G Protein–Coupled Receptor Kinase 2
A Link Between Myocardial Contractile Function and Cardiac Metabolism

Meryl C. Woodall, Michele Ciccarelli, Benjamin P. Woodall, Walter J. Koch

Abstract: Heart failure (HF) causes a tremendous burden on the worldwide healthcare system, affecting >23 million people. There are many cardiovascular disorders that contribute to the development of HF and multiple risk factors that accelerate its occurrence, but regardless of its underlying cause, HF is characterized by a marked decrease in myocardial contractility and loss of pump function. One biomarker molecule consistently shown to be upregulated in human HF and several animal models is G protein–coupled receptor kinase-2 (GRK2), a kinase originally discovered to be involved in G protein–coupled receptor desensitization, especially β-adrenergic receptors. Higher levels of GRK2 can impair β-adrenergic receptor–mediated inotropic reserve and its inhibition, or molecular reduction has shown to improve pump function in several animal models including a preclinical pig model of HF. Recently, nonclassical roles for GRK2 in cardiovascular disease have been described, including negative regulation of insulin signaling, a role in myocyte cell survival and apoptotic signaling, and it has been shown to be localized in/on mitochondria. These new roles of GRK2 suggest that GRK2 may be a nodal link in the myocyte, influencing both cardiac contractile function and cell metabolism and survival and contributing to HF independent of its canonical role in G protein–coupled receptor desensitization. In this review, classical and nonclassical roles for GRK2 will be discussed, focusing on recently discovered roles for GRK2 in cardiomycocyte metabolism and the effects that these roles may have on myocardial contractile function and HF development. (Circ Res. 2014;114:1661-1670.)

Key Words: G-protein-coupled receptor kinase 2 ■ heart failure ■ metabolism ■ myocytes, cardiac

Heart failure (HF) is the leading cause of death in developed countries and is caused by many different disease conditions, including coronary artery disease, high blood pressure, and diabetes mellitus. At its root, HF is the inability of the heart to pump blood adequately and meet the oxygen demands of the body. One of the first molecular events associated with HF is an increase in the G protein–coupled receptor (GPCR) kinase-2 (GRK2), a ubiquitously expressed kinase first discovered to regulate β-adrenergic receptors (βARs) and a prototypic member of a kinase family that phosphorylates and desensitizes agonist-occupied GPCRs.1 In the heart, this kinase is particularly important for triggering deactivation and downregulation of βARs, impairing the myocyte’s ability to contract. The upregulation of GRK2 occurs initially after cardiac injury or stress and is necessary to shutdown overactivated βARs that occur as a result of compensated increases in catecholamines to drive the impaired heart from the activation of the sympathetic nervous system (SNS). This is the start of a vicious cycle of adrenergic signaling impairment where excess catecholamines (norepinephrine and epinephrine) are produced to compensate for decreased βAR signaling that ultimately keeps contractility diminished. Two decades of research have overwhelmingly uncovered that inhibition of GRK2 is beneficial for restoration of inotropic reserve, and surprisingly, lowering GRK2 levels and activity in the hearts of several animal models has led to the prevention or reversal of HF.2 This is due, in part, to the normalization of βAR levels that leads to a neurohormonal feedback that acts as a sympathetic to undo the vicious adrenergic pathological cycle.3 Furthermore, targeting GRK2 seems complementary to βAR blockade because both can resensitize the receptor system over time, and GRK2 inhibition also has extra-βAR effects that seem to contribute to its therapeutic benefit in HF.3 Recently, there has been increased interest in novel, noncanonical roles of GRK2 where this kinase is a central molecule in a complex interactome that influences a wide variety of signaling networks and cellular functions.4 For example, GRK2 has been found to play a role in insulin signaling and also to be important for apoptosis induction in the injured heart. Contradicting the idea that GRK2 is/was primarily a cytosolic...
molecule, it was recently discovered that GRK2 also locates to the mitochondria and this localization is significantly increased after myocardial ischemic and oxidative stress. Below, we discuss in detail the influences that GRK2 has on glucose metabolism, ischemic injury, and mitochondrial health and function and how these diverse actions can affect myocardial contractility and HF development.

