EDITORIAL COMMENT

G Protein-Coupled Receptor Kinases: Gotta Real Kure for Heart Failure?*

Susan F. Steinberg, MD
New York, New York

With over five million patients in the U.S. alone carrying the diagnosis of chronic congestive heart failure, innovative treatments to delay its progression and/or ameliorate its symptoms remain a high priority of cardiovascular research. The G protein-coupled receptors (GPCRs) for catecholamines remain a centerpiece of heart failure research. As a result of research in several laboratories, the traditional notion that catecholamine actions are mediated by the predominant β1-adrenergic receptor (β1-AR) subtype has been broadened to encompass a β2-AR component, which also provides inotropic support and influences cardiomyocyte cell biology (particularly in the failing or aged myocardium where there is selective downregulation of β1-ARs).

For both β1- and β2-ARs, activation involves a conformational change that facilitates interactions with stimulatory heterotrimeric Gs proteins, leading to activation of adenylyl cyclase and formation of cyclic adenosine monophosphate (cAMP). However, recent studies identify differences in the pathways for β1- versus β2-AR activation of adenylyl cyclase, β-AR subtype actions that go beyond the generation of cAMP, and effects of β-ARs on cardiomyocyte gene expression, growth and survival ([1], and references cited therein). The distinct signaling properties of βγ dimers (liberated with guanosine triphosphate [GTP]-bound alpha subunits upon heterotrimeric G protein activation) also explain some of the complexities in β-AR subtype signaling.

One target of βγ dimers that has received considerable recent attention is the G protein–coupled receptor kinases (GRKs) that phosphorylate agonist-activated GPCRs.

Therapies targeted to β-ARs are cornerstones of heart failure management, despite lingering controversies regarding the role of β-AR signaling in the pathogenesis of heart failure. Traditionally, β-ARs were viewed as providing essential inotropic support for the failing heart. However, the evidence that chronic β-AR signaling leads to long-term adverse effects that accelerate the natural history of heart failure (offsetting any short-term benefit) has raised serious questions regarding the optimal therapeutic approach to the β-AR desensitization that develops in response to the chronically enhanced sympathetic tone of heart failure. Some investigators cite clinical evidence that long-term β-AR blockade reduces the combined risk of morbidity and mortality in heart failure as support for the notion that depressed β-AR signaling in heart failure is cardioprotective (2). According to this formulation, β-AR activation contributes to the progression of heart failure; pharmacologic strategies to interfere with β-AR signaling provide clinical benefit.

However, other investigators emphasize evidence that strategies designed to increase β2-AR activity or reverse β-AR desensitization (by reducing GRK levels) are well tolerated, improve myocardial performance and prolong survival in experimental models of heart failure. These results are taken in support of the diametrically opposite view that the depressed β-AR response (and the GRKs that desensitize β-ARs) constitute a convenient target for therapeutic intervention in heart failure. In this context, the study by Iaccarino et al. (3) in this issue of the Journal focuses specifically on the role of GRK2 (one GRK family member) in the pathogenesis of impaired β-AR responsiveness and cardiac failure that develops when transgenic mice that overexpress wild-type α1B-ARs (Tgα43) are subjected to treatment with phenylephrine (PE) (3). The study confirms a previous observation that β-AR activation of adenylyl cyclase is impaired in Tgα43 mice (4).

In the previous study (4), this was attributed to heightened GRK activity, which develops—without any detectable change in GRK2 protein expression—in the compensated hypertrophy displayed by Tgα43 mice (3,4). Phenylephrine induces moderate cardiac hypertrophy, with few biochemical abnormalities, in nontransgenic littermates. However, PE elicits a severe “maladaptive” hypertrophy with progressive biochemical markers of cardiac failure (impaired β-AR signaling, elevated GRK2 expression) and reduced survival in Tgα43 mice. Reduced cardiac neuropeptide Y stores in PE-treated Tgα43 mice is interpreted as evidence for chronic sympathetic nervous system activation, which is implicated in the induction of GRK2 expression. The investigators suggest that increased GRK2 expression contributes to the evolution of heart failure in this model. Given the growing enthusiasm for GRKs as a potential novel target for heart failure therapy, the comments that follow briefly review its structure and the elaborate/interconnecting networks of regulatory signals recently shown to influence its function. The aim is to evaluate critically the experimental basis for current concepts regarding the role of GRK2 in the evolution of cardiac dysfunction and consider potential long-term consequences of GRK2 inhibition.

The GRKs have emerged as a family of six members that

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From the Departments of Pharmacology and Medicine, College of Physicians and Surgeons, Columbia University, New York, New York. This work was supported by U.S.P.H.S.–N.H.L.B.I. grant HL-28958.

