

This Review is the first in a thematic series on **Novel Aspects of Cardiovascular G Protein-Coupled Receptor Signaling: Implications for New Therapeutics**, which includes the following articles:

Introduction to the Series [*Circ Res.* 2011;109:202–204]

Biased Ligands for Better Cardiovascular Drugs: Dissecting GPCR Pharmacology [*Circ Res.* 2011;109:205–216]

G Protein-Dependent and -Independent Signaling Pathways and Their Impact on Cardiac Function [*Circ Res.* 2011;109:217–230]

Compartmentalization of  $\beta$ -Adrenergic Signals in Cardiomyocytes [*Circ Res.* 2011;109:231–244]

G Protein-Coupled Receptor Kinases as Therapeutic Targets in Cardiovascular Disease

Regulators of G Protein Signaling in the Heart and Their Potential as Therapeutic Targets

*Howard Rockman, Guest Editor*

## G Protein Coupled Receptor Kinases as Therapeutic Targets in Cardiovascular Disease

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**Abstract:** G protein-coupled receptors (GPCRs) represent the largest family of membrane receptors and are responsible for regulating a wide variety of physiological processes. This is accomplished via ligand binding to GPCRs, activating associated heterotrimeric G proteins and intracellular signaling pathways. G protein-coupled receptor kinases (GRKs), in concert with  $\beta$ -arrestins, classically desensitize receptor signal transduction, thus preventing hyperactivation of GPCR second-messenger cascades. As changes in GRK expression have featured prominently in many cardiovascular pathologies, including heart failure, myocardial infarction, hypertension, and cardiac hypertrophy, GRKs have been intensively studied as potential diagnostic or therapeutic targets. Herein, we review our evolving understanding of the role of GRKs in cardiovascular pathophysiology. (*Circ Res.* 2011;109:309-319.)

**Key Words:** G protein-coupled receptors ■ G protein-coupled receptor kinases ■ heart failure ■ adrenergic receptors ■ cardiovascular disease

Diseases of the heart, including heart failure (HF), are the leading cause of death for both men and women in the United States, accounting for more than one in four deaths in 2006.<sup>1</sup> Roughly 5.8 million Americans have HF and 670,000 new cases are diagnosed annually, with associated health care and loss of productivity costs estimated at \$39.2 billion for 2010.<sup>2,3</sup> Though significant improvements in patient care have been realized with  $\beta$ -adrenergic receptor ( $\beta$ -AR) blockers, angiotensin receptor blockers, angiotensin converting

enzyme (ACE) inhibitors, aldosterone inhibitors, and diuretics, these standard HF treatments remain insufficient. Increasing our understanding of the molecular and cellular processes that contribute to HF pathogenesis, therefore, is of critical importance to developing improved therapeutic strategies.

It has long been appreciated that in response to the reduced cardiac output of the failing heart, the sympathetic nervous system (SNS) releases neurohormones to both stimulate the heart and retain salt and water.<sup>4,5</sup> Postganglionic and systemic

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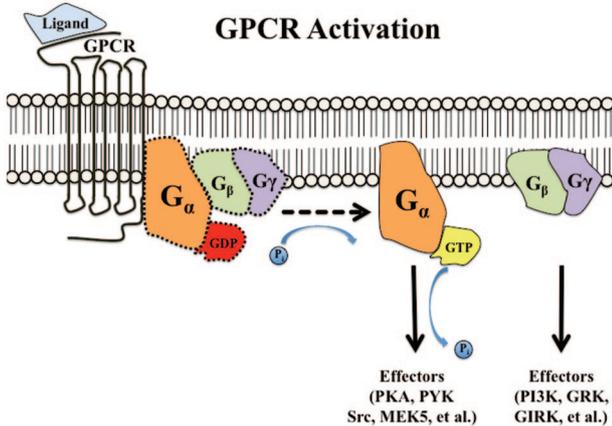
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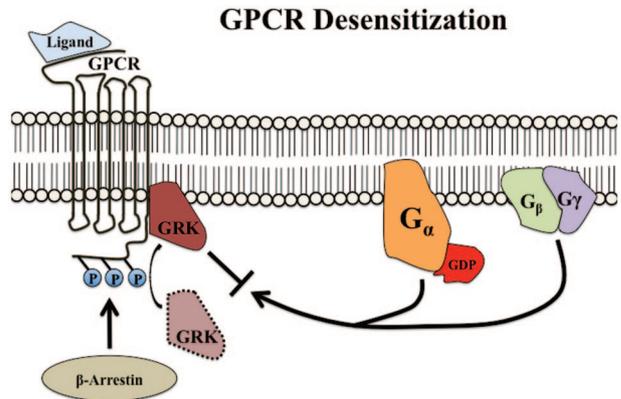
## Non-standard Abbreviations and Acronyms

AR	adrenergic receptor
ACE	angiotensin converting enzyme
ANF	atrial natriuretic factor
$\beta$ ARKct	$\beta$ adrenergic receptor carboxyl terminus peptide
$I_{Ca}$	calcium current
eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinase
G $\beta\gamma$	G protein $\beta\gamma$ subunit
GPCR	G protein-coupled receptor
GRK	G protein-coupled receptor kinase
HF	heart failure
IRS	insulin receptor substrate
IRES	insulin resistance
LV	left ventricle
LVAD	left ventricular assist device
MI	myocardial ischemia
PH	pleckstrin homology
PKA	protein kinase A
PKC	protein kinase C
RGS	regulator of G protein signaling
SNS	sympathetic nervous system
VSMC	vascular smooth muscle cell

release of catecholamines stimulate guanine nucleotide (G) protein-coupled  $\beta$ -ARs of the myocardium, increasing heart rate, enhancing contraction, and improving cardiac performance.<sup>6</sup> At the receptor level, agonist binding promotes dissociation of the heterotrimeric G protein into  $\alpha$  and  $\beta\gamma$



