination of subcellular localization is the major function of the carboxyl terminus of GRKs. Structural variations in this domain may account for differences in agonist-dependent translocation and receptor specificity. For example, the carboxyl-terminal domain of GRK2 contains a pleckstrin homology (PH) domain that binds the βγ-subunit of G proteins ($G_{\beta\gamma}$) (15, 16). Upon GPCR activation and dissociation of G_α from $G_{\beta\gamma}$, this interaction recruits GRK2 to the membrane from its basal location in the cytoplasm (17). Distinctively, GRK5 does not contain a PH domain, and remains bound constitutively to the plasma membrane via a carboxyl terminus amphipathic helix and phosphatidylinositol 4,5-bisphosphate (PIP_2) binding domains (13, 18) (Figure 1). In addition, GRK5 contains a functional nuclear localization signal (NLS) within its central catalytic domain (19, 20). GRK2 and GRK5 do share some properties that will be highlighted below including the fact that they themselves can be regulated by kinases such as PKC (21).

The pathway of homologous βAR desensitization is best demonstrated by the activity of the ubiquitously expressed GRK2, the major GRK isoform in the heart. GRK2 is primarily cytosolic under basal conditions and upon GPCR activation GRK2 translocates to where the receptor is located via binding to the membrane-bound $G_{\beta\gamma}$. Following GRK phosphorylation, β-arrestin binds to the receptor, sterically hindering interactions between the receptor and the G proteins and resulting in desensitization within milliseconds to minutes of GPCR activation (22). In addition, β-arrestin-bound receptors are internalized to intracellular lysosomes and degraded, leading to receptor downregulation at the plasma membrane (23) (Figure 2). Importantly, the $G_{\beta\gamma}$-mediated translocation of GRK2 has been exploited to generate a potent inhibitor of GRK2 activation as a polypeptide comprised of the last 194 amino acids of GRK2, known as the βARKct, has been shown to compete with endogenous GRK2 for $G_{\beta\gamma}$ binding, preventing GPCR desensitization both *in vitro* and *in vivo*

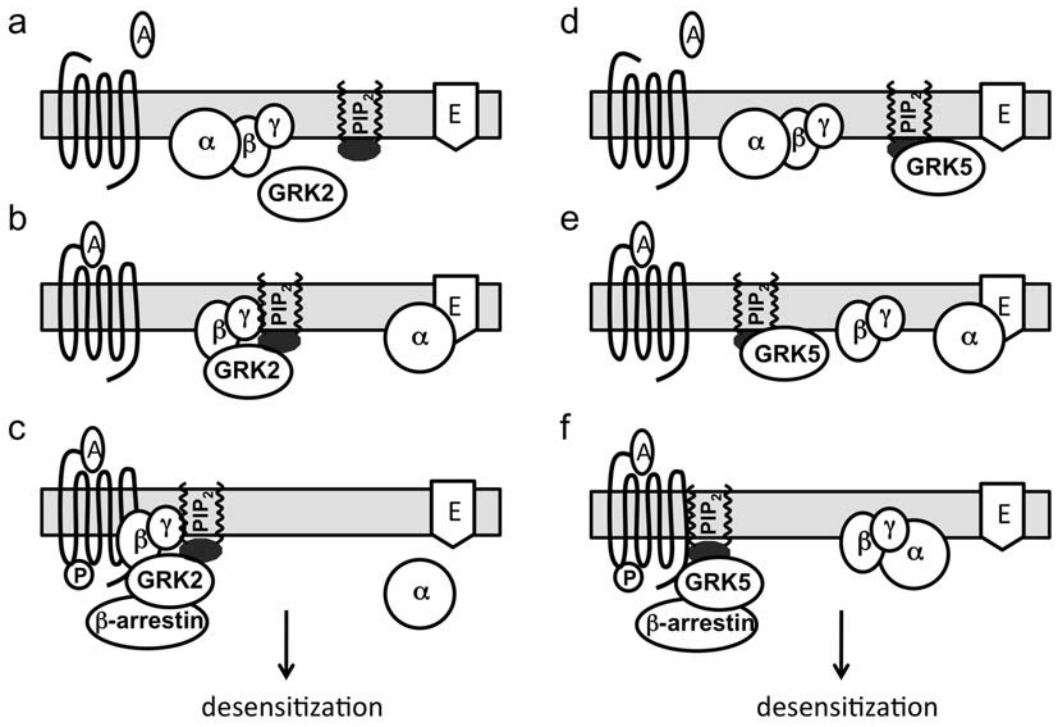

Figure 2. GRK2 (a, b, and c) and GRK5 (d, e, and f) mediated GPCR desensitization. a and d: basal condition. GRK2 is cytosolic and GRK5 constitutively bound to the membrane via binding of its polybasic domain to PIP2. b and e: GPCR activation by agonist (A) causes G protein dissociation into βγ subunits and GTP-bound α subunit, which binds to effectors (E) and activate downstream signal transduction. GRK2 binds to $G_{\beta\gamma}$ and PIP2 and translocates from cytosol to membrane. GRK5, on the other hand, does not need the assistance of $G_{\beta\gamma}$. c and f: GRKs phosphorylate GPCRs and β-arrestin binds to the complex, sterically blocking the activation of G protein, leading to receptor endocytosis and desensitization. The β-arrestins also induce novel signaling pathways distinct from G proteins through their kinase scaffolding (101).

