Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease

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Abstract

The G protein-coupled receptor kinases (GRKs) participate with arrestins in the regulation and signal propagation of multiple G protein-coupled receptors (GPCR) of key physiological and pharmacological relevance in the cardiovascular system. The complex mechanisms of regulation of GRK expression, degradation and function are being unveiled gradually. The levels of these kinases are known to change in pathological situations such as heart failure, hypertrophy and hypertension, and in animal models of these diseases. A better understanding of the mechanisms underlying these changes and of how these alterations participate in the triggering or progression of cardiovascular disease may contribute to the design of novel diagnostic and therapeutic strategies.

Keywords: G protein-coupled receptor kinases (GRKs); Heart failure; Protein kinases; Adrenergic receptors; Signal transduction

1. Introduction

The G protein receptor-coupled receptors (GPCR) are a superfamily of proteins with seven transmembrane domains, whose members interact with a wide range of chemical messengers. By way of interaction with heterotrimeric G proteins and other cell proteins, GPCR stimulation drives the initiation of multiple intracellular signalling routes. These include classical second messenger pathways controlled by adenyllyl cyclases, phospholipases and ionic channels, as well as different kinase cascades (ERK/MAPK, JNK, p38, ERK5) or the Akt/PI3K route. These pathways control cell proliferation, differentiation, survival, migration and other functions [1].

In addition to promoting the activation of G proteins, the stimulation of GPCRs triggers their phosphorylation by GRKs (G protein-coupled receptor kinases) [2–4]. Arrestins then bind to the phosphorylated receptors and impair communication with the G proteins even in the presence of a stimulus. Such rapid loss of receptor responsiveness is termed desensitization. GRKs and arrestins also promote the transient internalisation of GPCRs by mediating the coupling of the endocytic machinery [2]. In addition, recent data indicate that both arrestins and GRKs participate in signal propagation, cooperating in the assembly of macromolecular complexes in the receptor environment and interacting with different components of signal transduction pathways [2–4]. Arrestins and GRKs may also participate in signalling platforms regulating other receptor families, such as tyrosine kinase receptors [5], broadening even further the horizons of their cellular functions.

GPCRs mediate a number of essential events in cardiovascular function. The activation of α- and β-adrenergic, muscarinic, angiotensin II or endothelin receptors is central to cardiac contractility, vascular resistance, the development of the cardiovascular system and the...
growth and remodelling of different cardiovascular cell types. These receptors and their transduction systems are also the targets of many different drugs used in the treatment of angina, congestive heart failure and hypertension [6,7]. All these receptors are regulated by GRKs and arrestins.

Myocardial hypertrophy and an increase in the activity of the sympathetic nervous and renin–angiotensin systems are well known hallmarks of human heart disease. They are also seen in animal models in which cardiac performance is compromised [6,7]. In addition, end-stage heart failure is characterised by changes in the beta-adrenergic receptor signalling cascade. During the development of heart failure (in which severe loss of contractility and muscle remodelling occurs), β1AR density and responsiveness becomes severely compromised as a consequence of the down-regulation that takes place during the maladaptive response to chronic stimulation. In addition to a reduction in receptor density, a number of important consequences follow, including desensitization of the β1AR-dependent inotropy. However, fibroblasts, endothelial cells, smooth muscle cells and macrophages also form part of cardiac tissue, and although myocytes make up most of the adult myocardial mass, they represent only 30% of the total number of cells. This proportion is even lower in heart failure since remodelling involves fibroblast

immunohistochemical analyses of rat hearts shows high GRK2 levels in non-myocyte cardiac cells, while GRK3 is confined to cardiomyocytes. GRK5 is equally distributed among cardiac cell types [18]. Thus, the distribution of the different GRK isoforms in myocardial tissue might reflect their preferred physiological substrates and roles in cardiac pathologies. Although a detailed analysis of GRK distribution in heart failure is still to be performed, it is usually assumed that the increase in GRK2 levels is confined to cardiac contractile cells. This would explain the effect on β1AR-dependent inotropy. However, fibroblasts, endothelial cells, smooth muscle cells and macrophages also form part of cardiac tissue, and although myocytes make up most of the adult myocardial mass, they represent only 30% of the total number of cells. This proportion is even lower in heart failure since remodelling involves fibroblast

