The role of G protein coupled receptor kinases in neurocardiovascular pathophysiology

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Abstract

In coronary artery disease the G protein related kinases (GRKs) play a role in desensitization of β-adrenoreceptors (AR) after coronary occlusion. Targeted deletion and lowering of cardiac myocyte GRK-2 decreases the risk of post-ischemic heart failure (HF). Studies carried out in humans confirm the role of GRK-2 as a marker for the progression of HF after myocardial infarction (MI). The level of GRK-2 could be an indicator of β-AR blocker efficacy in patients with acute coronary syndrome. Elevated levels of GRK-2 are an early ubiquitous consequence of myocardial injury. In hypertension an increased level of GRK-2 was reported in both animal models and human studies. The role of GRKs in vagally mediated disorders such as vasovagal syncope and atrial fibrillation remains controversial. The role of GRKs in the pathogenesis of neurocardiological diseases provides an insight into the molecular pathogenesis process, opens potential therapeutic options and suggests new directions for scientific research.

Key words: autonomic, sympathetic, vagal, molecular signaling pathway.

Neurocardiovascular pathophysiology: sympathetic versus vagally mediated disorders

Neural control of the cardiovascular (CV) system is an integral part of CV physiology and consequently a part of CV pathological mechanisms. Two fundamental parts of the autonomic nervous system – the sympathetic and parasympathetic (vagal) components – play a role in the development and initiation of the pathological process. The classification of neurocardiological disorders by Goldstein [1] is as follows: 1) Sympathetic disorders – diseases in which activation of the catecholamine system worsens an independent pathological state (coronary artery disease, arrhythmias as long QT syndrome, sudden death, heart failure (HF)) as well as diseases in which abnormal catecholaminergic function is etiologic (sympathetic neurocirculatory failure, hypertension, cardiac necrosis and cardiomyopathy), and 2) Vagally mediated disorders – both neurally mediated syncope (vasovagal syncope, carotid sinus hypersensitivity) and vagally mediated atrial fibrillation. Neural regulation of the CV system can be studied by using different techniques [2-8]. G protein related kinases (GRKs) exist in 7 isoforms – GRK 1-7. They are serine-threonine protein kinases that are

ubiquitously expressed [9]. G protein related kinases are highly selective, mostly cytosolic proteins which phosphorylate G protein coupled receptors (GPCRs) [10]. GPCRs phosphorylation desensitizes these receptors [10]. G protein related kinases modulate both sympathetic [11] and vagal molecular signaling pathways [12]. Advances in both animal and human studies may lead to novel therapeutic approaches, especially through inhibition of GRKs [10].

In this narrative review we consider data from recent studies which focus on GRKs as a potential novel neurocardiovascular approach to various conditions, such as coronary artery disease (CAD).

G protein related kinases – general overview

The molecular signaling pathway of the sympathetic nervous system also involves GPCRs, α (α1-α2) and β (β1-β3) [13]. More than half of the drugs currently in clinical use target GPCR by either mimicking endogenous GPCR ligands, blocking ligand access to the receptor or by modulating ligand production [14], which also represents the mechanism of action of sympatholytic and sympathomimetic drugs. Agonist-dependent desensitization of GPCR is caused by the phosphorylation of the specific receptor by the GRK family [15-18]. GRKs have a central catalytic domain flanked by amino-terminal and carboxyl-terminal domains which contain specific regulatory sites (Figure 1) [10].

In the heart, GRK-2 is the most abundant isoform [19]. After ligand binding to the specific receptor and dissociation of G protein to G_s and $G_{\beta\gamma}$ subunits, G_s stimulates adenyl cyclase (AC), leading to cAMP synthesis and subsequent cAMP-dependent kinase (PKA) activation, while GRK-2 interacts with