**Figure 1. G protein-coupled receptor (GPCR) activation.** Generalized schematic for GPCR activation. On ligand binding to the GPCR, the receptor undergoes a conformational change whereby the  $\alpha$  subunit of its associated G protein is activated by exchanging bound GDP for GTP. The  $\alpha$  and  $\beta\gamma$  subunits of the G protein subsequently dissociate to activate their respective downstream signaling cascades. For a more comprehensive overview of the complex variety of GPCR signaling cascades, please refer to Neves et al.<sup>148</sup> PKA, protein kinase A; PYK, protein-rich tyrosine kinase; MEK5, mitogen/extracellular signal regulated kinase kinase-5; PI3K, phosphatidylinositol-3 kinase; GRK, G protein-coupled receptor kinase; GIRK, G protein-activated inward rectifying  $K^+$  channel.



**Figure 2. GPCR desensitization.** GRKs are recruited to, and phosphorylate ligand-occupied GPCRs on the cytoplasmic carboxyl-terminal tail. Beta-arrestins bind phosphorylated GPCRs with enhanced affinity, thereby creating a platform for: blocking recoupling of the dissociated G protein subunits to the GPCR, thus preventing further receptor activation (ie, desensitization); coordination of GRK and arrestin in assembly of macromolecular signaling complexes; and recruitment of endocytotic machinery as a precursor to receptor internalization (ie, down-regulation), whence the receptor may be dephosphorylated and recycled back to the membrane or targeted for lysosomal degradation. GRK, G protein-coupled receptor kinase; GPCR, G protein-coupled receptor.

subunits (see Figure 1), stimulating adenylyl cyclase to increase cAMP production, and activating protein kinase A (PKA).<sup>7</sup> Sustained  $\beta$ -AR stimulation is deleterious over time, however, causing receptor desensitization and downregulation, loss of responsiveness to catecholamines, and further contractile dysfunction.<sup>6,8,9</sup>

The harmful effects of chronic G protein-coupled receptor (GPCR) stimulation are initially mitigated by negative feedback via G protein-coupled receptor kinases (GRKs), originally named  *$\beta$ -adrenergic receptor kinases*. In a process termed *homologous desensitization*, GRKs phosphorylate agonist-bound receptors,<sup>10,11</sup> leading to  $\beta$ -arrestin recruitment to the receptor<sup>12</sup> (see Figure 2). Consequently, dissociated G proteins are sterically inhibited from coupling to the receptor/ $\beta$ -arrestin complex and further downstream signaling is inhibited.<sup>13</sup> Furthermore,  $\beta$ -arrestins target the receptor for clathrin-coated pits in the cell membrane that are internalized and either recycled back to the cell surface or degraded.<sup>14</sup>

This classic mechanism of regulating GPCR signaling and the primary role of GRKs in initiating this process have been extensively studied over the last 30 years.<sup>15–19</sup> Even so, evolving appreciation of the role of GRKs in both cardiac and noncardiac tissue, as well as the complex variety of non-GPCR substrates that make up the GRK “interactome,”<sup>20</sup> suggest GRK functions beyond GPCR desensitization and down-regulation may provide novel insights. In this review, we highlight our understanding of GRK physiology, with particular emphasis on recent findings with relevance to cardiovascular disease.

### GRK Family Members

Seven genes are known to encode the mammalian GRKs (1–7),<sup>15,21</sup> a family of serine/threonine kinases sharing common structural and functional features (see Table). All GRKs

**Table. Summary of Mammalian GRKs**

GRK	Tissue Expression	Primary Target GPCR(s)	GRK Modification	Functional Effect	Reference No.
1	Retina	Rhodopsin	Gene ablation	Prolonged photon response and rod apoptosis	39
2	All, heart	$\beta$ -AR, angiotensin II type 1	Gene ablation	Embryonic lethality	46
			Conditional myocardial gene ablation	Enhanced inotropic $\beta$ -AR sensitivity, blunted inotropic and lusitropic tachyphylaxis	48
			Myocardial overexpression	Enhanced desensitization to $\beta$ -AR- or angiotensin II-mediated effects on contractility and heart rate	50, 51
3	All, olfactory epithelium	$\alpha_1$ -AR, thrombin, $M_2$ and $M_3$ muscarinic	Vascular overexpression	Impaired $\beta$ -AR-mediated vasodilation	119
			Gene ablation	Loss of odorant-receptor mediated desensitization; enhanced airway smooth muscle constriction	42, 145
4	Testis, kidney, brain	Dopamine-1	Myocardial overexpression	Reduced $\alpha_{1B}$ -AR signaling and mitogen-activated protein kinase activation	53
			Overexpression	No effect with wild-type GRK4 $\gamma$ but A142V polymorphism yields impaired natriuresis, hypertension	137
5	All, heart	$\beta$ -AR, angiotensin II type 1, $M_2$ muscarinic	Gene ablation	Heightened response to cholinergic stimulation (eg, hypothermia, salivation, tremor, antinociception); diminished airway smooth muscle relaxation	43, 44
			Myocardial overexpression	Enhanced desensitization to $\beta$ -AR chronotropic and inotropic effects	51
6	All	Chemokine receptor 4, dopamine-2	Gene ablation	Impaired T-cell chemotaxis; enhanced sensitivity to locomotor-stimulating effects of cocaine, amphetamine	45, 146
7	Retina	Cone opsin	Inhibition (antibody)	Reduced termination of phototransduction	40, 41