2. GRK isoforms: molecular properties and cell type distribution

2.1. GRKs expressed in the heart

Seven GRK genes are known in mammals [3,11]. GRK1 and 7 are expressed in the retina and GRK4 in the testes; other GRKs are ubiquitously expressed though their levels depend on the tissue and cell type [3,11,12]. Several reports have shown that GRK2, GRK3 and GRK5 are clearly expressed in healthy human hearts, while GRK4, GRK6 and GRK7 are barely detectable or absent [13]. All GRKs share a central catalytic domain similar to other serine/threonine kinases. The C-terminal domain is of variable length and seems to facilitate interactions with lipids and other membrane proteins. Thus, the carboxy terminal domain of GRK5 binds phospholipids, allowing for its preferential membrane association, while GRK2 and GRK3 are mainly found in the cytoplasm. Membrane targeting of the latter kinases upon GPCR activation is facilitated by their specific interaction with Gβγ subunits released upon G protein stimulation (reviewed in [3,11,12]). The N-terminal region encompasses an RH domain (regulator of G protein signalling homology domain). In the GRK2/3 subfamily, this RH domain has been shown to interact specifically with Gαq family members [3,11].

The three GRKs expressed in the heart share certain characteristics but are distinct non-redundant enzymes with specific functional and regulatory properties, as demonstrated by the phenotypic characterisation of knockout or tissuespecific transgenic mice. GRK3 shows in vivo selectivity towards thrombin and α1B-adrenergic receptors, GRK5 shows the same towards angiotensin II receptors, and GRK2 and GRK5 are important in the myocardial βAR system (see recent reviews in [11,14–16]). Moreover, the specificity of GRK–GPCR interactions is suggested by the cellular and subcellular distribution of these kinases and their receptors (see below). In addition, the different GRKs are probably involved in distinct signalling pathways during the development of the heart, as suggested by the phenotypes of corresponding knock-out animals. GRK2 knock-out mice die at days 12–15 of embryonic life due to severe myocardial hypoplasia and heart failure [17]. However, mice lacking either GRK3 or GRK5 are viable [11], suggesting that GRK2 is the only critical isoform in heart development.

2.2. Cellular distribution of cardiac GRKs
proliferation and fibrosis. Therefore, it should be remembered that the upregulation of GRK2 may have important functional consequences in cells other than myocytes. In addition, the subcellular distribution of GRK, in contrast to total protein levels, might be key to understanding its importance in cardiac malfunction. Some evidence suggests that the subcellular distribution of GRKs is altered in heart failure. While GRK2 colocalises with α-actinin and G proteins in normal and diseased animals, in rats with heart failure it accumulates in the intercalated discs of the myocardium while GRK5 appears to show a nuclear pattern [19].

3. Mechanisms of regulation of GRK levels and functionality

3.1. Intramolecular interactions

The control of GRK2 activity and membrane targeting appears to involve the interaction of the N- and C-terminal domains of the kinase with different intracellular targets. Recently acquired biochemical and molecular modelling data suggest the existence of regulatory intramolecular interactions among the N-terminal, C-terminal and catalytic domains of GRK2 that would maintain the enzyme in a constrained, inactive state. The disruption of these intramolecular contacts would promote conformational changes, leading to the enzyme’s translocation and activation [20]. The structure of bovine GRK2 in complex with G protein βγ subunits has provided further insight into GRKs regulation. The RH domain interacts with both the kinase and the PH domains, suggesting this has an important role in the regulation of kinase activity. In addition, the PH domain interacts with the terminal subdomain of the RGS homology domain, suggesting allosteric regulation may occur between these two regions of the protein [21]. Moreover, the kinase appears capable of simultaneously interacting with Goq and Gβγ protein subunits, keeping them from signalling to downstream effectors and supporting a model of phosphorylation-independent desensitization mediated by GRK2 through the interference of signal propagation at the G protein subunit level.

3.2. Role of calcium binding proteins

Alterations in intracellular calcium levels are associated with the progression of heart failure. Cellular calcium levels appear to modulate GRK activity via the interaction of calcium-sensing proteins with different GRKs (see reviews [3,11,12]). GRK5 is very sensitive to the presence of calcium-bound calmodulin, which blocks membrane targeting and may increase its activity towards soluble substrates. GRK2, in contrast, is only affected at high concentrations. Due to its high affinity for calmodulin, GRK5 would be preferentially inhibited in most cell types when the calcium concentration rises upon activation of GPCR.