GRK2 Function, Regulation, and Role in the Heart and HF

GPCRs are a large superfamily of cell surface receptor proteins that are important for modulating a wide variety of physiological functions; together they constitute the most pharmacologically targeted protein family. On ligand binding, the receptors undergo conformational changes that ultimately result in the release of heterotrimeric G proteins that act as downstream signaling effectors of multiple pathways. GRKs are a small group of serine/threonine kinases that recognize only agonist-activated GPCRs, phosphorylating them and triggering the process of desensitization, which is marked by the loss of G protein coupling.5 In general, these kinases work in concert with β-arrestins to desensitize, internalize, and ultimately downregulate GPCRs (Figure 1). In addition, β-arrestins can also act as platforms to induce signaling outcomes in cells that can be independent of G protein activation (Figure 1).6

There are 7 mammalian GRKs that have been characterized to date, and they are divided into 3 subfamilies based on function, receptor specificity, and tissue distribution: GRK1 and GRK7, GRK2 and 3 (also known as βARK kinase [βARK] 1 and 2), and GRKs 4, 5, and 6. GRK1 and GRK7 are restricted to the retinal rods and cones; however, several of the other GRKs are ubiquitously expressed, including GRK2, which (along with GRK5) is the most highly expressed GRK in the heart. Despite their differences in tissue specificity, all GRKs share a similar overall structure, consisting of a central highly conserved catalytic domain similar to other AGC kinases flanked by variable N and C termini.7 These terminal domains in GRK2 contain binding sites for multiple proteins, including phosphoinositide 3-kinase (PI3K)/Akt, clathrin, and α-actinin, which are not present in GRK5. The C terminus of GRK2 also contains a pleckstrin homology domain that facilitates

Figure 1. The classical actions of G protein–coupled receptor kinase 2 (GRK2) on the cardiac β-adrenergic receptors ([βARs]) during physiological conditions and disease states. 

A, Under normal conditions, catecholamines (CA) activate the βAR, stimulating downstream signaling through Gαs that ultimately leads to increased contractility by activating adenylate cyclase (AC) and protein kinase A (PKA). B, As receptor activation occurs, the desensitization of the signal is triggered simultaneously when the normally cytosolic GRK2 translocates and anchors to the membrane by binding to Gβγ via its C terminus where it is able to interact with the agonist-occupied receptor and phosphorylate it, which begins the G protein uncoupling process. G protein activation is blocked by recruitment of β-arrestin molecules to the phosphorylated receptor. β-arrestins also start the receptor internalization and downregulation process, as well as initiate novel signaling events. C, After cardiac injury, GRK2 levels are increased because of enhanced βAR activation after stress-induced increases in catecholamines. The increase in GRK2 helps to prevent overstimulation of the βAR initially, but over time a vicious cycle of chronically increased βAR desensitization occurs, as well as increased pathological effects of GRK2, contributing to the progression of heart failure.
binding to the dissociated G\(\beta\gamma\) subunit of an activated GPCR. This is how the primarily cytosolic GRK2 (and the homologous GRK3) localizes to the sarcolemmal membrane where it can interact with and phosphorylate an activated GPCR. The C terminus of GRK2 anchors to membrane phospholipids, whereas the N terminus interacts with receptors, putting targeted Ser/Thr residues of the cytoplasmic tail of the GPCR in contact with the central GRK catalytic domain. This regulation of GRK2 ensures the fidelity of the activation/deactivation process because G\(\beta\gamma\) is not free to bind to and facilitate the membrane translocation of GRK2 unless a receptor system is activated (Figure 1).4 Peptides from this C-terminal domain of GRK2 can keep the kinase from localizing to the membrane and blocking GPCR desensitization: the entire C tail of GRK2 (known as the carboxyl terminus of \(\beta\)ARK (\(\beta\)ARKct)) has been used as an effective in vitro and in vivo GRK2 inhibitor.9

During HF, GRK2 is increased 3- to 4-fold in the myocardium. GRK2 upregulation seems to be one of the first molecular alterations in the myocyte after cardiac injury/stress, and this has been shown to precede other \(\beta\)AR and functional abnormalities. In fact, human studies have shown that GRK2 upregulation in failing myocardium may have novel diagnostic and prognostic value because its levels in the heart are mirrored by levels in white blood cells and so it can be measured peripherally.10–13 Levels in failing myocardium correlate with cardiac dysfunction, and improved cardiac function in HF is associated with lower GRK2 levels.14–16 These aspects of human heart disease are also mirrored in animal models of HF, where GRK2 levels are increased early in pathological cardiac hypertrophy and myocardial ischemia, which seems instrumental in HF development.17 Although initially adaptive to compensate for increased catecholamine stimulation, over time, the excess GRK2 causes dysregulation of the \(\beta\)AR system, leading to a loss of inotropic reserve and contributing to HF.

