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G Protein-Coupled Receptor Kinases and Hypertension

A Review of Disease Mechanisms

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Abstract

The understanding of molecular mechanisms of complex physiological phenomena, such as cardiac contractile function or blood pressure homeostasis, has generated the need for a new generation of scientists who must be able to conjugate intrinsic biological mechanisms and clinical manifestations of disease. From this body of knowledge new strategies of disease management or therapeutic tools are derived, creating the ground for translational medicine, which provides the bridge from basic science to the medical arena. The investigation of G protein-coupled receptor kinases in the cardiovascular system is one example of the successful transposition of basic science to the field of heart and vascular disorders.

This review attempts to assemble the currently available information in this continuing area of research for the class of scientists now referred to as 'translational researchers'.

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1. Classification of G Protein-Coupled Receptor Kinases (GRKs)

Guanine nucleotide binding protein (G protein)-coupled receptor (GPCR) kinases (GRKs) are a family of seven serine/threonine protein kinases (GRK1-7), each encoded by a single gene, that specifically phosphorylate the activated form of GPCRs, playing a primary role in mediating their desensitisation.[1,2] These seven transmembrane-spanning proteins form the largest known and most versatile family of integral membrane receptors.[3] GPCRs are involved in a multitude of signalling processes, which include neurotransmission, chemiotaxis, perception of light, smell and taste, and are stimulated by a wide range of ligands, such as hormones, lipids, chemokines, prostaglandins, leukotrienes and inflammatory mediators.[4,5]

Based on sequence and functional homology, GRKs, named numerically according to the chronological order of discovery, have been divided into three main subfamilies: (i) the rhodopsin kinase family (size: 62–63 kDa), which is comprised of GRK1[6] and GRK7,[7] and is responsible for phosphorylating rhodopsin, the prototypic 'light receptor', in retinal cells; (ii) the β-adrenergic

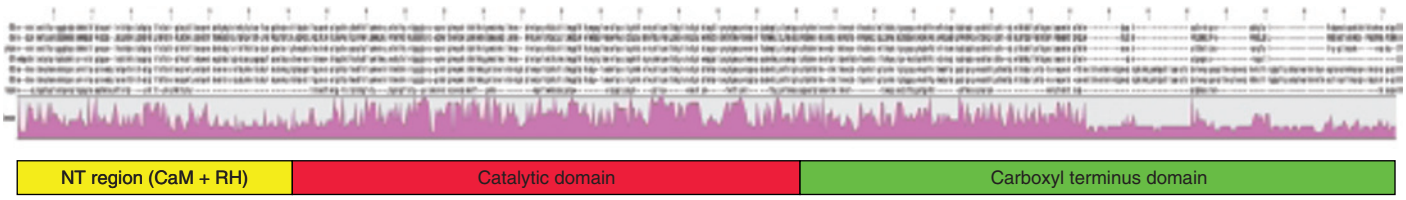

Fig. 1. Homology in the sequences of the seven members of the G protein-coupled receptor kinases (GRK) family (in pink). Each member contains an amino terminal (NT) domain, which is fairly conserved among GRKs, and contains at least one calmodulin-binding domain and a regulator of G protein signalling homology domain. The most conserved region is the catalytic domain, which is located in the middle of the protein sequence. The carboxyl terminus domain is the most different domain between GRKs and the domain that confers subcellular localisation to the kinase. GRK2 and GRK3 are localised in the cytosol, and GRK1 and GRK4 to GRK7 are localised in the membranes. Rhodopsin and GRK7 are only expressed in the retina and are kinases to the photon receptors.

receptor (βAR) kinase (βARK) family (size: 79–80 kDa), which is comprised of GRK2 and GRK3 (originally known as βARK1 and βARK2, respectively); and (iii) the GRK4 family (size: 66–68 kDa), which is comprised of GRK4 (four splice variants: α, β, γ, δ),[8] GRK5 and GRK6 (three splice variants: A, B, C)[9,10] [figure 1]. These GRKs are expressed in a quite wide variety of tissues (table I), with a more restricted expression showed by GRK4 (in testis),[8,11] GRK1 and GRK7 (in rod and cone photoreceptors in the retina).[12]