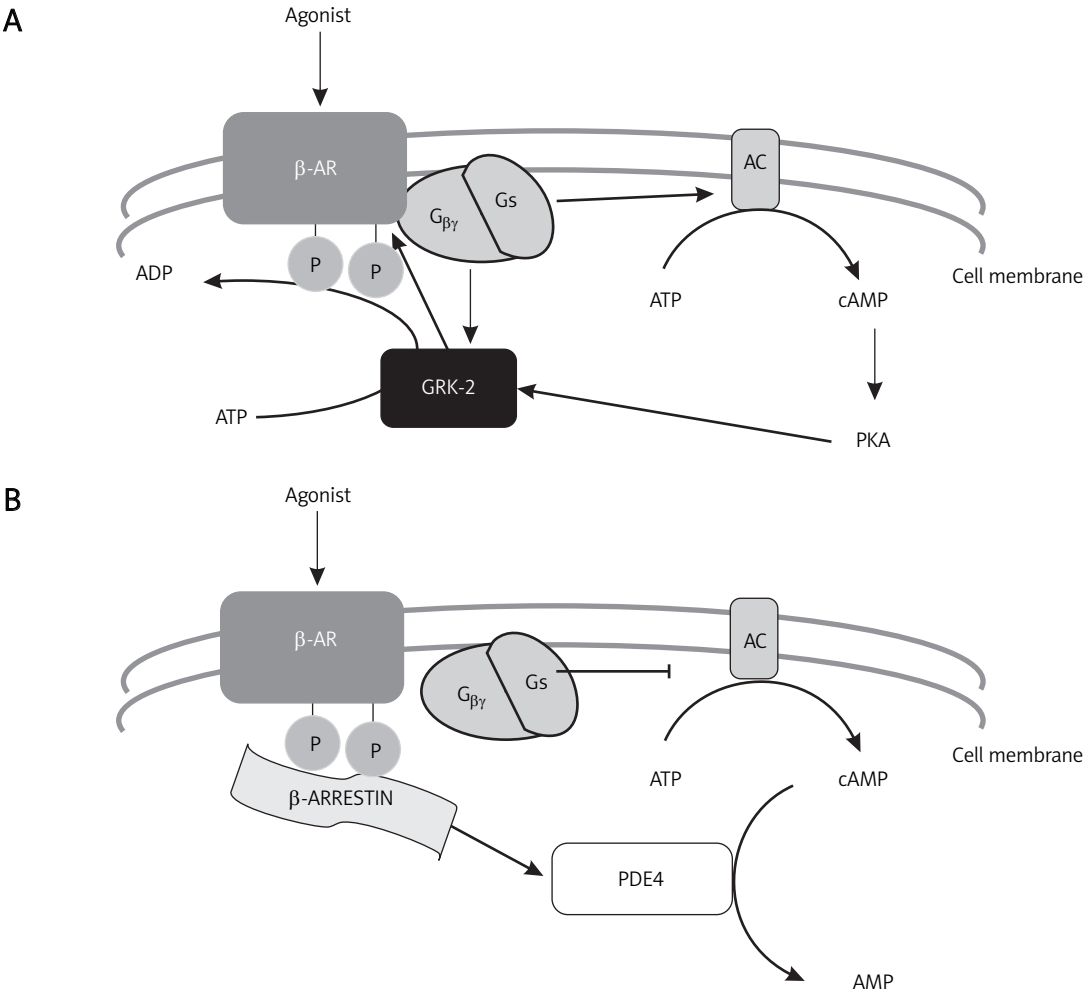

Figure 1. A schematic representation of events after agonist binding to β-AR. **A** – Agonist binding to β-AR and AC activation *via* G protein signaling, leading to PKA activation and GRK-2 mediated β-AR phosphorylation causing receptor desensitization. **B** – Subsequent recruitment of β-arrestin to the receptor and interactions with the signaling protein PDE4 resulting in the attenuation of receptor desensitization and internalization
β-AR – β-adrenoceptor, Gs – stimulatory G protein α subunit, $G_{\beta\gamma}$ – G protein βγ subunit, AC – adenylate cyclase, ATP – adenosine triphosphate, ADP – adenosine diphosphate, AMP – adenosine monophosphate, cAMP – cyclic adenosine monophosphate, PKA – cAMP dependent kinase, GRK-2 – GPCR kinase 2, PDE4 – phosphodiesterase-4

the $G_{\beta\gamma}$ subunit, and translocates to the plasma membrane where GRK-2 phosphorylates the intracellular domain of GPCR (Figure 1 A) [20]. Furthermore, PKA beside other cell functions also phosphorylates serine and threonine residues on GRK-2 which enhances the association of GRK-2 with a β-AR (Figure 1 A) [21]. The phosphorylation of GPCR enhances the affinity of the β-AR for binding to adapter proteins such as β-arrestin (Figure 1 B). In addition, β-arrestin sterically prevents G protein binding to the β-AR and further transduction of signaling [19]. Also, β-arrestin recruits PDE4, which attenuates local cAMP levels (Figure 1 B) and consequently the ability of PKA to further phosphorylate GRK-2, resulting in the attenuation of receptor desensitization and internalization [22]. It is important to note that GRK-2 participates not only in desensitization of β-AR, but also in desensitization of other GPCRs in the cell, such are the adrenergic and muscarinic receptor family [23].