3.3. Regulation of GRKs by interacting proteins

Apart from the potent stimulation upon Gβγ or activated receptor binding, the activity of GRKs is strongly modulated by their interaction with other cell proteins that usually keep the former’s catalytic activity at bay. Thus, their interaction with α-actinin, caveolin or with an undetermined microsomal component negatively modulates GRK kinase-dependent functions [3,11]. Caveolin interaction favours the appearance of GRK2 in the caveolin-rich fractions of cell membranes. Caveolin serves as a scaffold for a variety of signalling molecules including β2AR and different MAPK and G proteins, and may help to limit or compartmentalise signalling. GRK2 interacts with different caveolin isoforms present in the heart. Since reduced caveolin levels have been reported in patients with heart failure, and since caveolin knock-out mice develop cardiac hypertrophy and contractile dysfunctions [22,23], the caveolin–GRK2 interaction may have a role in cardiac function.

Another recently discovered GRK2 cellular partner is the Raf kinase inhibitor protein RKIP. Phosphorylation of RKIP by PKC displaces it from Raf and increases its association with GRK2. A reduced level of RKIP would lead to increased GRK2 activity and the impairment of the contractile response of myocytes [24].

3.4. Regulation of GRK by phosphorylation

The phosphorylation of GRK by a variety of kinases has emerged as an important mechanism for modulating its activity and protein stability. The second messenger-dependent kinases PKC and PKA modulate both the activity and membrane targeting of several GRKs. This crosstalk might be important in cardiac disease since both PKC and PKA are upregulated in human heart failure [25,26]. PKC phosphorylates both GRK2 and GRK5, but while phosphorylation of GRK2 leads to its increased activity at GPCRs (probably by relieving the inhibitory effect elicited by calmodulin), the phosphorylation of GRK5 strongly inhibits its catalytic activity (reviewed in [3,12]). GRK5 has been shown a good substrate for several PKC isoforms (α, β, γ, δ, η but not ε) in vitro, while GRK2 is only phosphorylated by PKCo, γ and δ. In addition, PKA activated by Gs-coupled receptors can directly phosphorylate GRK2. PKA phosphorylation does not affect kinase activity per se, but enhances the binding of GRK2 to Gβγ subunits, thereby facilitating membrane targeting of GRK2 and its interaction with activated receptors [27].

GPCR activation can also trigger the modulation of non-receptor tyrosine kinases, such as c-Src, and MAPK cascades. Our group has shown that c-Src can directly phosphorylate GRK2 in response to β2AR stimulation in a
process dependent on the ability of β-arrestins to recruit c-Src [28,29]. The activity of tyrosine-phosphorylated GRK2 towards a variety of substrates is thus enhanced [28].

GRK2 activity is also modulated by ERK1/ERK2 [30,31]. In vitro and in situ experiments have shown that ERK1 can phosphorylate GRK2 in its Goγ domain. MAPK phosphorylation strongly impairs the GRK2/Goγ interaction, thereby inhibiting kinase translocation and catalytic activity toward receptor membrane substrates. In addition, GRK2 phosphorylation by both c-Src and MAPK also triggers subsequent kinase degradation (see below). The possibility that other GRKs expressed in the heart, such as GRK3 or GRK5, are differentially modulated by these kinases deserves exploration.

### 3.6. Modulation of GRK degradation

The regulation of GRK stability may be an important mechanism for modulating their expression levels. GRK2 is a short-lived protein that undergoes polyubiquitination and is degraded by the proteasome [33]. Interestingly, kinase turnover is enhanced upon agonist stimulation of GPCR in heterologous systems and in nervous and immune cells [29,33,34]. Sustained GPCR stimulation results in a down-regulation of steady-state kinase levels, and interfering with GRK2 degradation potentiates GPCR desensitization [34]. The mechanisms triggering GRK2 degradation are complex and not completely understood. It is known that GRK2 degradation in response to GPCR activation requires β-arrestin to act as a scaffold for recruiting kinase c-Src, as well as tyrosine phosphorylation of GRK2 [29]. Moreover, MAPK-mediated phosphorylation also modulates GRK2 degradation by the proteasome pathway in concert with c-Src [34]. GRK2 modifications by c-Src and MAPK have to take place in a given subcellular context that involves β-arrestins. The latter are probably required for the recruitment of ligases such as MDM2 (Penela, Salcedo and Mayor, in preparation). Again, no information is available on the protein stability of GRK3 and 5 nor on their modulation by extracellular signals.