The overall importance of GRK2 to the heart has been demonstrated through studies with genetically engineered mice. The global loss of GRK2 by homologous recombination led to embryonic lethality with cardiac malformations and dysplasia18 that does not seem to be cardiomyocyte autonomous.19 Heterozygous GRK2 knockout mice with 50% less GRK2 in all tissues have increased cardiac function20 and have been shown to have a favorable phenotype in other aspects of cardiac and metabolic regulation,4 which will be discussed in more detail below. Furthermore, cardiac-specific GRK2 knockout mice where GRK2 is ablated after birth leads to improved cardiac function and prevention of HF development after a myocardial infarction.21 Moreover, when knock out of GRK2 is induced in the cardiomyocyte after HF development, there was active reverse remodeling and improved cardiac function.21 Both of these lines of GRK2 knockout mice also lead to significant cardioprotection acutely after myocardial infarction.22 In a manner fairly identical to loss of myocyte GRK2, transgenic expression of the \(\beta\)ARKct as a GRK2 inhibitor led to rescue of several mouse models of HF23,24 and also was cardioprotective.25 Of interest, when GRK2 is ablated in myocytes early in development, there were some remaining abnormalities into adulthood: mice exhibited supersensitivity to the negative effects of chronic catecholamine toxicity.19

This developmental importance of GRK2 may extend to the endothelium: deleting GRK2 in endothelial cells using a Tie-2-Cre mouse resulted in some abnormalities and changes in vasculogenesis because GRK2 is deleted prenatally in this murine model.26–28 Interestingly, the increased inflammatory and oxidative stress seen in the endothelium of Tie-2-GRK2 knockout mice is in contrast to vascular results found in heterozygous global GRK2 knockout mice with similar decreases in GRK2 expression in endothelium because these mice have improved endothelium function and also prevent the oxidative stress and dysfunction caused by angiotensin II treatment.28 Furthermore, studies using GRK2 inhibitors, albeit not as specific, also demonstrate prevention of endothelial dysfunction in diabetic and obese mice.29,30 More studies are needed to determine why endothelium-specific loss of GRK2 is detrimental, whereas simultaneous decrease of GRK2 in the endothelium and other tissues produces an opposite phenotype.

### Relationship Between Cardiac Function, HF, and Metabolism

The idea that changes in the energetics of the heart play an important role in the pathogenesis of HF is not novel. The heart expends more energy than any other organ, and to provide enough fuel to perform its functions, it must use energy derived from glucose and fatty acids to generate the force required for muscle contraction. As a result, metabolism and heart function are not mutually exclusive but intimately linked. There are 3 different components to energy metabolism: substrate use, oxidative phosphorylation, and ATP transfer and use. It is well established that after cardiac trauma there is a change in the relative amounts of substrate use to cover cardiac energy demands. In the healthy heart, fatty acid oxidation accounts for 65%, whereas glucose accounts for 30% of energy use. Nevertheless, the use of metabolic substrates can modify according to different physiological and pathological conditions and also to \(O_2\) availability. Healthy exercise, for example, increases glucose use according to its intensity and if it is prevalently aerobic or anaerobic. Under pathological conditions such as acute ischemia, the reduced \(O_2\) availability increases glucose use to reduce oxygen wastage while accumulation of free fatty acids is toxic and induces damage to the membrane and death of the cell.31–33 This metabolic flexibility is instead lost during a chronic condition such as HF as observed in positron emission tomographic studies where fatty acid use during cardiomyopathy is dramatically increased, whereas glucose extraction is decreased in the injured heart.34 Insulin signaling is the major regulator of cardiac glucose extraction and use. Cardiac insulin signaling is also important for the healthy and injured heart, independent of its ability to promote glucose extraction and use. Activation of the insulin receptor/insulin receptor substrate-1 (IRS1)/PI3K/AKT/mammalian target of rapamycin pathway is fundamental for the cardiac adaptive response to stress, where the short-term activation of this pathway promotes physiological hypertrophy and protection from myocardial injury;35 its long-term activation, however, causes pathological hypertrophy and HF.36,37 The correct balance of this signaling is, therefore, pivotal for the correct geometry and remodeling of the injured heart. Because
glucose metabolism is so crucial in the failing heart, the role GRK2 can play in myocyte insulin signaling, discussed below, becomes significant.