GRK indicates G protein-coupled receptor kinase; GPCR, G protein-coupled receptor.

possess a central catalytic domain, flanked by an amino terminus containing a regulator of G protein signaling (RGS) homology domain and a variable length carboxyl end.<sup>22</sup> The N-terminal region seems critical for receptor recognition and intracellular membrane anchoring,<sup>23,24</sup> and the C-terminal domain dictates subcellular localization and membrane association or translocation.<sup>25,26</sup>

On the basis of sequence homology and tissue expression, GRKs are further separated into 3 subfamilies: rhodopsin kinases (GRKs 1 and 7);  $\beta$ -adrenergic receptor kinases (GRKs 2 and 3); and the GRK4 subfamily (GRKs 4, 5, and 6). Rhodopsin kinases and GRK4 are generally restricted to retina and testes, respectively, even as the remaining GRKs are ubiquitously expressed, though to varying degrees depending on the tissue.<sup>16,18,20</sup> Essentially, only GRKs 2, 3, and 5 are appreciably expressed in the human heart, with GRKs 2 and 5 the most abundant in the myocardium.<sup>16,27,28</sup>

These 3 primary cardiac GRK isoforms display distinct structural and functional characteristics that likely shape their impact on cardiovascular disease. For example, the C-terminus of GRK5 binds phospholipids, promoting preferential membrane localization,<sup>29</sup> whereas GRK2 and GRK3 are primarily cytoplasmic. Unique to GRK2 and GRK3 is a C-terminal pleckstrin homology (PH) domain that binds  $G\beta\gamma$  subunits, thereby greatly enhancing GPCR phosphorylation through GRK plasma membrane translocation.<sup>30</sup> It should also be noted that the GPCR serine or threonine residue(s) phosphorylated by individual GRKs may influence which downstream signaling pathway is activated. For example,

GRK2 or 3 phosphorylation was required for angiotensin II receptor endocytosis, whereas the kinase activity of GRK5 or 6 directed extracellular signal-regulated kinase (ERK) activation in HEK293 cells.<sup>31</sup> It has been speculated that the GPCR phosphorylation pattern, or "barcode," may dictate the structural conformation assumed by bound  $\beta$ -arrestins, or recruit variable arrestin isoforms, either one of which may influence the functional outcome.<sup>31,32</sup>

Conventional dogma is that GRK activation in cardiac pathologies is mostly attributed to increased SNS stimulation, yet multiple molecular mechanisms of GRK regulation have been proposed. In HEK293 cells, PKA directly phosphorylates GRK2 on serine 685, enhancing  $G\beta\gamma$  subunit binding and promoting membrane translocation.<sup>33</sup> Other in vitro work has found that protein kinase C (PKC) phosphorylation inhibits GRK5,<sup>34</sup> yet activates GRK2 via disinhibition of tonic calmodulin regulation.<sup>35,36</sup> It has been recently reported that GRK2 activity can also be enhanced independently of circulating catecholamines. Equibiaxial mechanical stretch of neonatal rat ventricular myocytes activated GRK2 through angiotensin II type 1 receptor,  $G_{aq}$ , and PKC.<sup>37</sup> Furthermore, cardiac-specific PKC $\alpha$  activation in transgenic mice impaired ex vivo left ventricle (LV) systolic and diastolic function in response to  $\beta$ -AR agonism through enhanced GRK2 activity.<sup>37</sup> The method of producing mechanical stretch is apparently critical to deciphering the pathway(s) of GRK activation, because the use of an inflatable balloon in explanted hearts activated GRK5 and GRK6, but not GRK2.<sup>38</sup> This ligand-independent pathway necessarily entailed GRK-

mediated angiotensin II receptor internalization and  $\beta$ -arrestin directed prosurvival signaling through ERK.<sup>38</sup>

In vivo function of individual GRKs has been clarified by gene knockout studies. Such work defined the role of the rhodopsin kinases in terminating phototransduction in the retina,<sup>39–41</sup> as well as GRK3, GRK5, and GRK6 in regulating olfactory senses,<sup>42</sup> multiple cholinergic responses including airway smooth muscle tone,<sup>43,44</sup> and central nervous system psychostimulant responses,<sup>45</sup> respectively. Despite modest physiological alterations, animals featuring germline deletion of each GRK develop normally into adulthood, with the notable exception of GRK2. GRK2<sup>-/-</sup> mouse embryos develop myocardial hypoplasia and none survive past gestational day 15.5, suggesting that GRK2 may be critical in heart development.<sup>46</sup> Interestingly, GRK effects on mitogenic signaling are not without precedent, as GRK2 overexpression in smooth muscle cells attenuated cell proliferation induced by several GPCR agonists.<sup>47</sup> Systematic analysis of the developmental role of GRK2 was made possible by mating floxed GRK2 mice with mice expressing Cre recombinase under the control of the Nkx2.5 promoter, to specifically delete GRK2 in embryonic cardiomyocytes.<sup>48</sup> The fact that these animals developed normally, with essentially no adult basal cardiac phenotype, implied that embryonic lethality on global GRK2 deletion might entail extracardiac or noncardiomyocyte effects.