Although the signalling cascades known to trigger GRK2 degradation are activated in animal models of hypertrophy and heart failure, it appears that GRK2 turnover cannot counterbalance the increased transcription that occurs under these conditions. The result is increased GRK2 levels and activity. Whether additional factors required for triggering GRK2 degradation downstream of GRK2 phosphorylation

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**Fig. 1.** Mechanisms reportedly involved in the control of GRK2 expression at the transcriptional or the protein degradation level. The effects of the different signalling pathways have been reported in aortic smooth muscle cell lines (a), transgenic models (b), cardiomyocytes (c), neutrophils (d), HEK293, glial and myeloid cell lines (e). α1-AR, α1-adrenergic receptors; IL-1β and IL-6, interleukins 1β and 6, respectively; TGFβ1, transforming growth factor β1; TNF-α, tumor necrosis factor-α; INF-γ, interferon-gamma; β2AR, β2-adrenergic receptor; CXCR4, chemokine receptor; ROS, reactive oxygen species; TKs, unidentified tyrosine kinases; X, unidentified factor favouring GRK2 degradation; CK-II, casein-kinase II. See text for details.
are impaired during the course of the disease is still to be determined. The same is true of the potential participation of other mechanisms in the control of GRK2 stability in cardiovascular cells, such as the calpain-mediated proteolysis of GRK2 reported in lymphocytes in conditions of oxidative stress [35].

4. Changes in GRK levels and activity in cardiovascular pathologies

Increased left ventricular GRK2 mRNA and activity were reported in patients with ischemic and idiopathic dilated cardiomyopathy [36]. GRK2 expression and activity are also elevated in many conditions associated with the development of heart failure (see Table 1), including cardiac ischemia [37] and hypertension [38]. Interestingly, other GRKs also undergo changes in expression during pathological heart conditions. For example, GRK2 and GRK5 expression is increased in animal models of cardiac dysfunction [8,18,19], and in the left ventricle in human dilated cardiomyopathy and volume overload [13]. In contrast, GRK3 remains unchanged in dilated cardiomyopathy, and is only slightly increased in volume overload patients [13,18,36,37].

A common feature of these cardiac pathologies is the altered catecholamine/renin–angiotensin system overdrive. Certainly, GRK2 levels are increased in several experimental settings characterised by enhanced, chronic neurohumoral stimulation, regardless of the nature of the primary insult [39–41] (see Tables 1 and 2). When sympathetic tone is unmodified, GRK2 levels may remain unchanged (as observed in different transgenic mice displaying strong myocardial hypertrophy [42,43]) or even decrease (as observed in mice expressing a constitutively active Gq subunit [44]). In agreement with the notion of such sympathetic control, GRK2 levels were found to be reduced in mice lacking endogenous catecholamines due to the ablation of the dopamine β-hydroxylase gene (dbh) [45].

The question arises as to which are the signalling pathways involved in linking neurohormonal activity to altered transcription/activity of GRK2. In this regard, the data summarised in the previous section show a variety of potential molecular mechanisms. Enhanced neurohumoral activity in heart failure triggers profound alterations in the output signalling of both Gs/adenylyl-cyclase/PKA and Gq/PLC/PKC pathways, which emerge as potential candidates for controlling GRK2 levels and activity. As for GRK2 transcription, there is no evidence supporting a crucial role for a cAMP or PKA-dependent mechanism in GRK2 regulation; this is consistent with the absence of CRE sites in the GRK2 promoter. The expression of a dominant negative CREB transcription factor does not impede GRK2 upregulation in heart failure [41]. Interestingly, however, enhanced β1AR, but not β2AR signalling, results in increased GRK2 levels. This suggests that differences in the signalling pathways triggered by these receptors [7] are important in such regulation. In this regard, although β2AR and other Gi-coupled GPCRs promote an increase in the degradation rate of GRK2 [29], the question remains as to whether β1AR similarly regulates GRK2 stability. Increased PKA activity has been described in patients with dilated cardiomyopathy and volume overload [13,46]. Thus, elevated PKA functionality could contribute to an increase in GRK2 activity via its direct phosphorylation (see Section 3.4). β1AR desensiti-