Recently, novel insights in the connection between myocardium and global metabolism have been revealed. Interestingly, Gruter et al demonstrated the importance of the heart in regulating systemic energy consumption. Mice with cardiac-specific overexpression of mediator complex subunit 13, a protein that controls transcription by nuclear hormone receptors, were resistant to high-fat diet–induced obesity and demonstrated improved insulin sensitivity and glucose tolerance. These mice also exhibited higher levels of oxygen consumption, CO2 production, and suppression of thyroid hormone receptor transcriptional activation, suggesting a hypermetabolic state because of overexpression of mediator complex subunit 13 exclusively in the heart. The regulation of mediator complex subunit 13 was found to be controlled by a cardiac-specific microRNA. Therefore, there is now a precedent for cardiomyocytes having some regulatory control over global metabolism. GRK2 activity may be an important link in this process as discussed below.

**GRK2 and Insulin Resistance: Implications for Linking Cardiac Metabolism and Function**

Although a relationship between insulin resistance (IR) and β-adrenergic overstimulation has been observed for many years, the relationship between the two was not completely understood until the involvement and importance of GRK2 was elucidated. In addition to the classical role of GRK2 in cardiac βAR signaling regulation through the manipulation of downstream signaling that can have an indirect effect on insulin signaling, GRK2 can also directly regulate insulin signaling via different levels downstream of βAR signaling. In addition, GRK2 can influence insulin signaling independent of adrenergic signaling; GRK2 can regulate insulin receptor signaling in response to insulin itself as well. Novel aspects of GRK2 in its regulation of insulin signaling and myocyte glucose metabolism are highlighted in the next sections.

**Neurohormonal Activation and IR**

Cross talk between insulin and the SNS was noted several years ago. Insulin can affect a wide variety of cellular events; these include glucose transport, intermediary metabolism, DNA, RNA and protein synthesis rates, gene transcription, cell growth, and cellular differentiation. In the heart, neurohormonal overactivation of both the SNS and renin–angiotensin–aldosterone systems, which is a common feature of chronic HF, is accompanied by IR. Increased catecholamines confer myocardial damage and significant oxygen wasting by increasing the levels of ATP consumption. Norepinephrine also increases levels of plasma free fatty acids and promotes coronary vasoconstriction, which further increases oxygen consumption. Thus, central and peripheral activation of the SNS and also the renin–angiotensin–aldosterone system, both directly and through amplified plasma free fatty acids, correlates with systemic and cardiac IR. Consequently, IR in HF is associated with markers of SNS activation, both in patients and in large animal models of HF. In this scenario, therapies aimed to counteract neurohormonal activation would reduce IR and improve cardiac metabolism. Importantly, current frontline therapy for HF targeting overactive SNS and renin–angiotensin–aldosterone activities do not clearly reduce IR in the failing heart. A relative contraindication for βAR blockers in patients with diabetes mellitus is still present. Importantly, renin–angiotensin–aldosterone antagonism with angiotensin-converting enzyme inhibitors, AT1 (angiotensin type I receptor) blockers, and aldosterone antagonists can reduce peripheral IR caused by angiotensin II and aldosterone, particularly in the case of type II diabetes mellitus and obesity.

It is possible that neurohormonal activation causes several different cellular modifications, with particular consideration to the expression or activity of molecules involved in key pathways that regulate cell physiology and cardiac metabolism. Therefore, relative nonspecific receptor antagonism may not be a sufficient means to normalize some specific cellular modifications induced by persistent hormonal stimulation and could be alternatively treated using therapies that focus on one particular molecule. As detailed below, one molecule that is emerging as such a target is GRK2. In fact, a recent study has shown that GRK2 in brown fat suppresses thermogenic gene expression and its loss increases energy expenditure, which may be responsible for the prevention of obesity and metabolic syndrome in the heterozygous GRK2 knockout mice. There are other molecules implicated in IR during sympathetic stimulation that are beyond the scope of this review: Akt, a downstream effector of insulin signaling whose phosphorylation/activation is decreased in severe dilated cardiomyopathy, and also PTEN (phosphatase and tensin homolog), a phosphatase that is increased in cardiomyopathy and is known to prevent Akt phosphorylation. However, the fact that GRK2 is such a powerful regulator of myocardial function through GPCR-dependent mechanisms and its elucidation as having a key influence on cardiac metabolism seems to link contractility and metabolism via this kinase.