Utilizing the  $\alpha$ -myosin heavy chain promoter<sup>49</sup> to drive cardiac-specific expression in adult mice has proven invaluable in characterizing the in vivo function of GRKs in the heart. Indeed, this technique yielded the important discovery that GRKs 2 and 5 attenuate cardiac contractile responses to  $\beta$ -AR stimulation, but only GRK2 affects angiotensin II receptor-mediated contraction.<sup>50,51</sup> On the other hand, GRK3 selectively targets myocardial thrombin and  $\alpha_{1b}$ -adrenergic receptors,<sup>52,53</sup> buttressing the notion that GRKs expressed in the heart are not functionally redundant, but rather serve distinct physiological purposes that coincide with their unique structural and substrate properties.

### GRKs in Cardiac Pathologies

Diseases of the cardiovascular system, particularly HF, myocardial ischemia (MI), and hypertension are unified by the persistent strain placed on the heart muscle in such conditions. Whether faced with increased afterload (eg, HF, hypertension) or cardiac muscle damage (eg, MI), heart performance must adjust to the altered homeostasis in order to meet the body's energetic demands. This entails SNS activation to increase heart rate and contractility through catecholaminergic stimulation of cardiac  $\beta$ -ARs. In human myocardium, the  $\beta_1$  and  $\beta_2$  subtypes are the primary mediators of positive chronotropic and inotropic adrenergic effects through Gs coupling. Furthermore, the  $\beta_1$  subtype accounts for about 3 quarters of myocardial  $\beta$ -ARs in the nonfailing human heart, a distribution pattern that is fairly consistent in atrial and ventricular tissue.<sup>54</sup> Interestingly, chronic stimulation of  $\beta_1$ -ARs appears to be deleterious, whereas  $\beta_2$ -AR agonism may be cardioprotective.<sup>55</sup> Though historically thought to exist only in adipose tissue, functional cardiac  $\beta_3$ -ARs,<sup>56</sup> which signal through Gi proteins and are up-regulated in failing myocardium,<sup>57,58</sup> have also been reported.

Consistent up-regulation of the SNS leads to a number of biochemical and molecular alterations in GPCR pathways that have been extensively evaluated over the past 30 years. Perhaps most familiar is that failing human hearts demonstrate reduced  $\beta$ -AR density and responsiveness, primarily through down-regulation of  $\beta_1$ -ARs.<sup>59–63</sup> Dampened  $\beta$ -AR signaling is generally believed to be an early adaptation to protect the heart against cardiotoxicity from catecholamine overstimulation. Pathological  $\beta$ -AR down-regulation and desensitization, on chronic catecholamine exposure, prevents the desired increases in cardiac output from SNS stimulation. Thus, further SNS activation follows, leading to a self-reinforcing, pernicious cycle of progressively deteriorating heart function. For this reason, extended use of  $\beta$ -AR agonists such as dobutamine in HF is generally contraindicated, because clinical trials have associated their chronic (but not acute) use with increased mortality.<sup>64</sup>

In light of GRKs primary function of modulating GPCR signaling, it stands to reason that alterations in GRKs may be observed in cardiovascular diseases.<sup>55</sup> Of particular note, increased cardiac expression and activity of GRK2 and GRK5, the predominant GRK isoforms in the heart, have been manifestly associated with human and numerous experimental models of HF.<sup>27,62,63,65–74</sup> Enhanced GRK2 expression and activity are also linked to hypertension,<sup>75</sup> cardiac hypertrophy,<sup>76</sup> and MI.<sup>65</sup> Perhaps most intriguingly, GRK2 levels often increase prior to overt clinical HF,<sup>66,71</sup> and normalize in concert with improved  $\beta$ -AR signaling and ventricular function,<sup>77,78</sup> as seen in patients using left ventricular assist devices (LVADs).<sup>79–81</sup> Such findings have been interpreted to cast GRK2 as an alluring therapeutic target and potential biomarker of cardiac function.<sup>77</sup>

The SNS regulates glucose homeostasis in body fat, liver, and skeletal muscle; thus GRK2 has been implicated in insulin receptor regulation and the development of insulin resistance (IRES) associated with metabolic disorders such as diabetes. In vitro studies using adipocytes have shown GRK2-mediated dampening of insulin sensitivity (glucose transporter 4 membrane translocation) through Gαq/11 interference.<sup>82,83</sup> GRK2 also promotes enhanced insulin desensitization on chronic endothelin-1 treatment by phosphorylation-dependent insulin receptor substrate-1 (IRS1) degradation.<sup>83</sup> In HEK293 cells, chronic isoproterenol treatment or  $\beta_2$ -AR overexpression led to insulin resistance through GRK2-mediated IRS1 serine/threonine phosphorylation and inhibition of IRS1 tyrosine phosphorylation.<sup>84</sup> Concordant in vivo work showed that intravenous administration of a peptide containing the carboxyl terminus amino acid sequence of the antennapedia protein and modified from an existing GRK2 inhibitor<sup>85</sup> to promote intracellular localization, ameliorated glucose homeostasis and IRES in a hypertensive rat model.<sup>84</sup> Together, these findings point to a causal relationship between GRK2 activity and IRES, independent of canonical receptor phosphorylation and desensitization.