| Table 1 | Summary of changes observed in GRK levels and function in patients with different cardiovascular pathologies and in animal heart disease models |
|---|---|---|
| Change in GRK levels | Condition/experimental model | Reference |
| Elevated left ventricular GRK2 mRNA (3 fold) and activity (2 fold). | Human patients with the ischemic and idiopathic dilated forms of cardiomyopathy. | [36] |
| GRK activity increased (3 fold) in lymphocytes from hypertensive subjects, paralleled by an increase in GRK2 protein expression (30–40%). No alterations in GRK-5/6 expression were noted. | Lymphocytes from younger hypertensive subjects as compared with older and younger normotensive subjects. | [38] |
| Augmented GRK5 protein and GRK5 mRNA. No changes in GRK3. | Left ventricle of human dilated cardiomyopathy hearts and in volume overload patients | [13] |
| GRK2 mRNA (3 fold) and activity in the particulate fraction (2 fold) significantly induced. | Acute cardiac ischemia model based on stop-flow and low-flow ischemia in the isolated perfused rat heart. | [37] |
| 3-Fold increase in cytosolic and membrane GRK activity attributed predominately to GRK2 protein upregulation. | Pressure overload cardiac hypertrophy in the mouse was achieved following 7 days of transverse aortic constriction. | [43] |
| Increased GRK activity in the mild early phases of heart failure (no changes in GRK2, but rather in GRK5 were noted). | Pacing-induced congestive heart failure in Yorkshire pigs. | [8] |
| Biochemical abnormalities of heart failure and cardiac dysfunction were preceded by elevated GRK2 expression and activity. Myocardial GRK2 and GRK5 mRNA levels, but not that of GRK3, were induced in the failing hearts, as well as GRK2 (3 fold) and GRK5 (2.6 fold) protein. | Spontaneously hypertensive heart failure (SHHF) rats that develop left ventricular hypertrophy and progress to heart failure. | [9] |
| GRK2 activity and protein elevated only in the hypertrophic failing heart and not in hypertrophic but non-failing cardiac tissue. | Rats subjected to ligation of the left coronary artery or sham-operated. | [18] |
| | Rat model of heart infarction based on surgical occlusion of a coronary artery and tissue damage based on the existence of pulmonary edema. | [10] |

See text for details.
zation might also be promoted through direct receptor phosphorylation by PKA.

Signalling through the PLC/PKC pathway is also activated in both human heart failure and in animal models after pressure overload [6,7,47]. An increased activity of several PKC isoforms (such as PKCα, β, and ε) has been reported in these situations, with complex functional repercussions on cardiac performance. Thus, PKCα, the predominant form expressed in normal and failing hearts [47], plays an important role in heart failure, as does PKCβ, while PKCe seems to have a protective role [48]. As stated above, PKC activity positively modulates GRK2 function and expression in several ways (see Section 3.4). Therefore, it is tempting to suggest that the increase of either several or specific PKC isoforms might trigger changes in GRK levels and activity in heart failure. A better knowledge of the in vivo specificity of the different PKCs towards each GRK is needed, however, to understand their functional interplay.

The activation state of other kinases and signalling pathways, such as PI3K/AKT, c-Src, or MAP kinase cascades, in failing human hearts is controversial [49]. Given the complex modulatory mechanisms that some of these pathways elicit with respect to GRK2 function, expression and degradation (see above), a better knowledge of how they change during the progression of different cardiac diseases is desirable.

5. Functional relevance of GRK2 in heart failure and cardiac hypertrophy

The fact that GRK2 seems to be the main factor involved in diminishing βAR signalling, together with its alteration in a variety of cardiac dysfunctions of different aetiology, led to the concept that upregulation of GRK2 in heart failure was part of the pathological process contributing to the reduction of cardiac output. Therefore, both βARs and GRKs were identified as potential therapeutic targets for restoring cardiac function. Their expression was consequently subjected to genetic manipulation in murine models with the aim of increasing β-adrenergic input (Table 2).