(16, 24). GRK5, on the other hand, does not need to undergo translocation prior to receptor targeting as it is constitutively membrane associated (25). Despite similar roles in GPCR desensitization, studies of GRK2 and GRK5 in the heart using genetically engineered mice show distinct phenotypes, especially concerning maladaptive hypertrophy and HF. In fact, differences in their activity in the heart make both of these GRKs exciting targets for novel therapeutic strategies for heart diseases.

4. GRK2 AND ITS ROLE IN CARDIAC PHYSIOLOGY AND PATHOPHYSIOLOGY

4.1. GRK2-targeted engineered mice

The importance of GRK2 in the regulation of cardiac contractile function has been documented primarily by studies in genetically engineered mice (Table 1). When GRK2 was disrupted globally by homologous recombination in mice, no $GRK2^{-/-}$ animals survived beyond gestational day 15.5 (26). Interestingly, $GRK2^{-/-}$ embryos displayed pronounced hypoplasia of the ventricular myocardium, and *in utero* intravital microscopy showed that $GRK2^{-/-}$ embryos exhibited a >70% decrease in cardiac ejection fraction, suggesting cardiac failure (26). Importantly, heterozygous knockout mice (KO), with 50% levels of GRK2 in myocardium, showed increased contractile function in both whole animal and single isolated myocytes (26). More recently, conditional, cardiac-specific GRK2 KO mice using GRK2*floxed* mice and Cre recombinase driven by the Nkx2.5 promoter developed normally, which shows that the developmental defect in the global GRK2 KO mice was not cardiac autonomous (27). These cardiac-specific KO mice did have increased cardiac damage due to chronic treatment with the βAR agonist isoproterenol (Iso) demonstrating that under these non-physiological stress conditions, the loss of receptor desensitization can promote injury (27). GRK2 has been deleted in cardiac myocytes of adult mice using Cre recombinase driven by the αMHC promoter that is either constitutively active or inducible (MerCreMer) with tamoxifen (28). These mice have increased function consistent with heterozygous global KO mice and actually averted HF development (discussed in more detail below) (28).

Transgenic mice with altered GRK2 activity have also been developed. Cardiac-specific GRK2 overexpressing mice were generated over a decade ago. These mice displayed a loss of βAR-mediated inotropic reserve (24) and augmented AT_1R desensitization in their hearts (29). Cardiac-targeted βARKct mice have also been extensively studied. These were the first mice used to

Table 1. Genetically engineered mice with alterations in GRK expression or activity

Mouse Model	Cardiac Phenotype	Surgical Model	References
GRK2 global KO	KO embryo died before E15.5 Het: increased cardiac contraction		26
GRK2 cardiac specific KO	Enhanced sensitivity to Iso Impairment of tachyphylaxis	More catecholamine toxicity; Resistant to myocardial infarction	27, 28
GRK2 overexpression	More rhodopsin phosphorylation Less β_1AR in high affinity state Less adenylyl cyclase activation Blunted contractility in response to Iso	Decreased tolerance to MI	Koch WJ Unpublished data
βARKct overexpression	Less rhodopsin phosphorylation Increased contractility in response to Iso	Rescue of cardiomyopathy model; Less remodeling after TAC; Increased tolerance to MI	46, 55 Koch WJ Unpublished data
βARKnt overexpression	Increased βAR density Cardiac hypertrophy	Comparable hypertrophy and cardiac function to WT following TAC	30
GRK5 Overexpression	Decreases in Adenylate Cyclase activity and response to Iso stimulation No increased desensitization to AngII Partial Increased desensitization for αAR	Intolerant to pressure overload	29, 88, 20
GRK5 ΔNLS		Comparable hypertrophy and cardiac function to WT following TAC	20
GRK5 Q41L	Improved cardiac function and less cardiac remodeling following Chronic Iso stimulation		94

demonstrate the powerful effect of GRK2 activity on cardiac function as these mice has increased function throughout their lives without demonstrating any myocardial damage (24). These mice have also rescued several mouse models of HF (see below). Mice with cardiac overexpression of an amino-terminal peptide of GRK2 (βARKnt), which did not include the entire RGS domain, exhibited cardiac hypertrophy, even though there is no dampening of Gq-mediated hypertrophic signaling (30). Overall, these mouse models illustrate the importance of GRK2 in modulating myocardial contractile function and growth. As discussed in more detail below, these models provide excellent tools for investigating the regulation of βAR signaling and cardiac function under pathological conditions.