The nuclear localization of GRK5, as well as divergent receptor specificity and more gradual expression changes in HF than in GRK2, hint at unique cardiac regulatory functions for GRK5. For one, GRK5 seems to be directly relevant to myocardial hypertrophic gene transcription through its role as

a histone deacetylase kinase.<sup>86</sup> Subcellular distribution of GRK5 is apparently critical to this function, because overexpression of a nuclear excluded GRK5 construct does not produce the exaggerated hypertrophy observed with wild-type GRK5 overexpression.<sup>86</sup> Furthermore, intracardiac injection of adenovirus encoding the amino terminus of GRK5 (AdGRK5-NT) reduced cardiac hypertrophy, cardiomyocyte size, and apoptosis and improved cardiac ejection fraction and fractional shortening in SHR.<sup>87</sup> Mechanistically, the amino terminus of GRK5 contains the RGS homology domain that, through its interaction with I $\kappa$ B $\alpha$  and stabilization of I $\kappa$ B $\alpha$ /nF $\kappa$ B complex, reduces nF $\kappa$ B transcriptional activity.<sup>87</sup> In contrast to GRK2, which appears devoid of any nonsynonymous nucleotide polymorphisms, 4 such GRK5 polymorphisms have been discovered, the most common of which is a leucine substitution for glutamine at position 41.<sup>88,89</sup> This mutation is most abundant in African-Americans and mimics the effects of  $\beta$ -blocker therapy, thereby improving survival in individuals with the leucine 41 polymorphism, yet also rendering  $\beta$ -blockers as less efficacious in such populations.<sup>89</sup> These findings provide a clear example of how pharmacogenomics may be harnessed to optimize treatment results. Finally, GRK5 has been found to regulate the signaling of vascular endothelial growth factor receptor, a non-G protein-coupled substrate, in coronary artery endothelial cells, indicating that GRK functions beyond GPCRs also may be important.<sup>90</sup> Overall, our understanding of a central role for GRKs in a variety of cardiac diseases is amply supported by the extant literature.

### GRK2 Inhibition

Since the seminal finding that transgenic cardiac overexpression of GRK2 or a GRK2 inhibitor in mouse hearts reciprocally modulate myocardial  $\beta$ -AR signaling *in vivo*,<sup>50</sup> substantial work has been performed to investigate the effects of GRK inhibition in cardiac maladies. GRK2 inhibition is commonly achieved by utilizing  $\beta$ ARKct, a peptide comprising 194 amino acids of the carboxyl-terminal of GRK2 and containing the G $\beta\gamma$  binding site.<sup>30,50,91</sup> Whether the observed effects of  $\beta$ ARKct are due to GRK2 inhibition followed by resensitization of  $\beta$ -ARs, or by blocking G $\beta\gamma$  signaling,<sup>92</sup> or both, remains unresolved. Regardless, reports of functional cardiac improvement with  $\beta$ ARKct are legion.<sup>93</sup>

The  $\beta$ ARKct peptide has demonstrated efficacy improving cardiac performance in failing myocytes,<sup>94,95</sup> HF models,<sup>67,68,96</sup> and post-MI,<sup>97–99</sup> and in preventing myocardial dysfunction in the settings of cardioplegic arrest<sup>100</sup> and acute coronary ischemia.<sup>101</sup> Moreover,  $\beta$ ARKct normalized fetal gene expression changes in 2 murine models of HF.<sup>102</sup> The precise mechanism of action of  $\beta$ ARKct remains elusive, however. A recent report has proposed that reduced infarct size in  $\beta$ ARKct-expressing mice subjected to acute MI is attributed to activation of the prosurvival Akt and eNOS pathway, through enhanced  $\beta_2$ -AR signaling in cardiac myocytes.<sup>103</sup> Whether NO-mediated S-nitrosylation and inhibition of GRK2<sup>104</sup> figure in this observation, independent of  $\beta$ ARKct, remains to be determined. Another study found that viral delivery of  $\beta$ ARKct enhanced  $I_{Ca}$  in  $\beta$ -AR-stimulated normal and failing cardiomyocytes, presumably through

sequestration of G $\beta\gamma$ , which binds the  $\alpha_{1c}$  subunit of L-type Ca<sup>2+</sup> channels and modulates current.<sup>105,106</sup> More than likely,  $\beta$ ARKct has multiple actions that become more or less apparent depending on the model used and assay performed. Nevertheless, cardiac function was remarkably preserved and remodeling reduced on conditional cardiac GRK2 knockout either before or after MI,<sup>107</sup> confirming the beneficial effects of GRK2 inhibition.

Cardioprotection conferred by GRK inhibition in the setting of myocardial injury may seem paradoxical given the overwhelming evidence that suggests that continuous SNS stimulation of heart muscle is detrimental,<sup>108</sup> and the classical function of GRKs is to mitigate GPCR signaling. In agreement with this premise, Matkovich et al found that cardiac-specific GRK2 deletion actually hastens the progression of cardiomyopathy on chronic isoproterenol infusion in mice.<sup>48</sup> The miniosmotic pumps that deliver isoproterenol in this model are incapable of responding to afferent nerve input, and thus will continue to deliver the drug regardless of cardiac performance. This scenario contrasts with genetic or myocardial injury models, in which SNS activation is presumably lessened on improvement of heart function. Such negative feedback prevents incessant  $\beta$ -AR stimulation, and the deleterious effects on cardiac muscle, perhaps explaining the apparently discrepant results observed on GRK2 inhibition or deletion.