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specifically phosphorylate GPCRs, but only following agonist activation (homologous desensitization). The overall topology of GRKs is characterized by a somewhat conserved central catalytic domain flanked by C- and N-terminal regulatory sequences ([5,6] and references cited therein). The GRKs are classified into three categories, based upon divergence in their C-terminal domain architecture and membrane-targeting mechanisms. These comments are confined to the three GRKs (GRK2 [βARK1], GRK3 [βARK2], and GRK5) that fall into two subclasses and are the most abundant isoforms expressed by cardiomyocytes. Both GRK2 and its structural homologue GRK3 are primarily cytosolic in resting cells; they become targeted to the membrane as a result of an interaction between their C-terminal pleckstrin-homology domains, membrane PIP2, and membrane-anchored G protein βγ dimers.

Recent studies identified sequence homology between the N-terminus of GRK2 or GRK3 and regulators of G protein signaling (RGS) family proteins, proteins that serve as GTPase-activating proteins (GAP) by binding to the transition state of Ga subunits and accelerating their intrinsic rates of GTP hydrolysis (i.e., limiting their half-lives of activation). Indeed, the N-terminus of GRK2 or GRK3 binds tightly to Gaq/11 proteins (but not Gai, Gaiq/12/13 or Gap). However, GRK2 and GRK3 display only weak GAP activity for Gaiq (relative to RGS proteins); Gq inhibition is due to the sequestration of active Gq subunits (7,8). Hence, GRK2 and GRK3 exert multidomain regulation of GPCR signaling; phosphorylation of agonist-occupied GPCRs by the catalytic domain leads to homologous desensitization, whereas phosphorylation-independent binding interactions inhibit the bifurcating Gq signaling pathway by sequestering Gq subunits (GRK C-terminus) and Gβγ dimers (GRK N-terminus).

Despite their high degree of structural homology, GRK2 and GRK3 display unanticipated in vivo specificity toward certain GPCRs. For example, GRK2 desensitizes β-ARs and angiotensin II type 1A receptors, whereas GRK3 does not (6). Conversely, GRK3 (but not GRK2) desensitizes α1B-ARs and protease-activated receptor-1 (PAR-1, the receptor for thrombin and other proteases, which elevates calcium and promotes cardiomyocyte hypertrophy [6,9–11]). The structural basis for differences in GRK2 and GRK3 substrate specificity has been mapped to a region of substantial sequence divergence in their Gβγ-binding domains (the region critical for GRK-Gβγ interactions). This suggests a model wherein specificity in GPCR–GRK interactions is dictated by the distinct pools of structurally distinguishable Gβγ dimers released by various GPCRs that recruit different GRK isoforms to the plasma membrane.

Cardiomyocytes also express the structurally distinct GRK5 enzyme that phosphorylates agonist-occupied GPCRs, but does not bind Gaiq/11 proteins and does not require membrane translocation for activation; it is constitutively membrane-anchored through a cluster of positively charged residues located at the C-terminus. The GRK5 desensitizes β1-ARs, PAR-1 and (to a lesser extent) α1B-ARs, but not angiotensin II receptors (6).

Recent studies identify an inhibitory interaction between the N-termini of GRK2 and GRK5 and caveolin, the structural protein that drives formation of caveole membrane subdomains (12). The evidence that caveolin expression is highly regulated, and reportedly decreases during chronic β-AR stimulation (as occurs in heart failure [13]), has fueled speculation that this interaction might influence β-AR signaling. According to this formulation, decreased inhibitory caveolin provides an ancillary mechanism to elevate GRK and impair β-AR responsiveness in heart failure, as the bulk of the cardiomyocyte β-ARs reside in caveole (12,14).

The GRK-dependent phosphorylation of agonist-occupied GPCRs facilitates the binding of β-arrestin, which sterically hinders the interaction between agonist-occupied GPCRs and their cognate G proteins, thereby initiating agonist-induced receptor desensitization and internalization. Also, β-arrestins function as adapter proteins to promote the stable association of other molecules with GPCRs. The β-arrestin docks Src tyrosine kinase, an upstream element in ERK and JNK cascades, and components of the endocytic machinery (AP-2, clathrin). In this manner, β-arrestin plays a role in GPCR trafficking, desensitization/resensitization, and initiates a “second-wave” of effector responses. The contribution of β-arrestin binding to ERK/JNK activation by GPCRs has received intense recent scrutiny in heterologous expression systems. In theory, β-arrestin binding should carry similar significance for GPCR (and β-AR) signaling in cardiomyocytes. However, given ample evidence for the contextual nature of GPCR signaling (with differences in the molecular machinery between cells markedly influencing GPCR signaling phenotype), any conclusions regarding the importance of this mechanism in the heart await specific experimental validation.