4.2. βARs and GRK2 in failing myocardium

In the heart, β_1ARs are the predominant isoform, comprising about 75-80% of total βARs. β_2ARs and β_3ARs are also expressed (31). The principle function of βARs in the heart is to increase the rate and force of cardiac myocyte contraction following activation by the catecholamines epinephrine (Epi) and norepinephrine (NE). Consequently, pharmacological interventions to stimulate βARs have been used to increase cardiac function in conditions of pump failure. However, long-term usage of β-agonists in conditions such as HF has proven lethal due to catecholamine toxicity (32, 33). On the other hand, and initially counter-intuitive, βAR antagonists can improve mortality and function of the failing heart and are currently standards of care of this condition (32, 34).

The negative outcomes of β-agonists could be due to differential signaling between β_1ARs and β_2ARs in myocardium. To address this point, transgenic mouse models have been used and directly demonstrate that β_1AR signaling in the heart is generally toxic while β_2AR signaling can promote myocyte survival, interestingly, through Gi coupling (35-39). Alternatively, β-agonists may be toxic due to the dysfunctional state of βARs in failing myocardium. In HF, myocardial β_1ARs are selectively downregulated by 50% and remaining β_1ARs and β_2ARs are desensitized (31, 40). This leads to the characteristic loss of inotropic reserve and functional capacity of the failing heart (40). The loss of βAR function is no doubt initiated through heightened sympathetic nervous system activity characteristic of HF as catecholamine levels are increased in efforts to drive the failing pump (41).

The mechanistic link between hyperactive sympathetic nervous system activation and βAR dysfunction is apparently increased GRK2 expression (as well as GRK5 perhaps – see below). GRK2 is increased at the mRNA, protein and activity level in failing human myocardium (7), and is shown to be increased almost immediately after cardiac injury such as myocardial infarction (MI) by coronary artery ligation (42), global ischemia (43), and pressure overload induced hypertrophy (44, 45). In all cases, heightened GRK2 activity causes βAR desensitization. A model to explain this - as the heart fails, desensitization of the βARs by GRK2 leads to insufficient cardiac output activating compensatory mechanisms that chiefly increase catecholamine excretion. This further increases GRK2 expression and facilitates βARs desensitization (44, 46, 47). The vicious cycle eventually leads to deterioration of heart function and facilitates HF progression. The success of β-blockade in treating chronic HF in clinical practice supports the maladaptive nature of βAR desensitization. Indeed, chronic treatment of mice with β-blockers decreases GRK2 expression in the heart (46). Combined with the generally favorable effects of GRK2 lowering in HF models, (see below) this could represent a novel action of β-blockers that contributes to their success in HF.

Since GRK2 is elevated after myocardial injury and remains elevated chronically in HF, studies have begun to address whether this could be a novel biomarker to evaluate ventricular dysfunction in human disease. Importantly, levels of GRK2 in peripheral lymphocytes

have been shown to mirror cardiac levels (48, 49). Indeed, lymphocyte levels of GRK2 are elevated in human HF patients (49) and interestingly, in human hypertensive patients, mimicking GRK2 up-regulation during cardiac stress (50). The mirrored levels of GRK2 in white blood cells and cardiac tissue were best illustrated in our study where LV and blood samples from the same patient were examined for GRK2 (48). In this study, patients with chronic HF undergoing surgery for a LV mechanical assist device (LVAD) were evaluated for blood and cardiac GRK2 levels before and 2-3 months after mechanical unloading (48). LVAD implantation was found to lower GRK2 levels in myocardium as well as in lymphocytes, and corresponded with mechanical unloading of the heart improving βAR signaling and contractile function (48). Further, there was a significant positive correlation between myocardial and lymphocyte GRK2 levels in both HF and LVAD samples. Thus, lymphocyte GRK2 may serve as a surrogate marker of myocardial GRK2 in these patients (48). In a more recent study, blood from 24 HF patients before and after heart transplantation was analyzed and followed up to 1 year. Lymphocyte GRK2 significantly dropped, remained low, and correlated with improved cardiac function in the transplanted heart, indicating the potential of using lymphocyte GRK2 to predict cardiac function at end stage HF, and to possibly evaluate the effectiveness of the therapy regimen (51).