Because it is a large peptide,  $\beta$ ARKct application requires a vehicle (typically a virus) for delivery to the target organ, which is not optimal. An alternative approach is to find small molecules that can be administered systemically, thus precluding immune response and cytotoxicity often associated with viral use. By screening a small molecule library, promising compounds that bind to the G $\beta\gamma$  site modulating protein–protein interactions were identified.<sup>109</sup> Our group recently assessed 2 of these compounds, M119 and gallein, for efficacy in treating 2 murine HF models.<sup>110</sup> Small-molecule G $\beta\gamma$  inhibition mitigated cardiac dysfunction and enhanced  $\beta$ -AR signaling, at least in part because of reduced GRK2 expression and membrane recruitment, in either new-onset or preexisting heart failure<sup>110</sup> (recently reviewed by Kamal et al<sup>111</sup>). Whether these compounds exert significant extracardiac effects, or regulate other G $\beta\gamma$ -signaling pathways, such as hypertrophic ERK1/2 activation,<sup>112</sup> remains to be clarified.

### Vascular GRKs

Numerous GPCR agonists—including angiotensin, endothelin, norepinephrine, and epinephrine—provide the neurohormonal inputs that modulate blood pressure. More specifically, vasoconstriction through angiotensin II, endothelin, and  $\alpha$ -adrenergic receptor activation is counteracted by  $\beta$ -AR-mediated vasodilation, fine-tuning vascular tone. As approximately 1 out of 3 of adults in the US have hypertension,<sup>113</sup> increasing heart disease risk, and >70% of HF patients have antecedent hypertension,<sup>2</sup> GPCR dysregulation in the vasculature can have a profound impact on cardiovascular health.

Impairment of  $\beta_2$ -AR-mediated vasodilator response in vascular smooth muscle cells (VSMCs) has been described in hypertensive patients<sup>114</sup> and in animal models of hyperten-

sion.<sup>115,116</sup> Assessment of  $\beta$ -AR functionality in humans is made possible by the use of human lymphocytes, in which  $\beta$ -AR properties mirror changes in  $\beta$ -ARs from less accessible tissues.<sup>117,118</sup> Using lymphocytes from hypertensive and normotensive subjects, Gros et al established a link between defective  $\beta$ -AR responsiveness in hypertension and altered GRK2 activity.<sup>75</sup> Hypertensive patients displayed elevated GRK2 activity and protein expression without concomitant changes in GRK5, GRK6, PKA,  $\beta$ -arrestin 1, and  $\beta$ -arrestin 2, suggesting selective variation of GRK2 in this condition.<sup>75</sup>

Support for the hypothesis that increased GRK activity may underlie reduced  $\beta$ -AR responsiveness characteristic of the hypertensive state was supplied by the generation of transgenic mice with VSMC-targeted GRK2 overexpression driven by a portion of the SM22 $\alpha$  promoter.<sup>119</sup> These animals display elevated resting blood pressure, reduced isoproterenol-mediated drop in diastolic blood pressure, vascular wall thickening, and myocardial hypertrophy.<sup>119</sup> It should be noted that VSMC GRK2 overexpression curiously attenuated blood pressure rise on vasoconstricting angiotensin II challenge. Enhanced GRK2-mediated phosphorylation and desensitization of angiotensin II receptor signaling, as has been observed in vivo in the heart,<sup>51,53,120</sup> was the authors' proposition, yet the apparent primacy of  $\beta$ -AR signaling defects in regulating blood pressure highlights the need for continued investigation into GRK substrate specificity.

In vivo analysis of GRK substrate selectivity using hybrid transgenic mice with myocardium-targeted overexpression of GRK2, 3, or 5, and constitutively activated mutant or wild-type  $\alpha_{1b}$ -ARs, revealed that GRK2 has no effect on cardiac  $\alpha_{1b}$  adrenergic signaling, as assessed by diacylglycerol production, myocardial hypertrophy, and atrial natriuretic factor (ANF) expression.<sup>53</sup> Alone among these isoforms, GRK3 reduced myocardial diacylglycerol and either GRK3 or GRK5 reduced hypertrophy and ANF expression.<sup>53</sup> These results indicate differential substrate targeting by various GRK isoforms, proof of which had been limited in various in vitro cell culture system studies.<sup>121–124</sup> More recently, GRK3 was found to be highly selective for endothelin receptors and  $\alpha_1$ -ARs of adult rat cardiac myocytes, more so than GRK2, which displayed greater potency and efficacy at  $\beta$ -ARs.<sup>125</sup> Cardiac-specific expression of a GRK3 inhibitor in mice raised blood pressure and cardiac output through overactive  $\alpha_1$ -AR signaling, substantiating the concept of preferential regulation of this receptor subtype by GRK3.<sup>126</sup>