**GRK2 and Cardiac Insulin Signaling**

Our group and others have explored the role of GRK2 in cardiovascular disease and have described its role in the pathophysiology of HF. As previously mentioned, GRK2 upregulation brought about by catecholamine stimulation is a hallmark of failing myocardium; this phenomenon has also been observed during conditions with chronic insulin stimulation. The connection between GRK2 and insulin signaling has been convincingly demonstrated by experiments showing that treatment with insulin can swiftly increase the cellular content of GRK2, within a time frame of 15 to 30 minutes, suggesting that increased transcription or translation is not the causal mechanism. Interestingly, studies have shown that insulin-like growth factor-1 (IGF-1) can induce cellular accumulation of GRK2 by inhibition of murine double minute 2 (mdm2), an ubiquitin ligase responsible for GRK2 ubiquitination and degradation. IGF-1 stimulation also recruits GRK2 to the membrane where it seems that GRK2 can desensitize this receptor tyrosine kinase. This is certainly plausible because earlier studies have shown that the IGF-1 receptor can activate G proteins and especially Gβγ-dependent signaling that can bind the C terminus of GRK2. These data with IGF-1 and mdm2 suggest that this could also be the mechanism for how GRK2 levels increase after chronic insulin treatment. This has significant pathophysiological
significance because, as described below, GRK2 is a negative regulator of insulin signaling and alone can induce IR.

Several lines of evidence lead to the conclusion that GRK2 is a crucial modulator of IR, both systemically and in the heart.54,55 Early data implicated GRK2 increases after chronic catecholamine exposure as being responsible for the well-known βAR-mediated IR.56 Recently, GRK2 has been directly implicated in pathologies associated with metabolic disorders with characteristics of elevated insulin levels and IR including aging and obesity.56 Of note, the expression of GRK2 is increased in important tissues, including myocytes, in different experimental models of IR.56 Interestingly, lowering GRK2 systemically by 50% protects mice against tumor necrosis factor-α, aging, or high-fat diet–stimulated negative modifications in glucose homeostasis and insulin signaling.56 These novel findings were shown by using the heterozygous global GRK2 knockout mice and indicate a critical role for GRK2 in the alterations of insulin sensitivity in physiological and disease conditions.56 Exercise has also been shown to lower GRK2 levels, which improves insulin sensitivity in spontaneously hypertensive rats; this was also accomplished by targeted GRK2 silencing in endothelial cells.57 Moreover, using small peptides to inhibit GRK2 restored glucose tolerance in animal models of IR.50,58 Therefore, GRK2 promotes IR and reduces glucose metabolism and metabolic syndrome, which are conditions associated with lower cardiac contractile function and add to the potential mechanisms for the apparent therapeutic benefit in the heart with GRK2 inhibition. It seems that targeting GRK2 will ameliorate both detrimental pathways; although because both are closely associated and linked, the improvement of one could influence the other.

Data from our laboratory have recently emerged showing how GRK2 can directly promote IR and lowered glucose metabolism in the cardiomyocyte, directly implicating the kinase activity of GRK2 in this process. Data had existed in other cell types that GRK2’s influence as a negative regulator of insulin signaling was because of nonkinase actions of GRK2 through protein–protein interactions. First, in liver cells and adipocytes, GRK2, through a regulator of G protein signaling domain present in its C tail, was shown to block Gαq/11 signaling downstream of insulin stimulation and its receptor tyrosine kinase activation.59-61 GRK2 has also been shown to bind to and inhibit Akt, which would have a negative effect on glucose transporter 4 translocation to the membrane to increase glucose uptake into cells.62 However, neither one of these occurs in myocytes because insulin treatment induces the loss of downstream insulin signaling (Akt activation and glucose transporter 4 membrane translocation) as a result of the direct interaction with IRS1.41 In fact, when GRK2 is elevated to levels seen in human HF, there is a significant defect in myocardial glucose uptake and impaired insulin signaling in myocytes.41 GRK2 directly phosphorylates IRS1 at the inhibitory Ser307 residue, promoting dissociation of the insulin receptor signaling complex and attenuating signaling to downstream effectors such as Akt and glucose transporter 4 (Figure 2).41