4.3. Cardiac GRK2 as a Therapeutic Target

Since mice with 50% cardiac GRK2 expression and βARKct transgenic mice had enhanced cardiac contractile function, the hypothesis that targeting GRK2 may improve HF was tested. In fact, the first rescue of a genetic mouse model of HF was demonstrated using the βARKct mice (52). In this study, KO mice for the muscle-LIM protein, which present with a dilated cardiomyopathic phenotype, were bred with βARKct transgenic mice. The resultant hybrid mice had no signs of HF (52). Other genetic models of HF were also rescued by βARKct expression (53, 54). One interesting study involved the combined use of βARKct expression with β-blockade in a model of hypertrophic HF. βARKct mice were crossed with transgenic mice with cardiac overexpression of the sarcoplasmic reticulum Ca^{2+}-binding protein calsequestrin (CSQ) (53). CSQ mice have severe cardiomyopathy and shortened survival (9±1 weeks). The βARKct/CSQ hybrid mice exhibited significant enhancement in survival age to about 15 weeks (46). Cardiac βARKct expression attenuated cardiac remodeling of CSQ mice, as demonstrated by smaller LV end diastolic dimension, LV end systolic dimension, and maintenance of LV wall thickness, accompanied by improved LV fractional shortening. Biochemically, despite decreased membrane βAR density, βARKct induced an increase in the percentage of βARs in the high-affinity state (46). More importantly, βARKct and β-blocker metoprolol had an additive effect with regard to cardiac function (46). These data demonstrated that inhibition of GRK2 is beneficial in established HF models, and could provide additional benefit to β-blocker therapy. The beneficial mechanism of βARKct is sequestering $G_{\beta\gamma}$ and thus inhibiting GRK2 activity. This was directly tested in βARKct transgenic heart lysate. Addition of $G_{\beta\gamma}$ showed significantly less rhodopsin phosphorylation, namely lower GRK2 activity, as compared to without $G_{\beta\gamma}$ (24). Whether βARKct has other effects independent of $G_{\beta\gamma}$ warrants further investigation.

The βARKct mice themselves have been shown to exert therapeutic effects in surgically induced HF models. In fact, a positive gene-dosage effect has been seen with cardiac βARKct expression. Transgenic mice with low βARKct expression developed severe HF 12 weeks after LV pressure overload induced by transverse aortic constriction (TAC), whereas mice with high βARKct expression showed significantly less cardiac deterioration than WT mice (55). Importantly, high levels of myocardial βARKct preserved Iso-stimulated adenylyl cyclase activity and βAR densities in cardiac membranes (55), demonstrating that the level of GRK2 inhibition determines cardiac function. In a different mouse model, βARKct transgene expression was driven under the control of the cardiac ankyrin repeat protein (CARP) promoter, which is active during cardiac development and quiescent in normal adult heart (45). CARP is a fetal gene that is activated during cardiac diseases, like hypertrophy. As a result, when the CARP-βARKct mice underwent TAC, βARKct expression was acutely induced, preventing the loss of βAR responsiveness during hypertrophy, despite upregulated GRK2, as well as ameliorating HF development (45). These results demonstrated that acute GRK2 inhibition could restore lost myocardial βAR responsiveness *in vivo* (45).

Since βARKct works via $G_{\beta\gamma}$ sequestration, there could be non-GRK2 effects contributing to the remarkable HF rescue data presented above. Other potential $G_{\beta\gamma}$ targets include activation of phosphatidylinositol 3–kinase (PI3K) (56) and acetylcholine activated K^+ channels ($I_{K,Ach}$) (57). In addition, it is possible that βARKct inhibits the phosphorylation of other GPCRs since GRK2 has many receptor targets in the heart. Accordingly, to further elucidate that GRK2 is the critical molecule mediating ventricular maladaptation, cardiac-specific GRK2 KO mice were subjected to MI to induce HF (28). In one set of experiments, mice with cardiomyocyte GRK2 loss at birth were observed for 28 days after MI to note any phenotypic changes. GRK2 KO mice showed better survival, despite a similar infarct size (28). Analysis of hemodynamics showed that GRK2 KO mice had better preserved βAR signaling post MI, as compared to control mice. GRK2 KO mice also had less remodeling as reflected by heart weight to body weight ratio, and less fetal gene activation. The protective phenotype was explained at isolated single cell level, since GRK2 KO myocytes from post MI heart displayed better contraction (28). The results directly demonstrated that minimizing GRK2 activity prevents or delays the occurrence of HF (28). In a second set of experiments, inducible cardiac GRK2 KO mice were studied. Mice were subjected to MI producing LV dysfunction and remodeling. Next, GRK2 gene deletion was induced (28). Following the loss of GRK2 in

myocytes, post-MI mice stopped dying, had significantly improved cardiac function and underwent an active reverse remodeling process (28). This data clearly shows that in the setting of ischemic-HF, GRK2 is pathological and its loss actively reverses LV dysfunction. At least in the small animal setting, GRK2 is a profound target for HF therapy (28). Moreover, it indicates that βARKct apparently targets GRK2 inhibition as its primary mechanisms of action since KO and βARKct phenotypes in HF are so similar. Thus, βARKct delivery and expression in the heart appears to represent a potential therapeutic strategy in HF that has indeed been tested using gene therapy approaches (discussed below).