In a murine renal artery stenosis model of hypertension, in which plasma norepinephrine and VSMC GRK2 expression is increased, inhibition of VSMC GRK2 via genetic ablation or peptide ( $\beta$ ARKct) failed to reduce hypertension, even though  $\beta$ -AR mediated vasodilation was functionally improved.<sup>127</sup> The authors attributed this finding to increased  $\alpha_{1D}$ -AR vasoconstricting activity upon GRK2 antagonism, although concerns about the pharmacological specificity of agonists used preclude discounting a role for  $\alpha_{1a}$ -AR signaling.<sup>127</sup> Blocking  $\alpha_{1b}$ -AR signaling, moreover, did not affect enhanced  $\alpha_1$ -AR constriction of GRK2-inhibited vessels, suggesting that this receptor subtype is not involved.<sup>127</sup> In rat mesenteric arterial smooth muscle cells (resistance arteries), inhibition of GRK2 (but not GRK3, 5, and 6) with siRNA or

dominant negative mutants reduced desensitization of endothelin-induced Ca<sup>2+</sup> and IP<sub>3</sub> signaling.<sup>128</sup> GRK2 has also been implicated in disrupting nonadrenergic (endothelial cell nitric oxide synthase [eNOS]) vasodilation via Akt inhibition in portal hypertensive rats.<sup>129</sup> GRK2 knockout in this animal model restored NO production and normalized portal pressure.<sup>129</sup> Together, these findings implicate GRK2 in vascular adaptations of the hypertensive state, yet the antagonistic effects on constriction and relaxation merit further investigation to effectuate therapeutic benefit.

A large cohort study of 133 Black Americans reported GRK2 mRNA expression and activity, but not that of GRK5, correlated with blood pressure and plasma norepinephrine levels.<sup>130</sup> Furthermore, GRK2 protein expression doubled and GRK2 activity rose more than 40% in hypertensive subjects.<sup>130</sup> In contrast, 1 group has reported that lymphocyte mRNA levels for both GRK2 and GRK5 increase on isoproterenol injection in a rat HF model.<sup>131</sup> The discrepancy in results can likely be attributed to the obvious physiological differences between humans and rodents, as well as the respective conditions studied.

Another clinical study identified a negative correlation between GRK3 mRNA and systolic and diastolic blood pressure, although corresponding GRK3 protein levels were not assessed.<sup>132</sup> Oliver et al have shed new mechanistic light on hypertension etiology with a systematic analysis of  $\alpha$ - and  $\beta$ -AR subtype expression, as well as ex vivo contraction of aortic rings from spontaneously hypertensive rats (SHR).<sup>133</sup> To wit,  $\alpha_{1D}$ - and  $\beta_3$ -ARs are the subtypes most resistant to GRK2-mediated desensitization, thereby enhancing their functional importance in the setting of hypertension.<sup>133</sup> Coupled with the observation that  $\alpha_{1D}$ -ARs are most sensitive and  $\beta_3$ -ARs least sensitive to agonists, greater vasoconstrictor tone prevails in the hypertensive state.

Whether increased GRK2 contributes to a rise in peripheral resistance through desensitization of  $\beta$ -AR-mediated vasodilation remains to be definitively resolved in the clinical setting. An alternative hypothesis is that GRK2 up-regulation merely reflects overactive SNS activity on the vasculature, because the GRK2 promoter activity is stimulated by Gq and  $\alpha_1$ -AR signaling.<sup>134</sup> It must also be emphasized that cardiovascular disorders involve the complex interplay of many tissues and systems, with multiple potential etiologies. Although initially thought to be confined to the testes, mRNA for each of the 4 GRK4 isoforms<sup>135,136</sup> have been identified in the renal proximal tubule.<sup>137</sup> Transgenic expression of a naturally occurring single nucleotide polymorphism of GRK4 $\gamma$  in mice enhances GRK4-mediated phosphorylation of D<sub>1</sub> dopamine receptors in the kidney, thus dampening urinary sodium excretion and producing hypertension.<sup>137</sup> Such findings highlight the still incompletely understood pathophysiological role of GRKs in cardiovascular diseases.

### Adrenal GRK2

Modulating GRKs in myocardial tissue has produced many exciting findings with the potential to improve human health. However, the compensatory SNS response to diminished cardiac output involves the coordination of GPCR activity in various cell types and tissues beyond the heart. Systemic

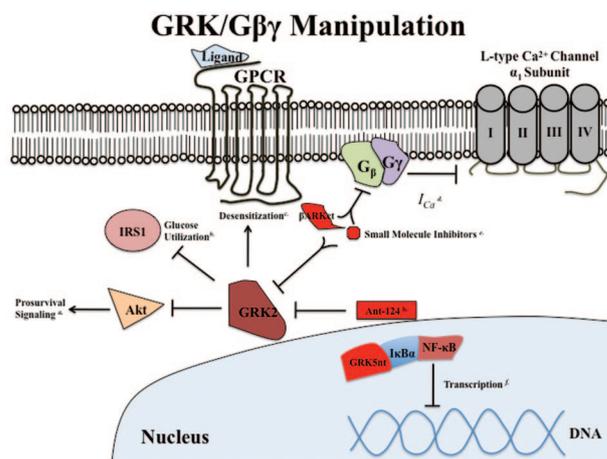
release of catecholamines, primarily epinephrine from the adrenal gland and norepinephrine from presynaptic nerve terminals, provides the initial stimulus to enhance cardiac contractility and maintain adequate perfusion of blood to the body's tissues.<sup>138</sup> As was noted previously, however, continued sympathetic activation damages the heart, partially explaining the beneficial effects of myocardial  $\beta$ -AR antagonism in HF. Indeed, a classic prognostic indicator of heart failure is increased plasma norepinephrine, which is highly correlated with mortality.<sup>139,140</sup>