Structurally, GRKs share a similar tridomain functional organisation[13] with a central catalytic domain, which mediates serine/threonine phosphorylation of target proteins and contains the adenosine triphosphate-binding site, flanked by variable amino N-terminal and carboxyl C-terminal (C) domains. The N-terminal domain of the GRKs is 183–188 amino acids in length and shares a certain degree of conservation as this region has been proposed to be involved in substrate recognition.[14] Moreover, within the N-terminal domain lies a regulator of G protein signalling (RGS)-like domain or RGS-homology (RH) domain that appears to have important regulatory functions. Indeed, the crystal structure of the RH domain of GRK2 can support binding to the α-subunit of the G protein Gq;[15] however, the recent elucidation of the structure of GRK6 shows that this RH domain would probably not support this interaction,[16] and there are distinct differences between the structure of these two GRKs that support the subfamily classification mentioned earlier.

Table I. Tissue localisation of G protein-coupled receptor kinases (GRKs) in mammals

Name	Size (kDa)	Main localisation
GRK1	63	Retina (rod outer segment), pineal gland
GRK2	79	Leukocytes, cerebral cortex, heart
GRK3	80	Olfactory tubercle, brain, spleen
GRK4	66	Testes, brain
GRK5	68	Heart, lung, skeletal muscle, liver
GRK6	66	Brain, skeletal muscle, heart
GRK7	62	Retina (cones)

The C-terminus of the GRKs is highly variable[17] and represents the site of several post-translational modifications that contribute to the regulation of protein-protein interactions and to the specific targeting of these kinases.[18] GRK1 and GRK7 are farnesylated at the cysteine residue of a CAAX motif (where C is cysteine, A is a small aliphatic residue and X is an uncharged amino acid); GRK4 and GRK6[19] are palmitoylated at cysteine residues.[20] The C-terminal domains of GRK2 and GRK3 do not undergo post-translational lipid modifications but contain a βγ-subunit binding domain[15,20,21] that exhibits a sequence similar to a pleckstrin homology domain.[20,22,23] The GRK5 carboxyl-terminal domain contains a stretch of highly basic amino acid residues (547–560) that mediate electrostatic interactions with plasma membrane phospholipids.[24,25]

2. Regulation of GRKs

Continuous exposure to a stimulus promotes GPCR pathways of desensitisation, which serve to decrease receptor signalling (figure 2). The current model of GPCR desensitisation is characterised by events that contribute to the uncoupling of heptaelical receptors from their heterotrimeric G protein through means of two different mechanisms.[26] Heterologous (or non-agonist-specific) desensitisation is mediated by the second messenger-stimulated protein kinases: cyclic adenosine monophosphate-dependent protein kinase A (PKA), activated by G_s-coupled receptors, and protein kinase C (PKC), activated by G_q-coupled receptors.[27] GPCR phosphorylation by these kinases is followed by internalisation via a caveolae pathway. Homologous (or agonist-specific) desensitisation consists of a two-step process in which the agonist-occupied GPCRs are phosphorylated in serine and threonine residues within the intracellular loop and C-terminal tail domains by a GRK and then recruit and bind stoichiometrically an arrestin protein, visual (1 and 4 or X) or non-visual (2 and 3, most often known as β arrestins 1 and 2),[28] which sterically