4.4. Cardiac GRK2 Inhibition with Gene Therapy

To make targeting GRK2 more relevant to clinical practices, βARKct delivery has been used in larger animal models of HF. Early studies used adenoviral vectors. For example, in failing myocytes isolated from spontaneously hypertensive heart failure (SHHF) rats, adenovirus-mediated βARKct overexpression led to significant increases in basal and βAR-stimulated cAMP production, at rates even higher than non-failing myocytes (47). Single cell shortening, relaxation, and contraction were also improved by βARKct. Importantly, more recent studies have shown similar results in failing human ventricular myocytes (58). The acute therapeutic efficacy of adenoviral-delivered βARKct has also been shown in several other HF models (rats, rabbits and hamsters) during MI (59), post cardioplegic arrest (60), HF induced by pacing (61), or cardiomyopathic mice with overexpression of a myosin heavy chain gene mutant (54).

The studies using adenovirus demonstrated positive results but had limitations, since adenoviruses support only short-term βARKct expression in the heart *in vivo*. Therefore, to determine clinical relevance, new vectors were needed. This has recently been solved through the use of adeno-associated viral (AAV) vectors, which appear extremely amenable for human use in chronic diseases, such as HF, because they support chronic transgene expression with limited immune problems (62, 63). Recently, a clinically relevant HF study was carried out in rats where AAV6-βARKct was delivered to the post-MI failing rat heart (64). Echocardiography confirmed compromised heart function and remodeling 12 weeks after a cryo-infarction. AAV6-βARKct was then directly delivered to the heart, and rats were followed chronically for 3 months. Echocardiography showed that βARKct improved cardiac contractility and even reversed LV remodeling, while hearts received control virus continued deteriorating (64). The protection by βARKct was accompanied by a normalization of the neurohormonal (catecholamine and aldosterone) status of the chronic HF animals including normalization of βAR signaling (64). These results demonstrate the potential for βARKct gene therapy, which is currently being tested by us in large animal pre-clinical models of HF.

4.5. Novel regulation of GRK2 activity

Since GRK2 appears to play a crucial role in cardiac physiology and pathophysiology, it is of great importance to elucidate the cellular mechanisms that regulate GRK2 function. Attention has been given to the post-translational modification of GRK2, primarily via phosphorylation and S-nitrosylation. There are three major phosphorylation sites on GRK2, a PKC site at Ser29, an ERK site at Ser670 (65), and a PKA site at Ser685 (66). PKC phosphorylation activates GRK2 (67, 68) by abolishing the inhibitory effect of Ca^{2+}/camodulin on GRK2 (69). It has recently been reported that PKC phosphorylation of GRK2 at Ser29 accounts for impaired βAR signaling after mechanical stretch, which is upstream of the Gq pathway (70). The crosstalk between hypertrophic Gq and Gs pathways might be an important mechanism for the transition from hypertrophy to HF. ERK phosphorylation of GRK2 at Ser670, on the other hand, inhibits its membrane translocation and hence its GPCR activity (65).

It was reported recently that GRK2 could be S-nitrosylated, with the primary site being Cys340 (71). Thus, in addition to multiple GPCRs (such as the β_2AR (72) and AT_1R) (73) that are also targets of nitrosylating agents, Targeting GRK2 adds complexity to nitric oxide synthase (NOS) – NO system regulation of cellular signaling. Interestingly, S-nitrosylation of GRK2 attenuates its activity on βAR signaling and other down-stream targets (71). In mice, reduced NO production by the NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) accelerated βAR desensitization in the heart *in vivo* as determined by LV dP/dt_{max} within 30 min of isoproterenol treatment (71). Further, NO bioavailability preserves βAR signaling in the heart through GRK2 inhibition. Consistent with this, mice with more endogenous NO due to deficient breakdown of active GSNO, display increased membrane βAR density (74). Also, mechanistic studies in cells demonstrated that NO donors inhibited GRK2 mediated events, including βAR phosphorylation and subsequent β-arrestin binding to the receptor as measured by FRET, cAMP production, and receptor internalization (71). In addition to GRK2, other proteins involved in endocytosis, like β-arrestin (75) and dynamin (76), are also S-nitrosylated. This novel form of post-translational regulation has important therapeutic significance, as during HF, there is both deficiency of NO and desensitized βAR signaling. Thus, supplementation of NO donors may also serve to inhibit GRK2 and restore βAR signaling. On the other hand, the deficiency of NO bioactivity and, thus, less endogenous GRK2 inhibition, may be another mechanism for disturbed GPCR signaling during HF. Studies investigating the functional effects of S-nitrosylation on GRK2 during diseased states will provide more insights into the benefits of manipulating this regulation (71).

4.6. GPCR independent functions of GRK2 in the heart

In addition to its classical GRK activity, emerging new data suggests that GRK2 may have functions independent from its actions on GPCRs. The recent concept of an extensive "GRK2 interactome" points to the possibility that GRK2 may exert its functions by interacting with other intracellular proteins (77). For example, GRK2 interacts and phosphorylates tubulin in bovine brain (78), which facilitates the polymerization of tubulin into microtubules (79). It has been reported that an increased

ratio of microtubules to tubulin in the heart is pro-hypertrophic (80). Whether the regulation of tubulin by GRK2 in the heart has functional significance merits further investigation. Emphasizing the potential importance of GRK2 in cardiac hypertrophy, it has been shown that GRK2 can influence the cell cycle of certain cells and that cell cycle kinases can regulate GRK2 itself (81). This has yet to be seen in myocytes but presents an intriguing scenario if this is true.