Importantly, elevated myocardial GRK2 levels enhanced negative cardiac glucose metabolism after ischemic injury, and these effects precede GRK2-mediated ventricular contractile dysfunction.41 This was the first report to link adrenergic control of contractility and metabolism; GRK2 seems to be this nodal link and the metabolic dysfunction that occurs first after cardiac injury may contribute to pump failure. Importantly, to strengthen this nodal link of GRK2 further, cardiac-specific GRK2 knockout mice have more myocardial glucose uptake that is maintained even after ischemia and insulin signaling remains intact with significantly decreased IRS1 phosphorylation.41 This improved glucose metabolism in the face of decreased GRK2 remains normalized out to 28 weeks after myocardial infarction in mice. It is accompanied by HF prevention because cardiac contractility is not adversely affected as it is in wild-type mice or GRK2-overexpressing mice post–myocardial infarction.41

Interestingly, the interaction between GRK2 and IRS1 is dependent on an intact C terminus of GRK2 because introduction of the βARKct peptide inhibits insulin-mediated GRK2-dependent IRS1 phosphorylation, whereas βARKct expression improves Akt activation and glucose transporter 4 membrane translocation in response to insulin (Figure 2).41 Moreover, βARKct gene delivery to the hearts of rats using adeno-associated virus serotype 6 before ischemic injury prevented IR, and myocardial glucose uptake remained high.41 These results could mean the direct interaction between GRK2 and IRS1 takes place within the C tail of GRK2 or that activation of the insulin receptor stimulates a pool of G proteins, such as the IGF-1 receptor, and the Gβγ released recruits GRK2 to the membrane where it can interact with IRS1 (Figure 2). Overall, given the higher efficiency of glucose in ATP production and the lower effect in oxidative stress with respect to other substrates, these data argue strongly that the role of GRK2 in the pathogenesis of HF is due at least, in part, by negative alterations in cardiac metabolism.

GRK2 and Mitochondrial Function: Relevance to Cardiac Metabolism and Myocyte Survival

GRK2 Localizes to Mitochondria

An interesting idea that arises from these data is that GRK2 may be a master regulator of cellular metabolism by controlling signal transduction from multiple receptors (GPCRs and receptor tyrosine kinases) in addition to the cellular production and expenditure of energy through the regulation of βAR-mediated contractile function. In an interesting finding that can support this hypothesis, GRK2 has been found to localize in/on mitochondria. This was first observed in the brain53 and more recently in other cells including cardiac myocytes.64,65 During basal states or resting conditions, the substrates, binding partners, or functions of GRK2 within myocardial mitochondria are not known; however, a study in noncardiomyocytes did show GRK2 to be in involved in ATP production.64

What is known for myocytes is that when levels of GRK2 are elevated 3- to 4-fold as is found in human HF, there is more GRK2 associated with mitochondria as well, leading to reduced calcium tolerance of the mitochondrial permeability transition pore basally,65 cytochrome c release, and increased apoptotic signaling after ischemic stress (Figure 3).22 As discussed more below, cardiac-specific GRK2 knockout mice had...
reduced ischemic injury because of lower cytochrome c release from mitochondria and decreased apoptotic signaling, consistent with the hypothesis that GRK2 mediates these events.22

As detailed in the next section, it is clear that GRK2 is a prodeath kinase, and the activity of this kinase is dependent, at least in part, on its mitochondrial localization. Importantly, in the setting of chronic HF development, the increase in cellular and mitochondrial GRK2 has negative effects on βAR and insulin signaling, resulting in impaired cellular metabolism and survival that eventually lead to contractile dysfunction. More studies are needed to determine the GRK2 targets in mitochondria, if any, that may affect basal metabolic functions.