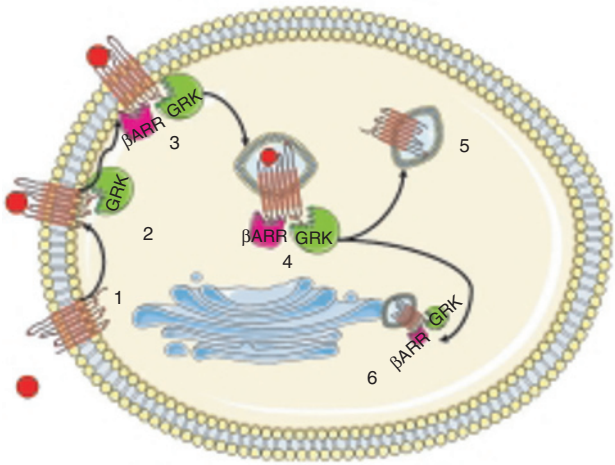

Fig. 2. Schematic regulation of receptor desensitisation. (**1**) Agonist released in the intercellular space engage the extracellular portion of the G protein-coupled receptor (GPCR), starting cell signalling; (**2**) the very same interaction causes a change in receptor conformation that allows recognition by the GPCR kinase. (**3**) Following receptor phosphorylation, the βArrestin (βARR) complexed to the GPCR is associated with a multi-component complex that has been involved in a series of intracellular events. (**4**) In particular, βARRs start the aggregation of clathrin-coated pits, which, by invaginating in clathrin-coated vescicolae, remove the receptor from the cell membrane. The fate of this receptor is now decided through means of processes that involve receptor ubiquitination or intravesicular acidification. (**5**) Receptors can be either recycled to the membrane or (**6**) sent for degradation.

interrupts signalling to the G protein. GRK phosphorylation of a GPCR directs receptor internalisation through a clathrin-coated pit pathway. GRK function is a tightly regulated process and this could explain how only seven proteins (identified to date in mammalian cells) are able to regulate the responsiveness of numerous GPCRs in a coordinated manner.

There are many molecules capable of interacting with GRKs for the purpose of targeting them to substrate or modulate their catalytic activity: GPCRs, which serve not only as substrate but also as GRK activators,[29] second messenger-regulated kinases (PKA,[30] PKC[31,32]), βγ subunits of heterotrimeric guanosine triphosphate (GTP)-binding proteins,[33,34] lipids such as phosphatidylinositol-4,5-bisphosphate (PIP_2) or phosphatidylserine,[25,35] actin and α-actinin,[36] extracellular signal regulated kinases (ERK1/2),[37,38] soluble tyrosine-kinase Src,[39] the Raf kinase inhibitor,[40] and calcium-binding proteins such as calmodulin[27,41] and recoverin.[42] Thus, different mechanisms modulate the activity and subcellular targeting of GRKs. Moreover, their activity appears to be regulated by a complex series of protein phosphorylation events. This point could provide the basis for the potential pleiotropic roles of the GRKs and their involvement in several human diseases.

3. GRKs in Hypertension

The most important hallmark of essential hypertension is the increase in peripheral resistance, which can be due to both structural and functional mechanisms. Overall vascular tone is regulated by the adrenergic system, and an important role has been reported for the β_2-adrenergic receptor (β_2AR), which leads to a vasodilator response.[43] Impaired vascular β_2AR responsiveness is thought to play an important role in the development of hypertension. Indeed, an impairment in β_2AR-mediated vasodilatation has been described in both human and animals models of hypertension.[44-46] This impairment may be due to an alteration in GRKs and their activity on βARs as well as other important GPCRs regulating vascular tone. In particular, much attention has been focused on GRK2, which is highly expressed throughout the cardiovascular system. Of note, in 1997 Gros et al.[47] first reported that GRK activity is selectively increased in lymphocytes from younger hypertensive subjects. Furthermore, they then identified lymphocyte GRK2 protein as being increased in hypertension.[48] This increase in GRK2 and GRK activity may underlie the reduction in β-adrenergic responsiveness that is characteristic of the hypertensive state.

One major issue that was important to document was to demonstrate that increased lymphocyte GRK2 is relevant to the increase in blood pressure. To address this issue, Gros and colleagues[49] analysed the parallelism of GRK2 expression in vascular smooth muscle (VSM) cells and lymphocytes in a genetically determined model of hypertension, the spontaneously hypertensive (SHR) rat. Importantly, they found that the increase in lymphocyte GRK2 mirrors the increased levels observed in VSM cells of SHR rats. Of interest, increases in VSM GRK5 levels, a second GRK highly expressed throughout the cardiovascular system, has been found in a rat model of hypertension induced by long-term angiotensin II infusion.[50]

It remains to be determined conclusively that increases in GRK2/GRK5 and vascular GRK activity cause hypertension. However, an important study by Eckhart et al.[51] has documented in transgenic mice that VSM-targeted overexpression of GRK2 alone can cause a hypertensive phenotype. Their study showed that vascular GRK2 overexpression caused a significant elevation of resting blood pressure that was accompanied by vascular thickening and cardiac hypertrophy, complications often observed in human hypertension.[51] Interestingly, this group also showed that VSM-targeted transgenic overexpression of GRK5 leads to hypertension in the mouse; however, this phenotype differed from GRK2-induced hypertension as there was no vascular or cardiac hypertrophy.[52]