Firstly, GRK2 activity and expression levels were reduced with the kinase for Gq/11 activation, in normal and failing hearts. In fact, overexpression of Gq11 receptors and downstream effectors. The Gβγ binding domain of GRK2, which has been proposed to inhibit endogenous GRK2 by competing with the kinase for Gβγ-mediated membrane translocation [50]. Secondly, cardiac inotropy was modulated by overexpressing β-receptors and downstream effectors. The results, however, point to a more complex involvement of GRK2 function, and of the physiological consequences of chronic β1 activation, in normal and failing hearts. In fact, there is compelling evidence that a sustained increase in β1-AR stimulation induces heart damage [14,26,51]. In line with these findings, β1-AR polymorphic variants which show enhanced coupling to Goα correlate with increased risk of heart failure and more severe disease [52]. Similarly, the overexpression of Goα or of the catalytic PKA subunit promotes severe cardiomyopathy [16].

In this scenario, the induction of mechanisms that specifically help attenuate β1AR signalling, such as GRK2 upregulation, might be considered an initial compensatory process in response to adrenergic overdrive. Consistent with this concept, higher GRK2 levels have been found in transgenic-β1AR animals and in other models

### Table 2

<table>
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<tr>
<th>Mice model</th>
<th>βAR-dependent cardiac function</th>
<th>βAR density</th>
<th>βAR coupling efficiency</th>
<th>Adenylyl cyclase activity</th>
<th>GRK2 levels</th>
<th>Cardiac hypertrophy</th>
<th>Cardiomyopathy/heart failure</th>
<th>Phenotypic rescue by cross-breeding with GRK2ct mice</th>
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The phenotypic features of the different mice models and the effect of GRK2ct expression are indicated. See text for details. n.c., no change; ND, not determined; Tg, transgenic; KO, knock-out; HMC, heavy myosin chain; CQs, calsequestrin; TFG-β1, transforming growth factor; MLP, muscle lim protein; dbh, dopamine β-hydroxylase.
characterised by a detrimental excess of β1AR-dependent signalling [14–16]. However, the chronic maintenance of such a βAR desensitized state in a compromised heart leads to further deterioration. Delivery of an inhibitory GRK2ct construct results in the prevention of cardiac dysfunction and increased survival in several experimental animal settings (see Table 2 and below). Interestingly, such effects are synergistic with those achieved with β-blocker treatments, suggesting that interference with upregulated GRK2 function and a reduction in receptor occupancy cooperate in preventing the development of heart failure.

5.1. GRKct and βAR desensitization-dependent functions

GRK2ct mice show enhanced basal and isoproterenol-stimulated contractility, suggesting that the level of GRK2 activity directly modulates βAR-dependent cardiac responses in vivo. This was confirmed by the increase in isoproterenol-dependent contractility assessed in GRK2 deficient heterozygous mice models, which display reduced GRK2 levels [53]. In contrast, in transgenic GRK2 mice with raised kinase levels, the cardiac contractile response to isoproterenol, as well as β-agonist-dependent adenylyl cyclase activity, were significantly depressed [50]. These findings imply that raised GRK2 levels in human cardiac disease affect myocardial β1AR desensitization and signalling, and contribute to the lack of β1AR responsiveness in heart failure. A criticism of these conclusions is that the manipulation of GRK2 levels in these murine models was performed in myocytes. Under normal conditions, this kinase is only modestly expressed in this cell type compared to other GRKs [18], and the cell type distribution of upregulated GRK2 in failing human hearts is not well known. Clearly, a better knowledge of the role of different GRKs and other kinases (such as PKC and PKA) in β1AR desensitization and their regulation in myocytes during heart failure is needed.

It must be stressed that increased GRK2 protein expression in transgenic GRK2 mice attains levels comparable to those detected in patients with heart failure and in several animal heart failure models. Despite these similarities, GRK2 transgenic mice have no overt cardiomyopathy symptoms, although no long term follow-up of these animals has been reported. Thus, cardiomyocyte GRK2 upregulation per se might not play a causal role in cardiac diseases.