An interesting recent finding in liver endothelial cells shows GRK2 inhibiting the kinase, Akt (82). Akt is an important survival kinase and a strong activator of endothelial NOS (eNOS) (83, 84). Whether GRK2 could induce changes in Akt and eNOS activity in the heart could explain the phenotypic changes in GRK2 transgenic mice. This obviously has additional importance due to the novel effects of S-NO on GRK2 discussed above. Other potential binding partners of GRK2 reported so far include: caveolin (85), clathrin (86) and ERK1/2 (65). Overall, several of the proteins shown to be present in a dynamic GRK2 interactome may be important in cardiac physiology and pathophysiology, indicating that the beneficial effects of GRK2 targeting in HF extend well beyond the reversal of βAR dysfunction. Future experiments will deal with the mechanistic potential of GPCR independent effects of GRK2 in the heart.

4.7. Adrenal GRK2 and HF

As mentioned above, one of the prominent features of HF is increased circulating catecholamines including Epi and NE, due to sympathetic nerve system hyperactivity. While NE is secreted by sympathetic nerve terminals, Epi is mainly secreted by the chromaffin cells of adrenal medulla. The secretion of Epi is tightly regulated by α_2ARs, which serve as a presynaptic negative feedback mechanism. Recent work in our lab demonstrated that in animal models of HF, adrenal GRK2 upregulation led to downregulation and uncoupling of α_2ARs and elevated circulating catecholamines as a result of losing the inhibitory feedback (87). Adrenal βARKct gene delivery post-MI rescued both the adrenal and cardiac dysfunction (87, 88). Furthermore, chromaffin cell-specific GRK2 KO exhibited decreased circulating catecholamines post-MI and better cardiac function, suggesting the potential of adrenal GRK2 as a therapeutic target for HF (89).

5. THE ROLE OF GRK5 IN MALADAPTIVE HYPERTROPHY AND HF

5.1. GRK5-Targeted Engineered Mice

Although GRK5 has not been as extensively studied as GRK2 in myocardium, it has also been found to be upregulated in HF, including in humans (8-10). Studies in cardiac-targeted GRK5 overexpressing transgenic mice have found that this GRK can desensitize distinct receptors compared to GRK2, although they both share in the regulation of cardiac βARs (90, 91). Indeed, GRK5 transgenic mice have blunted *in vivo* inotropic responses to βAR stimulation similar to GRK2 overexpression (29). However, in contrast to reduced cardiac AT_1R signaling in GRK2 transgenic mice, GRK5 mice had normal *in vivo* functional responses to angiotensin II, showing the first GPCR selectivity by these two GRKs (29). Divergent receptor specificity was also demonstrated for the α_{1B}AR *in vivo*, with GRK5 showing partial desensitization and GRK2 displaying no inactivation effects (91). The ability of GRK2 and 5 to desensitize specific GPCRs suggests overlapping, but distinct roles of these predominant cardiac GRKs.

5.2. Role of GRK5-mediated non-cannonical signaling in HF

Characterization of GRK distribution in SHHF rats uncovered novel nuclear localization of GRK5 (92). Cardiomyocytes isolated from SHHF rats display significant redistribution of GRK5 in the nucleus, while myocytes from wild-type Wistar rats show diffuse distribution throughout the cytoplasm (75). This nuclear accumulation is unique to GRK5 as staining for GRK2 shows a diffuse cytoplasmic distribution in both the SHHF rats and the Wistar rats (92, 93). It was then discovered that GRK5 has a functional NLS within its catalytic domain, as well as a putative nuclear export sequence (NES) (19). To demonstrate the functional capacity of the catalytic domain NLS to guide GRK5 into the nucleus, a mutant GRK5 was generated by mutating the basic residues within its NLS to alanines. This mutant GRK5 was unable to enter the nucleus (19). Additionally, the NLS of GRK5 shows homology to the DNA binding NLS of homeobox transcription factors, and is able to selectively bind single-stranded DNA, suggesting a possible role of GRK5 as a transcription factor (19).