Regarding the relevance of GRK2 in hypertension, an important step in proving a key role in pathogenesis is to demonstrate that reducing GRK2 activity can reduce the alterations observed in the vasculature of hypertensive patients. This observation has been produced indirectly in another model of impaired βAR vasorelaxation, the aged rat. In this model, long-term exercise leads to reduction in the GRK activity of the vasculature, in particular in the endothelium, and this reduction parallels an ameliorated vasodilatation.[53] The relevance of these observations to the human condition is still to be clarified and better understood.

Another important theory about hypertension pathogenesis targets functional kidney alteration as a principal mechanism that leads to an impairment in hydroelectrolytic balance.[54] The renal dopaminergic system exerts a pivotal role in maintaining fluid and electrolyte balance. Dopamine has an antihypertensive function because it is both vasodilatory and natriuretic.[55] Dopamine via D_1-like receptors is responsible for >50% of incremental sodium excretion when sodium intake is increased. This receptor is a GPCR and its function is regulated by GRKs. Some studies have demonstrated that basal GRK activity and serine phosphorylation of the D_1 receptor in renal proximal tubule cells is higher in hypertensive subjects than in normotensive subjects.[56] In the same study, the authors evidenced messenger RNA (mRNA) of all of the reported GRK4 isoforms (α, β, γ, δ) in renal proximal tubules. Previous work has showed that GRK4 locus is linked with essential hypertension.[57] Felder et al.[56] have demonstrated an association between a single nucleotide polymorphism (SNP) of GRK4γ and human essential hypertension. GRK4γ-isoform derives from an alternative splicing of GRK4.[8] The hypothesis is that the SNP (A142V) of GRK4-γ produces a higher activity of GRK with stronger receptor desensitisation and a reduced sodium excretion.

Indeed, the overexpression of the GRK4γ-A142V in transgenic mice gives a hypertensive phenotype compared with mouse overexpressing the wild-type GRK4γ.[56] Similarly, Speirs et al.[58] found an association with hypertension for a GRK4γ-SNP A486V but not for A142V. Altogether, this evidence suggests an important role of GRK4 in the kidney handling of sodium and volume, leading to the development of hypertension.

Finally, a recent publication has highlighted the possible role of GRK2 in the regulation of a key molecule for endothelial regulation of vascular tone, endothelial nitric oxide synthase (eNOS).[59] In a model of portal hypertension GRK2 is increased in the endothelium, and this increase results in the inhibition of eNOS. Interestingly, the regulation of eNOS appears to be not related to the catalytic activity of the GRK but rather to a physical interaction of the two molecules.[59] This interesting observation opens the field of the investigation to the ability to regulate cellular function through means of non-phosphorylation events by the GRKs. Given the large heterogeneity between the kinases in the non-catalytic domain, it is possible that more specialised features will be revealed for individual kinases.

4. Hypertension and Cardiac Hypertrophy

High blood pressure causes a cardiac overload that leads to left ventricular (LV) hypertrophy. Moreover, it is also one of the main risk factors for the development of heart failure (HF). The role of GRKs in this process is not fully understood; however, they appear to be involved and important. As mentioned previously, VSM-GRK2-induced hypertension in mice is accompanied by cardiac hypertrophy,[51] whereas increased blood pressure induced by VSM-GRK5 overexpression does not have a cardiac hypertrophy component.[52]

Of interest, GRK activity has also been investigated in cardiac pressure overload in both humans and in mouse models. The strongest data to date actually involve uncovering the importance of GRK2 in cardiac hypertrophy based on the actions of a GRK2 inhibitor, the C-terminus of β-adrenergic receptor kinase (βARKct). The βARKct is a peptide comprised of the last 195 amino acids of GRK2 and it competes for $G_{\beta\gamma}$-mediated translocation and activation of endogenous GRK2, thus limiting GPCR desensitisation.[60] We have shown previously that mouse hearts harbouring transgenic expression of the βARKct preserves βAR responsiveness in the hypertrophied heart when mice are exposed to thoracic aortic constriction (TAC) and LV pressure overload.[61,62] More recently, the βARKct was found to have a dose-dependency in preserving long-term cardiac function and limiting HF in a chronic TAC model.[63]