Nevertheless, GRK2ct overexpression exerts a notable protective effect in different transgenic mouse heart failure models with profound β1AR signalling abnormalities (see Table 2), preventing hypertrophy and the progressive deterioration of cardiac function, and improving exercise tolerance and survival. Surprisingly, the ability of GRK2ct to ameliorate heart dysfunction does not always correlate with the restoration of βAR signalling, as observed in severe cardiomyopathy induced by a dominant negative form of CREB [41]. GRKct neither improves cardiac function nor prevents β1AR desensitization in transgenic mice overexpressing Gaq [54], consistent with the fact that GRK2 is not upregulated in this model [44].

Overall, these results indicate that the potential benefits of gene therapy based on GRK2ct delivery would depend on the aetiology of the heart failure, and on the fact that GRK2ct effects are not limited to the modulation of the β1AR/adenylyl cyclase signalling module.

5.2. GRK2ct functions other than βAR desensitization

Modulation of GRK2 activity may also affect the function of other cardiac GPCRs likely to be desensitized by GRK2 and critically involved in the pathophysiology of myocardial hypertrophy and failure (such as angiotensin II receptors) [55]. In addition, the effects of GRK2ct might be explained by mechanisms independent of GPCR desensitization, but related to its ability to bind and sequester Gβγ subunits. Adenoviral delivery of phosducin (a known Gβγ-binding protein) or GRK2ct to failing rabbits hearts [56] has similar effects on the prevention of cardiac damage. The Gaq pathway is critical in triggering heart failure in response to pressure overload [57] and PLCβ2 is an important downstream effector of Goq, which activity is also modulated by Gβγ subunits and, interestingly, depressed by the expression of phosducin or GRK2ct [56]. Therefore, it is tempting to suggest that the improvement observed in mice expressing GRK2ct might be mediated not only by β1AR desensitisation”, but also by decreased PLCβ activity.

Other Gβγ-dependent processes important in cardiac function might also be modulated by GRK2ct. The importance of the PI3K/PTEN pathway in the development and maintenance of cardiac dysfunction has clearly been established [58]. Cardiac PI3K activity is associated with GRK2 protein [59], and a role for the GRK2/PI3K interface in the proper internalisation and downregulation of the βARs and their implication in the subsequent development of heart failure has been reported [60]. βAR downregulation induced by pressure overload is accompanied by an increase in Gβγ-dependent PI3K activity. Further evidence of PI3K contribution to cardiac dysfunction is seen in that the expression of an inactive p110 catalytic subunit prevents βAR desensitization and heart damage after pressure overload [61]. Moreover, a role for GRK2 in the Gβγ-dependent membrane translocation of cardiac PI3K has been reported [60] and the disruption of the GRK2/PI3K interaction prevents βAR downregulation. Thus, it is possible that the cardioprotective effect of inactive p110 kinase could at least in part be due to the displacement of endogenous PI3K from GRK2.

Recently, a new player has been described in the PI3K modulation of the βAR signalling pathway [62]. PI3K associates with and activates a cAMP-degrading enzyme, PDE3B, in a catalytic-independent manner [62]. Therefore, the PI3K upregulation observed in patients with heart failure...
could constitutively reduce cAMP levels, thereby contributing to the deterioration of catecholamine-dependent contractile function.

5.3. GRK2 and myocardial hypertrophy

Myocardial hypertrophy can develop independently of GRK2 levels/activity in several experimental settings involving the activation of the Gq/MEKK1/JNK pathway [43,44,54]. Although GRK2ct expression does not reverse hypertrophy in every heart failure model, it is interesting to note that it does so in those murine models in which it also efficiently blocks GRK2 upregulation (see Table 2). It is thus tempting to suggest that GRK2 upregulation may play some undefined role in physiological or stress-induced hypertrophy. Interestingly, transgenic mice overexpressing an amino-terminal region of GRK2 that interacts with Gq (β-ARKnt, residues 50–145) develop hypertrophy and show higher βAR densities than do control animals [63]. This GRK2 region may act by blocking Gq signalling, although the β-ARKnt construct used does not comprise the complete RGS domain that may be necessary for affecting Gq function. However, it is still unknown how β-ARKnt alters the receptor complement without affecting βAR signalling, or why there is no evidence of physiopathological consequences despite the clear myocardial hypertrophy. It is also possible that β-ARKnt expression might disrupt endogenous GRK2/receptor interactions, or that it acts as a scavenger of relevant endocytic molecules, thereby impairing normal receptor downregulation and favouring cardiac hypertrophy [63]. More experiments are needed to fully understand the functional roles of the different GRK2 domains, and to determine how such functions are altered when the full-length protein is upregulated in pathological situations.