In regards to HF, a very exciting and potential significant role for nuclear GRK5 has recently been elucidated. Following TAC surgery in mice, or Gq-stimulation, nuclear accumulation of GRK5 increases (20). In fact, in cardiac-targeted GRK5-overexpressing transgenic mice TAC-induced hypertrophy and subsequent HF was significantly exaggerated and accelerated (20). Interestingly, nuclear GRK5 accumulation leads to increased cardiomyocyte growth via augmented activity of myocyte elongation factor 2 (MEF2), a transcription factor critical for cardiac development and growth (94). We found that GRK5 has novel histone deacetylase (HDAC) kinase activity in the nucleus and phosphorylates HDAC5, a class II HDAC that represses MEF2 activity in the heart (95, 96). Thus, increased activity of GRK5 in the nucleus of myocytes enhances MEF2 activity and appears to participate in maladaptive cardiac hypertrophy. Interestingly, GRK5 nuclear accumulation and HDAC kinase activity lie downstream of activated Gq. The post-Gq and hypertrophic signaling pathways that induce GRK5 accumulation in the nucleus remain to be determined (Figure 3). The role of nuclear GRK5 in promoting cardiac hypertrophy and HF was recapitulated *in vivo* with the generation of a transgenic mouse expressing a mutant GRK5 devoid of a functional NLS (ΔNLS). Compared to wild-type mice, the ΔNLS mice show no difference in cardiac hypertrophy or function at four weeks post-TAC (20). Recent data from our lab reinforces this injurious role of cardiac GRK5, as global GRK5 KO mice, which have no

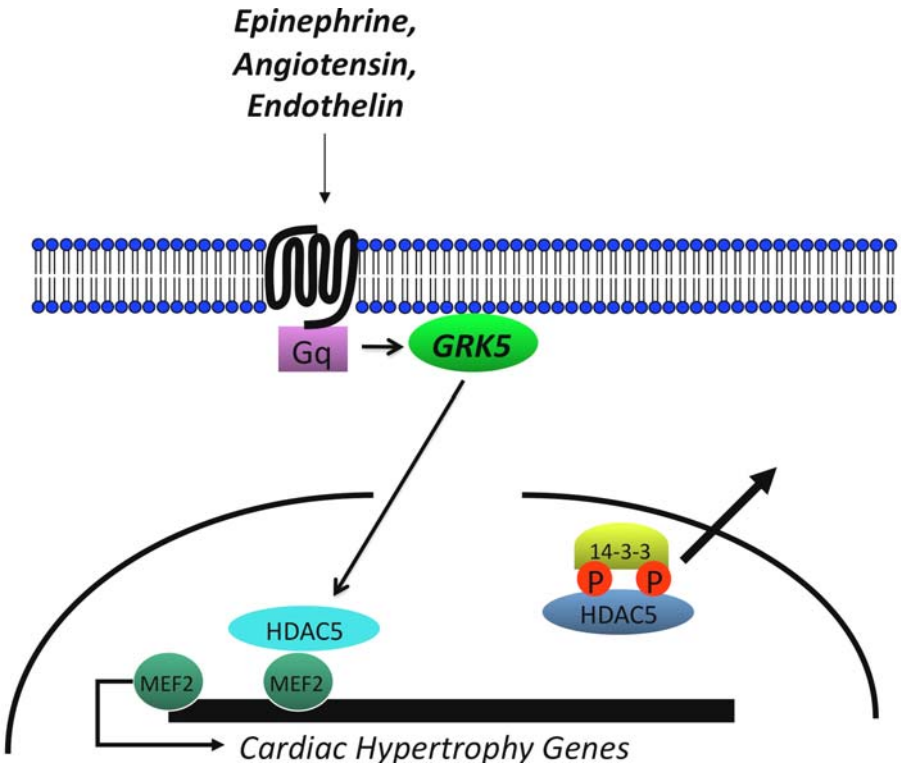

Figure 3. GRK5 functions as a HDAC kinase. During the stage of pathological hypertrophy that precedes HF, increased signaling activity occurs through GPCRs that couple to Gq. Examples of these GPCRs and their agonists are the αAR (Epinephrine), AT_1R (Angiotensin II), and ET_1R (Endothelin). GRK5 nuclear accumulation is downstream of activated Gq (20). Upon entering the nucleus, via its catalytic domain-located NLS, GRK5 can phosphorylate HDAC5, targeting it for nuclear export and releasing its repression on MEF2, a transcription factor crucial in regulating cardiomyocyte growth.

basal cardiac phenotype, demonstrate attenuated hypertrophy and normal cardiac function when compared to WT at 12 weeks post-TAC (Koch lab, unpublished data). However, the exact role of cardiomyocyte-specfic GRK5 deletion remains uncharacterized.

5.3. Human Polymorphisms of GRK5

A recent discovery has strengthened the clinical significance of GRK5 in HF. Liggett et al. recently uncovered a single nucleotide polymorphism (SNP) in GRK5 that protects against catecholamine toxicity associated with HF (97). Common among African Americans, this amino terminal SNP creates a glutamine to leucine switch at amino acid position 41 of GRK5. Transgenic mouse models with low and comparable levels of cardiac GRK5Glu41 and GRK5Leu41 reveal a protective effect of GRK5Leu41 following chronic infusion of Iso via mini-osmotic pump (97). The GRK5Leu41 transgenic mice show less cardiac remodeling with smaller heart weights and decreased alterations in LV end diastolic diameter. These beneficial effects in mice appear to be translated to African American HF patients. Without β-blocker treatment, patients with the GRK5Leu41 allele show prolonged survival or time to cardiac transplant compared to β-blocker naïve patients expressing the GRK5Glu41 allele (97). The effects of the GRK5Leu41 allele mirror the effects of β-blocker treatment in patients with the GRK5Glu41 allele, yet there is no additive effect of β-blocker treatment in the GRK5Leu41-expressing patients (97). This "genetic βAR blockage" expounds the importance of decreased βAR stimulation in HF. Interestingly, the mutation's position, at amino acid 41, abuts an important calmodulin regulatory region (5, 98, 99). Calmodulin has been shown to decrease GRK5's plasma membrane association and increase GRK5's kinase activity on cytoplasmic substrates (98). Decreased affinity for calmodulin by the Q41L mutation may cause increased plasma membrane association and heightened desensitization of βARs.