Another approach to alleviating excessive SNS burden is to inhibit adrenal catecholamine release. Under normal circumstances, catecholamine release by chromaffin cells of the adrenal medulla is under feedback inhibition by  $\alpha_2$ -ARs expressed on the membranes of these cells. In 2 different animal models of HF, calsequestrin-overexpressing mice and rats subjected to MI, Lympopoulos et al showed significant down-regulation and desensitization of adrenal  $\alpha_2$ -ARs, correlated with increased adrenal GRK2 expression and catecholamine secretion.<sup>141</sup> Furthermore, GRK2- $G_{\beta\gamma}$  inhibition via adenoviral-mediated delivery of  $\beta$ ARKct to adrenal glands of HF rats restored  $\alpha_2$ -AR signaling, resulting in lowered plasma catecholamine levels, and improved  $\beta$ AR-mediated cardiac contractility and relaxation after 7 days.<sup>141</sup>

In a separate study, adrenal-specific transgene expression of GRK2 in rats produced enhanced plasma catecholamine levels in comparison with control animals, whereas  $\beta$ ARKct effected the opposite result.<sup>142</sup> In vitro results revealed that  $\alpha_2$ -ARs from GRK2-infected chromaffin cells failed to inhibit catecholamine secretion, in contrast to  $\beta$ ARKct-infected cells, providing proof of principle that catecholamine secretion from the adrenal gland can be manipulated through adrenal GRK2 regulation of  $\alpha_2$ -AR signaling.<sup>142</sup>

These findings were extended by utilizing adrenal-specific genetic knockdown of GRK2 in mice. Fifty-percent reduction of adrenal GRK2 protein precipitated a significant, though modest, reduction in circulating catecholamines at 4 weeks post-MI in these animals.<sup>143</sup> Moreover, GRK2 knockdown was associated with increased adrenal membrane  $\alpha_2$ -AR density, reduced adrenal gland size, and diminished catecholamine biosynthetic capacity.<sup>143</sup> Interestingly, cardiac GRK2 protein also went down, improving ejection fraction and isoproterenol-induced contractility in the failing hearts.<sup>143</sup> Though it is not clear why adrenal GRK2 expression increases in HF, nevertheless adrenal GRK2 inhibition highlights the potential therapeutic benefit of a comprehensive approach to regulating catecholamines. Furthermore, concomitant inhibition of cardiac and adrenal  $G_{\beta\gamma}$ -GRK2 with systemic inhibitors, such as those described recently,<sup>110,111</sup> may provide dual clinical efficacy in heart failure.

Adrenal GRK2 levels and activity may, in part, provide the molecular mechanism underlying the observed benefits of moderate exercise training in ameliorating cardiotoxic SNS hyperactivity in chronic HF. Indeed, rats that began a treadmill exercise regimen for 10 weeks at 4 weeks post-MI demonstrated significantly reduced circulating catecholamines and gene markers of cardiac remodeling (ANF, collagen type 1, and transforming growth factor- $\beta$ 1 mRNA levels in heart) in comparison with sedentary animals.<sup>144</sup>



**Figure 3. GRK/ $G_{\beta\gamma}$  manipulation.** Pictorial representation of putative regulation of GRK/ $G_{\beta\gamma}$  functions from recent literature. See text for details. GRK, G protein-coupled receptor kinase. a. Reference 103. b. Reference 84. c. References 99, 107, 125, 127, 141, 142, 143. d. Reference 106. e. Reference 110. f. Reference 87.

Importantly, adrenal and cardiac GRK2 protein expression, as well as adrenal  $\alpha_2$ AR membrane expression, were also normalized in the exercise group.<sup>144</sup> Despite improved LV contractile response to  $\beta$ -AR stimulation, consistent with increased cardiac  $\beta$ -AR density, the post-MI exercise-trained group showed no functional improvement in ejection fraction, suggesting that the primary advantage of physical activity may be inhibition of adverse cardiac remodeling.

## Conclusions

GPCR signaling is a ubiquitous means of effecting physiological processes throughout the body; hence GPCRs are the most common target of pharmacotherapy today. Investigation of GRK function is, therefore, a logical extension of efforts to uncover improved treatments for heart diseases afflicting Western societies. Modulation of GRK activity has yielded promising results in alleviating cardiovascular dysfunction in a wide variety of animal models and cell culture systems, the most recent of which are depicted in Figure 3. The concept of “functional selectivity,” that divergent downstream signaling pathways can be activated by a single ligand–receptor interaction,<sup>147</sup> highlights the emerging notion that long-held principles regarding GPCR signaling will no longer be sufficient to generate the next generation of therapeutic drugs (see other articles in this special review series for *Circulation Research*). Nevertheless, the overwhelming data implicating GRKs in cardiovascular diseases suggest that GRK regulation will continue to be an important target of investigation in multiple aspects of not only cardiovascular disease, but also of its comorbidities (eg, diabetes), new diagnostics (eg, elevated GRK2), and novel therapeutics (eg, small molecules, stem cells).

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## Disclosures

None.

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