Outside of mouse models, the role of cardiac GRK2 has been investigated in the spontaneously hypertensive heart failure (SHHF) rat.[64,65] This model is characterised by an impairment of βAR signalling with decreased receptor density and coupling to adenylyl cyclase as well as increased GRK2 levels and activity.[64,65] In fact, increased myocardial GRK2 is an early abnormality preceding maladaptive hypertrophy and failure in this model, suggesting that this abnormality may be critical for the pathogenesis of disease.[64,65] Importantly, the contractile dysfunction seen in ventricular myocytes taken from late-stage SHHF rats was reversed with adenoviral-mediated βARKct addition.[65]

Park et al.[66] have shown that the increased GRK2 expression and GRK activity in lymphocytes does appear to parallel associated human cardiac hypertrophy in patients with hypertension. However, the relationship between lymphocyte GRK2 levels and alterations in the cardiac β-adrenergic system in hypertension requires further study. Although it has not been unequivocally established

whether expression of GRK2 in lymphocytes is related to that in the heart, both may change comparably in certain conditions, such as hypertension and HF through activation of the sympathetic nervous system.[67]

5. GRKs and Heart Failure

The original observation showing increased GRK2 expression and GRK activity in the failing human heart[68] opened the field of the investigation of GRKs and cardiac dysfunction. These alterations in GRK2 appear to contribute to pathogenesis of HF[69] and certainly contribute to the loss of βAR responsiveness in HF. GRK2 may also be the trigger for the loss in βAR density in HF.[69] In HF, β_1AR mRNA and receptor numbers are decreased by 50%, whereas these levels are unaltered for β_2ARs.[70] A possible triggering mechanism for the increase in GRK expression and activity in HF is thought to be enhanced sympathetic nervous system activity,[67] which has been demonstrated in HF.[71] Along these lines, we have shown previously that long-term stimulation of mice with the βAR agonist isoproterenol produces an increase in cardiac GRK2 activity with a reduction in β-adrenergic signalling, whereas long-term treatment with a β-blocker (β-adrenoceptor antagonist) leads to a selective reduction in GRK2 levels with a rescue in β-adrenergic function.[67]

To substantiate the pivotal role of the sympathetic nervous system we also showed that experimentally increasing sympathetic activity, through a severe dietary salt deprivation in rats, could cause the up-regulation of GRK2 in the heart.[72] Salt deprivation during concurrent administration of the β_1AR blocker atenolol did not result in GRK2 upregulation.[72]

Elevated levels of GRK2 in HF have been observed in several animal models and appeared to be critical.[69] One such model is a mouse model of dilated cardiomyopathy caused by the ablation of the gene for the muscle LIM domain protein (MLP). MLP knockout mice have increased myocardial GRK2 expression and activity[73] that is intimately involved in the disease since these mice were rescued by cardiac expression of the βARKct.[73] It is important to point out that there have been other models of HF that do not have associated increases in cardiac GRK2 levels, although this is a minority of reports. One noted in particular is mice with HF due to cardiac transgenic overexpression of Gαq.[74] These mice develop a severe cardiac hypertrophy and eventually HF. They do not present with increased cardiac GRK2, although the myocardial βAR system is dysfunctional.[74] Interestingly, cardiac GRK2 in these animals is downregulated.[74] This somewhat variable GRK2 association in animal models of HF suggest that a similar gradient and diversity of GRK2 upregulation may occur in humans.