With these comments as background, the following questions consider the role of GRKs in cardiac failure: Does an increase in GRK2 expression provide a generalized mechanism to explain reduced cardiac β-AR responsiveness; is it a general marker of cardiac hypertrophy? A potential role for GRK2 in the regulation of β-AR sensitivity was first suggested based upon evidence that steady-state levels of GRK2 mRNA are elevated in a wide range of human and animal models of cardiac dysfunction, where β-AR activation of adenyl cyclase and modulation of contractile function is impaired (reviewed in [6]). However, impaired β-AR responsiveness without a detectable change in GRK2 expression is reported in some syndromes of cardiac hypertrophy/dysfunction (e.g., mice that overexpress Gqq proteins or the constitutively active α1B-AR mutant [6,15]). The GRK5 rather than GRK2 is elevated in tachycardia-induced heart failure in pigs (16). Cardiac β1-AR and β2-AR responsiveness is reduced, without any change in
steady-state GRK2 or GRK5 mRNA levels or GRK activity, during normal aging of the rat ventricle (17).

The prevailing evidence from the current study by Iaccarino et al. (3) as well as previous studies (5,6) is that increased GRK expression results from the enhanced sympathetic nervous system activity in heart failure. Increased GRK expression is not detected in models of cardiac hypertrophy that bypass the β-AR signaling pathway (hypertrophy induced by α1-ARs, Gaq, or Ras); it is not a requisite feature of hypertrophy itself. This is most convincingly demonstrated by a previous study showing that iso-proterenol infusion leads to cardiac hypertrophy with increased GRK2 mRNA, protein, and activity; PE infusion, at a concentration that induces a similar degree of hypertrophy, does not alter GRK2 expression (6).

Are changes in GRK in heart failure optimally detected as changes in mRNA/protein expression or enzyme activity? In the studies cited above, disease-dependent alterations were monitored as changes in GRK expression or activity (variably both). The precise method used to detect disease-dependent changes in GRK may be pertinent, as recent studies identify distinct mechanisms that regulate GRK expression and activity. The observation that α1B-AR activation rapidly translocates GRK2 to the plasma membrane provided the first evidence that GRK2 function can increase as a result of enhanced activity, without induction of GRK2 protein/mRNA expression. Subsequent studies identified specific regulation of GRKs by protein kinase-C (PKC) and calcium. The PKC phosphorylation increases GRK2, but decreases GRK5, activity (5,6). The GRK5 (and to a lesser extent GRK2) activity is inhibited by calcium as a result of an interaction between its N-terminal regulatory domain and the calcium-binding protein calmodulin. However, PKC-dependent phosphorylation of GRK2 relieves calmodulin-dependent inhibition of GRK2 (18). Hence, signaling by α1-ARs tends to favor GRK2 activation (while turning off GRK5). This mechanism might explain the increased GRK activity (without an increase in GRK protein expression) that is reported to impair β-AR responsiveness in TGα43 mice. The predicted consequences of activation of the α1-AR/PKC pathway include decreased signaling by β-ARs (which are regulated by GRK2 and GRK5), decreased signaling by angiotensin II type 1A receptors (which are regulated by GRK2, but not GRK5), but persistent signaling by PAR-1 (which is regulated by GRK5, but not GRK2). These newer concepts regarding GRK regulation would effectively implicate GRK more broadly in the impaired β-AR responsiveness that develops in various experimental models of cardiac hypertrophy (including potentially in the Gaq and α1B-AR overexpressing mice, where β-AR responsiveness is impaired without an increase in GRK2 expression). However, these newer studies also introduce a fundamental uncertainty regarding the role of GRK in the pathogenesis of heart failure. Iaccarino et al. (3) in the current study suggest that increased GRK2 expression is key for the transition from myocardial hypertrophy to cardiac failure. Does the enhanced GRK activity in TGα43 mice with stable, compensated hypertrophy hold similar significance?

Current published reports focus on elevated GRK as the mechanism for impaired β-AR responsiveness in heart failure, but Serikov et al. (19) recently demonstrated that lowering extracellular calcium to relieve the calcium load restores β-AR responsiveness in several transgenic murine models of hypertrophy and heart failure. These investigators speculate that β-AR responsiveness is impaired in cardiac failure as a result of an alternative mechanism involving calcium-dependent inhibition of cardiac adenyl cyclase isoforms (19). The relative importance of GRK versus calcium overload must be considered in future studies.