6. CONCLUSIONS

Despite the apparent similarities in structure and function of GRK2 and GRK5, these kinases fulfill non-redundant roles in the heart. Distinct phenotypes displayed in genetically engineered mice and surgically acquired models of cardiac injury, in addition to differential regulation of GRK2 and GRK5 in HF, demonstrate that both of these GRKs deserve to be considered as potential therapeutic targets for alleviating maladaptive cardiac hypertrophy and HF. To note, an interesting divergence between GRK2 and GRK5 lies in their subcellular localization. As discussed above, GRK2 displays diffuse cytoplasmic distribution basally. GPCR activation targets GRK2 to the plasma membrane via various interactions at

its carboxyl terminal. On the other hand, GRK5 is constitutively membrane-associated via its carboxyl terminal. However, agonist binding to specific GPCRs, likely receptors that couple to Gq, promotes membrane dissociation of GRK5 and translocation into the nucleus. In both cases, the GRK's subcellular locale relates to its role in HF pathology. Decreasing GRK2 recruitment to the membrane by expression of βARKct rescues cardiomyocytes from dysregulation, and ameliorates functional loss after cardiac injury. The relationship between GRK5 localization and cardiac pathology appears to mirror that of GRK2, however, instead of increased GRK5 at the plasma membrane being pathological, the accumulation of GRK5 in the nuclear compartment of myocytes is detrimental to cardiac function. Additionally, plasma membrane-associated GRK5 may be beneficial for preserving function in the face of cardiac insult. Recent data has suggested that GRK5 plays a role in EGFR transactivation, protecting the heart against ischemic injury through increased EGF signaling (100). In a similar vein to βARKct, the development of a therapeutic intervention that prevents the nuclear accumulation of GRK5 may show beneficial effects in the treatment of HF.

In dissecting the differences between GRK2 and GRK5, it is also important to note discrepancies in their regulation by kinases and other effector molecules. These molecules likely participate in the differential subcellular domain targeting and modulation of kinase activity of the GRKs. For example, both GRK2 and GRK5 contain calmodulin binding sites at their amino termini, and both GRKs show decreased GPCR phosphorylation following calmodulin binding. The extent of calmodulin's inhibition is significantly greater for GRK5 than GRK2 (69, 98). Carboxyl terminal phosphorylation by PKC relieves this inhibition for GRK2, while further decreasing GRK5's kinase activity on membrane-associated and cytoplasmic substrates (21, 69). Furthermore, GRK5 displays the ability to autophosphorylate at its carboxyl terminal, following stimulation by either phospholipids or calmodulin (12, 98). The differences in regulation outlined above, among others, may help explain the non-overlapping roles of the two primary cardiac GRKs.

As outlined in this review, GRK2 and GRK5 are intriguing targets for future HF therapy. Experiments with βARKct demonstrate an extremely promising role for inhibiting GRK2 in order to prevent HF and rescue diseased cardiomyocytes. Further study into the mechanism of GRK5 nuclear accumulation will allow for development of intervening agents. Overall, future research on the classical and non-cannonical roles of GRK2 and GRK5 in HF as well as delving deeper into the translational significance of the ever increasing "interactomes" will likely continue to uncover the importance of these two GRKs in the pathogenesis of cardiac disease.

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Abbreviations: HF: Heart failure, GPCR: G protein-coupled receptor, GRK: GPCR kinase, βAR: β-adrenergic receptor, AT_1R: angiotensin II receptors, ET_1R: endothelin-1 receptor, PKA: protein kinase A, PKC: protein kinase C, PIP_2: phosphatidylinositol 4,5-bisphosphate, CSQ: calsequestrin, MI: myocardial infarction, TAC: transverse aortic constriction, CARP: cardiac ankyrin repeat protein, PI3K: phosphatidylinositol 3–kinase, $I_{K,Ach}$: acetylcholine activated K^+ channels, AAV: adeno-associated virus, eNOS: endothelial nitric oxide synthase, MEF2: myocyte elongation factor 2, HDAC: histone deacetylase, NLS: nuclear localization sequence, NES: nuclear export sequence, SNP: single nucleotide polymorphism, EGF: epidermal growth factor

Key Words: Beta-adrenergic receptor, heart failure, G protein-coupled receptor kinases, βARKct, desensitization, cardioprotection, Review

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