5.4. Novel functions for GRKs

A number of laboratories have recently established that GRKs interact with and phosphorylate proteins other than the GPCRs, broadening the variety of their cellular functions (Fig. 2). In addition to the regions involved in Gqg and Gβγ binding, GRK2 contains several other protein–protein interaction domains. This accounts for its direct interaction with other molecules involved in signalling, such as the regulator of focal adhesions GIT1 or the endocytic molecule clathrin [2,3,11,24]. The significance of these functional interactions is only partially known, but they add to the potential of GRK2 to control the activity, location, and stability of the proteins that interact with it in a phosphorylation-independent manner.

GRK2 can also phosphorylate non-receptor substrates such as phosducins, synucleins, tubulin and ribosomal factor P2 (reviewed in [3,11]) with different functional effects. The phosphorylation of phosducins by GRK2 markedly reduces their interaction with Gβγ, thus facilitat-

Fig. 2. Possible effects of altered GRK2 expression levels on signalling pathways related to cardiac function.
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rearrangements of the actin cytoskeleton and modulate microtubule polymerisation or tubulin binding to microtubule-associated proteins, respectively. Recently, βγ-dependent modification of ezrin by GRK2 has been described as critical for both GPCR-dependent membrane clustering as well as receptor internalisation [65]. The phosphorylation of ribosomal factor P2 by GRK2 might favour an increase in protein synthesis. In this regard, GRK5 has been found in the nucleus upon GPCR activation [66], suggesting new potential nuclear functions and targets for this kinase. Overall, these results suggest that, following GPCR activation, GRKs may have an “effector” function by phosphorylating non-receptor substrates. GRK2 also phosphorylates membrane proteins such as PDGF receptors [5] (thereby promoting its desensitisation) and epithelial Na+ channels [67] (which are required for blood pressure regulation). Phosphorylated Na+ channels are resistant to ubiquitination and endocytosis, and this might underlie the known association between increased GRK2 and essential hypertension in humans [38]. Although the potential impact of such GRK2 substrates on cardiac pathophysiology is unknown, it is tempting to suggest that they could play a role in heart failure by impairing the balance of protein synthesis (hypertrophy) and receptor endocytosis (downregulation) as a result of aberrant GRK2 levels and function (Fig. 2).

6. Conclusions

The functional complexity of the proteins with which GRKs interact (the GRK “interactome”) shows these kinases are central to a number of different signalling pathways. This suggests novel physiological implications for GRKs in different cell types, including those of the cardiovascular system. The important role of GRK2 and other GRKs in the modulation and transduction of GPCR signalling, and perhaps of other receptors with tyrosine kinase activity, suggests that changes in GRK protein levels or activity might affect the efficiency or characteristics of signal transduction routes and, therefore, may be of considerable pathophysiological importance.

However, the important question of whether increased GRK2 expression is a precipitating factor in congestive heart failure or a biochemical consequence of the remodelling that occurs in this condition still remains to be answered. The observation that GRK2 levels are increased only in the failing heart and not in hypertrophic non-failing cardiac tissue may be an interesting clue [68]. An initial event might trigger different regulation of GRK2 expression in different individuals. Those who respond with a low GRK2 expression are less prone to heart failure; those who respond with an increased GRK2 expression are destined to develop heart failure with time.

Alterations in the levels and/or functionality of GRKs in pathological circumstances are the consequence of an imbalance in their normal synthesis, degradation and control. A more detailed knowledge of the specific regulation of such processes in the different types of cardiac cells is needed. On the other hand, the functional consequences of GRKs alterations should be investigated in the context of their increasingly complex “interactome”, including Gαq, PI3K and non-GPCR substrates. Such knowledge, combined with the use of animal heart failure models in which the functional effects of modulating GRK activity can be assessed in vivo, would help answer whether changes in GRK levels are a normal consequence or a precipitating factor in heart failure, and whether raised GRK levels are beneficial or detrimental (or indeed whether this depends on the stage of the illness). An improved understanding could translate into novel diagnostic procedures using GRKs as potential markers, or new therapeutic strategies based on the modulation of their activity, levels or specific interactions.

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