However, it is important to point out that in humans there have been a number of reports to date documenting an association between cardiac dysfunction and increases in myocardial GRK2. Ungerer et al.[68] originally demonstrated that GRK2 mRNA levels and protein were increased almost 3-fold in both forms of HF in patients with dilated cardiomyopathy or ischaemic cardiomyopathy, and GRK2 activity was similarly enhanced. More recently, Dzimiri et al.[75] demonstrated that GRK2, 3 and 5 in lymphocytes of patients with LV volume overload were increased not only at the mRNA level but also in protein. Moreover, the elevations in the three GRK were associated with an increase in β-arrestin2 mRNA.[75] This group further demonstrated in myocardial biopsies obtained at the time of surgery, a different pattern of expression of GRK2 and GRK5 in human myocardium during LV volume overload, suggesting a different functional role of GRKs in heart disease.[76] In this study, elevation in GRK2 was manifested globally in the heart, whereas GRK5 changes appeared to be localised primarily in the LV.[76] The changes in GRK mRNA and protein levels were accompanied by a global reduction in myocardial β_1AR expression as well as receptor-mediated adenylyl cyclase activity.[76] The global nature of the increase in myocardial GRK2 expression correlates more closely with the decrease in myocardial β_1AR than the increase in GRK5. This evidence points to a greater role for the GRK2 in the alterations in β_1AR signalling in cardiac muscle disease.

Another study, which increases the complexity of the cardiac GRK expression profile, reported that GRK2 changes in right atrial appendages obtained from HF patients with coronary artery disease undergoing coronary artery bypass grafting had only transient increases in GRK2 levels.[77] Right atrial GRK activity was elevated in early (New York Heart Association [NYHA] I and II) HF stages but reduced to nearly control values in late (severe) HF (NYHA III and IV).[77] We do not know the reason for the transient increase of GRK activity in the failing human heart and, therefore, can only speculate. One possibility is that locally increased catecholamines activate GRK via βAR stimulation and simultaneously initiate the βAR desensitisation process in early HF. In advanced HF βARs are markedly desensitised and now the increase in catecholamines is not sufficient to stimulate the desensitised βAR and regulation of GRK2 levels are lost. Alternately, right atrial levels do not mirror what is found in the LV as previous studies, including ours, have shown that in end-stage HF, there is increased levels of GRK2.[68,78]

The intriguing result that GRK2 upregulation in the heart appears to be also found in lymphocytes of HF patients raises the possibility that blood GRK2 may be used as a novel biomarker in HF. Before this can occur, proof of an association between cardiac and lymphocyte levels is needed in patients with HF. We have

recently shown in different HF groups that cardiac levels are mirrored in lymphocytes.[79,80] Moreover, GRK2 expression levels and activity in lymphocytes is associated with the loss of βAR responsiveness and higher GRK2 levels are associated with more severe HF.[79] Consistent with this, lower levels of GRK2 in the heart and blood appear to be associated with improved βAR signalling and function, since samples (blood and LV biopsies) taken before and after a period of cardiac unloading with LV assist devices (LVADs) showed that LVADs induce a significant decrease in GRK2 levels.[80] Importantly, LVAD use is associated with reverse remodelling of the heart and a normalisation of cardiac structure and function, and it appears that GRK2 lowering is part of this process.[80] Therefore, lymphocytes may provide a surrogate for monitoring cardiac GRK2 in human HF, and possible measuring of GRK2 in the blood of HF patients may become a surrogate marker to monitor changes in LV function and, importantly, may monitor response to therapy.

6. Conclusions

Increases in GRK expression appear to be an early event in the natural history of hypertension, which is probably initially localised to the vasculature. In a later phase, GRK expression and activity increases in the cardiac myocyte, associated with pressure overload hypertrophy caused by chronic elevations in blood pressure. This enhanced GRK activity in the myocardium, which appears to be primarily due to GRK2 upregulation, participates in and causes impairment of the βAR response and the evolution of LV hypertrophy and eventual HF. Once HF is clinically manifested, increased GRK levels can be used as a molecular prognostic marker to be followed in peripheral blood lymphocytes. Experimental data suggest that treatment can indeed reduce GRK2 expression, in particular by β-blockers and mechanical unloading.

It remains to be determined whether GRKs can become a large-scale tool for cardiovascular disease management either as therapeutic targets or biomarkers. However, the pathophysiological implications of GRKs suggest that they could well be a therapeutic target for hypertension and HF. New classes of small-molecular GRK inhibitors, which are currently in the pharmaceutical pipeline and will be available in the near future, will shed light on this issue. In addition, and alternatively, strategies for gene therapy-targeted GRKs are being pursued.

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