Myocyte Survival and the Role of Mitochondrial Localized GRK2

Studies using a model of in vivo ischemia–reperfusion (I/R) injury have shown unequivocally that increased GRK2 expression in the heart enhances cardiac injury, whereas inhibition with βARKct expression is robustly cardioprotective.23 In addition to the above potential effects of mitochondrial GRK2 in cardiac metabolism, we have found a definitive mitochondrial-dependent mode of prodeath kinase action in the myocyte after ischemic injury and the pursuing oxidative stress via reactive oxygen species.65 Interestingly, like Akt and protein kinase C mitochondrial translocation, GRK2 movement from the cytosol to mitochondria in response to reactive oxygen species is mediated by the chaperone protein heat shock protein 90.65 This protein–protein interaction is mediated by the phosphorylation of GRK2 itself at Ser670 by mitogen-activated protein kinases (presumably extracellular signal–regulated kinase; Figure 4). This residue resides in the C tail and is also present in the GRK2 inhibitor, βARKct; reactive oxygen species results in the phosphorylation of the βARKct peptide when it is expressed in myocytes, and the binding of phosphorylated βARKct prevents endogenous GRK2 from binding to heat shock protein 90 and translocating to the mitochondria.

Figure 2. The role of G protein–coupled receptor kinase 2 (GRK2) in cardiac insulin receptor signaling and myocyte glucose uptake. A, Normally, insulin binding to its receptor activates a downstream signaling process involving insulin receptor substrate-1 (IRS1) that ultimately results in the movement of glucose transporter 4 (GLUT4) from the cytosol to the membrane, allowing for increased glucose uptake. B, Excess GRK2, as in heart failure, attenuates the insulin response via phosphorylation of IRS1 and inhibition of GLUT4 membrane translocation. The net result of GRK2’s action on the insulin signaling pathway in the myocyte is insulin resistance and glucose intolerance. GRB2 indicates growth factor receptor-bound protein 2; GSK, glycogen synthase kinase; and mTOR, mammalian target of rapamycin.
Moreover, expression of a Ser670Ala mutant of βARKct can no longer prevent myocyte death after oxidative stress because it cannot compete with GRK2 for binding to heat shock protein 90.65 We have previously found the βARKct to be cardioprotective in the ischemic heart, and Chen et al65 determined that this mechanism of protection involves preventing the localization of GRK2 to the mitochondria. Recent studies in cardiomyocyte-specific GRK2 knockout mice have also hinted at the prodeath actions of GRK2 at the level of mitochondria because loss of myocyte GRK2 after I/R injury decreased cytochrome c release and increased levels of antiapoptotic Bcl-2 (B-cell lymphoma 2) proteins.22 Lowering GRK2 is cardioprotective, and it resembles βARKct-mediated protection because both molecular manipulations decrease mitochondrial levels of GRK2. Prevention of the prodeath actions of GRK2 at the level of the mitochondria provides a novel mechanism to add to the therapeutic potential of GRK2 inhibition as a clinical therapy for HF. There are still many questions to be answered about the role of GRK2 in the mitochondria during both healthy and disease states, including the identification of potential substrates for GRK2 in the mitochondria, the precise function of mitochondria-localized GRK2 under basal conditions, and last, if GRK2 either directly or indirectly hinders prosurvival functions of heat shock protein 90.

Other Cell Survival Roles for GRK2: Regulation of Cellular Redox States Through Endothelial Nitric Oxide Synthase

In addition to the above mitochondrial-dependent mechanism of GRK2-mediated myocyte apoptosis, we have uncovered another key mechanism of GRK2 in relation to the redox state of the myocyte that seems to have crucial importance in regulating cardiac injury after ischemia. In the original study demonstrating the prodeath actions of elevated myocardial GRK2 and the cardioprotective effects of βARKct expression after I/R, we found that inhibition of GRK2 in the myocyte was accompanied by increased Akt and endothelial nitric oxide synthase (eNOS) activity, which led to increased nitric oxide (NO) production in the ischemic heart.25 Consistent with this, GRK2 overexpression decreased eNOS–NO.25 Myocyte death is a consequence of I/R injury and has detrimental effects on left ventricular function, contributing to the development of HF. Protective pathways, such as PI3K/Akt, and increased levels of NO allow for the preservation of myocardial...
contractility adding to the importance of GRK2 in not only influencing contractility directly through βAR regulation but by other means including cardiac metabolism and cell survival.