Do changes in GRK expression influence β-AR signaling to growth regulatory pathways? Studies to date have focused on GRK desensitization of pathways leading to cAMP accumulation and inotropic support. However, ample evidence shows that individual β-AR subtypes differ in their coupling to this and other signaling mechanisms that contribute to the pathogenesis of hypertrophy and the transition to cardiac failure. The most striking evidence for complex regulatory actions of β-AR subtypes comes from studies in transgenic mice, where β2-ARs increase adenyl cyclase activity and elevate contractile function without obvious evidence of cardiotoxicity (unless β2-AR expression is forced to very high levels or maintained for protracted...
intervals). In marked contrast to the relatively wide “therapeutic window” for $\beta_2$-AR overexpression (6,22), even low levels of transgenic $\beta_1$-AR overexpression rapidly lead to a dilated cardiomyopathy with fibrosis and cardiomyocyte apoptosis (6). These results emphasize the very distinct cellular actions of $\beta_1$-versus $\beta_2$-ARs.

The signaling properties of individual $\beta$-AR subtypes have been deciphered largely in cardiomyocyte cultures, where $\beta$-AR activation induces morphologic and molecular features of hypertrophy (23). In initial studies, the anabolic response was attributed to $\beta$-AR activation of the ERK cascade via pathways that involve both Gs/cAMP/PKA and Gi protein $\beta\gamma$ dimers/Src/Ras/PI-3 kinase/AKT (and not PKA-dependent phosphorylation of $\beta$-ARs, which switches $\beta$-AR coupling from Gs to Gi, and leads to ERK activation by a $\beta\gamma$ dimer pathway, was proposed (24) and references cited therein). The implicit assumption of these studies was that ERK activation is mediated by $\beta_2$-ARs (PKA-dependent modulation of G-protein-coupling preference has only been shown for $\beta_2$-ARs). However, other studies place ERK downstream from both $\beta_1$- and $\beta_2$-AR subtypes (25,26), and two recent studies implicate $\beta_1$-ARs in cardiomyocyte hypertrophy (27,28). Hence, other mechanisms, including a pathway initiated by GRK-dependent phosphorylation of $\beta$-ARs, which facilitates $\beta$-arrestin binding as a scaffold for ERK activation also must be considered in future studies.

Consistent with the marked cardiotoxic properties of $\beta_1$-ARs (relative to $\beta_2$-ARs) in transgenic mice, other studies identify an effect of $\beta_1$-ARs to induce cardiomyocyte apoptosis (28) and an effect of $\beta_2$-ARs to counter the pro-apoptotic actions of $\beta_1$-AR via a Gi-dependent pathway involving phosphoinositide 3-kinase/AKT (and not ERK (24)). Recent studies also identify $\beta$-AR activation of calcineurin (via a calcium-dependent pathway) and Src family protein tyrosine kinases (via direct cAMP-independent actions of Gs subunits); both of these molecules have been implicated in the induction of hypertrophy and apoptosis (29,30). Hence, many different effectors (some with opposing actions) have been implicated as downstream targets for $\beta$-ARs in the intact heart will be defined by the balance of signaling through these highly integrated molecular signals. Current knowledge provides little guidance to predict whether GRK-dependent desensitization of cAMP responses is accompanied by a global loss of $\beta$-AR signals. For example, increased GRK2-dependent phosphorylation of GPCRs ($\beta$-ARs or others) might actually facilitate arrestin binding and signaling via the ERK pathway. A previous observation that cardiomyocytes isolated from ventricles of mice that overexpress GRK2 display increased resting cell length (relative to wild-type controls [6]) would be consistent with the notion that increased GRK2 contributes to the acquisition of certain morphological features of hypertrophy; however, more direct studies are required.

Finally, current published reports focus largely on GRK regulation of $\beta$-AR signaling to adenyl cyclase. The challenge of future studies will be to broaden the analysis to consider the role of GRKs in signaling by individual $\beta$-AR subtypes to effectors that are predicted to influence cardiomyocyte structure, gene expression, and survival during hypoxic insults (MAPK cascades, the PI-3K/AKT pathway, Src). These trophic effects of $\beta$-ARs ultimately are likely to be as important as cAMP in determining the relative contribution of $\beta$-AR activation to the progression of heart failure and/or survival.

Reprint requests and correspondence: Dr. Susan F. Steinberg, Department of Pharmacology, College of Physicians and Surgeons, Columbia University, 630 West 168 Street, New York, New York 10032. E-mail: ssf1@columbia.edu.

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