Recently, we have uncovered that this is a result of a direct interaction between GRK2 and eNOS because we were able to communoprecipitate GRK2 and eNOS from the mouse heart, as well as myocytes; ischemia or oxidative stress increased the interaction.66 Interestingly, within this interaction, GRK2 and eNOS negatively regulate one another. The negative regulation of GRK2 by eNOS occurs via the direct S-nitrosylation of GRK2 at Cys340 as previously found.67 Thus, when NO bioavailability is high, GRK2 is inhibited and cardiac contractile function and cell survival are enhanced. Studies have shown that increased S-nitrosylation of GRK2 at this site enhances βAR-mediated cardiac contractility.67 GRK2 inhibits eNOS activation by a yet unknown mechanism; however, Ser1177 is involved (although this does not seem to be the target of direct GRK2 kinase activity).66 Therefore, in HF or after myocardial injury, the increased expression of GRK2 suppresses eNOS activity preventing increased NO that ultimately feedbacks to less GRK2 inhibition through S-nitrosylation, creating a vicious cycle of the negative actions of this kinase. Additional studies using a novel GRK2-Cys340Ser knockin mouse demonstrate that the lack of dynamic S-nitrosylation control of GRK2 can directly influence cardiac ischemic injury because these mice have more GRK2 activity and greater injury after I/R.66 Interestingly, we also found that the full cardioprotection of βARKct expression is dependent on eNOS activity,66 which suggests that the GRK2–eNOS interaction may also influence GRK2 mitochondrial localization, and this is the subject of ongoing investigations. Nitrates and β-blockers are some of the most commonly prescribed drugs for HF, and the molecular mechanisms underlying their effectiveness are not clearly understood; elucidating the reciprocal relationship between GRK2 and eNOS lends insight into this and underscores the pathological role of GRK2, implicating that direct inhibition may be a more effective treatment in HF.

Conclusions

Overwhelming evidence revealed over the course of the past 2 decades has convincingly demonstrated the benefits of preventing excessive GRK2 expression activity in the cardiomyocyte after cardiac injury and during HF. Although initially this therapeutic benefit of GRK2 inhibition was attributed to increases in contractility resulting from enhanced or restored βAR signaling, recent data have now come to light focusing on the harmful adrenergic-independent activities of GRK2 displayed during cardiac injury including cardiac metabolism and cell survival. These latter effects of GRK2 are not only βAR independent but are GPCR independent and also include a novel cellular localization of GRK2 in/on mitochondria and novel binding partners (ie, eNOS). Although specific targets and potential substrates still need to be elucidated, the mitochondrial localization of GRK2 points toward this kinase being a nodal link between cellular metabolism, cell survival, and cardiac contractility. Based on the vast amount of research demonstrating the potential therapeutic effects of GRK2 inhibition during HF in animal models, it is interesting to speculate whether the benefits are because of the classical effects of GRK2 activity or the novel actions of GRK2. It is clear that the altered βAR signaling alterations found in the failing heart with GRK2 inhibition support and improve cardiac contractile function; however, recent data on cardiac metabolic defects (ie, IR) being improved with GRK2 inhibition lends a novel perspective to the overall therapeutic landscape.41 Moreover, given the importance of the mitochondria as the cellular powerhouse and the initiator of apoptosis during basal and disease states, the significance of this localization and its inhibition could be considerably important in the pathological development of HF. In addition, the interaction with and inhibition of eNOS, the catalyst of NO production, adds another dimension to the importance of regulating GRK2 levels to maintain NO levels because NO is important to maintain vascular tone and cardiac function. Therefore, it is probable that inhibition of both classical and novel aspects of GRK2 localization and function contributes to the beneficial effects seen in the heart after injury/stress. Keeping these critical cellular events in mind, these novel discoveries have placed even more emphasis on the importance of inhibiting GRK2 in multiple disease models, including HF and conditions associated with metabolic syndromes, hypertension, and type 2 diabetes mellitus. More studies are needed that specifically target the different roles of GRK2 in the failing heart to discern alterations in GPCR desensitization from these novel and exciting roles of GRK2 in the cardiomyocyte.

Acknowledgments

W.J. Koch is the William Wikoff Smith Chair in Cardiovascular Medicine.

Sources of Funding

W.J. Koch is supported by the following National Institutes of Health grants: R37 HL061690, R01 HL085503, P01 HL091799, P01 HL075443 (project 2), and P01 HL108806 (project 3).

Disclosures

None.

References


W. Joseph Koch


G Protein–Coupled Receptor Kinase 2: A Link Between Myocardial Contractile Function and Cardiac Metabolism
Meryl C. Woodall, Michele Ciccarelli, Benjamin P. Woodall and Walter J. Koch

Circ Res. 2014;114:1661-1670
doi: 10.1161/CIRCRESAHA.114.300513

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/114/10/1661

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/