Role of G protein related kinase in coronary artery disease – evidence from animal studies

Neurohormonal activation occurs early in the progression to HF, as reflected by increased catecholamine levels and adrenergic drive immediately after myocardial infarction (MI) and before progression to end-stage HF [24]. Therefore, sympathetic nervous activity has been investigated as a possible early trigger for increasing GRK activity in the failing myocardium. Excessive catecholamine stimulation modulates β-AR signaling and damps sympathetic signaling [24]. This is considered to be an adaptive mechanism to sympathetic overstimulation. With the progression of HF, chronic exposure to increased levels of catecholamines results in pathologic desensitization of β-AR and their down-regulation [23].

Mice have been engineered to overexpress myocardial GRK-2 [25], GRK-3 [26] and GRK-5 [27]. Left ventricular function in response to a β-AR agonist was significantly decreased [25]. This was the first time that this GRK was shown to functionally uncouple β-ARs in the heart [23, 25]. In the same study, the effect of low level of GRK-2 was investigated by targeting GRK-2 derived peptide, β-ARKct, the protein inhibitor of GRK-2 translocation and activation. In these mice, the heart had an improved function both in baseline conditions as well as in response to adrenergic stimulation [25].

Besides using transgenic mice to investigate the role of GRK-2 in cardiac development and function, the knockout mice model has also been used. The investigations have shown that embryos lacking the GRK-2 gene develop major cardiac anomalies incompatible with life [28].

Furthermore, it has been reported that β-AR sensitivity increases 30 min after coronary artery ligation (CAL) [29]. There are few studies of β-AR transduction sensitivity in animal models of MI during the sub-acute period extending from 6 h to 24 h after CAL [30, 31]. This period roughly coincides with an observed second peak for susceptibility and development of β-AR-sensitive ventricular arrhythmias and sudden cardiac death in animal models [30]. GRK-2, but not GRK-5, activity in the sub-epicardial border zone was reduced 24 h after CAL compared with the non-ischemic subepicardial tissue [30]. This corresponds with the fact that in the ischemic tissue there is a loss of the ability to desensitize to β-AR stimulation 24 h after CAL. This is in temporal correspondence with a second peak in sudden cardiac death observed between 6 h and 24 h in dog and rat models of MI [30]. Raake *et al.* [31] generated a mouse model where cardiac myocyte GRK-2 was ablated after birth or after application of tamoxifen and the consequences of GRK-2 ablation before and after CAL on cardiac remodeling after MI were observed. GRK-2 ablation was beneficial for survival, enhanced cardiac contractile performance and preserving effect on cardiac remodeling. Their results confirmed the causal role of GRK-2 in generation of HF and the potential importance of targeting of this protein in order to prevent or slow down cardiac remodeling [31].

Transgenic and adenoviral expression of a peptide inhibitor of GRK-2 translocation, β-ARKct, has proved useful in experimental HF models [27, 32]. The genetic manipulations at different levels of β-AR signaling demonstrate that the right point of intervention is important to the function of the heart and suggest that it is more desirable to circumvent β-AR desensitization than to simply facilitate β-AR activation [33]. Results [33] show that targeted deletion and lowering of cardiac myocyte GRK-2 activity by β-ARKct gene therapy leads to a novel protective and inotropic phenotype, which prevents post-ischemic HF and rescues a phenotype of established HF. The same authors reported that the inhibition of GRK-2 by β-ARKct had a beneficial effect, not only on the hemodynamic parameters of heart function, but also on normalization of the catecholaminergic neurohormonal axis [33].

Role of G protein related kinase in coronary artery disease – evidence from human studies

Increased cardiac GRK-2 levels have been described in chronic HF and are associated with elevated sympathetic activity [23, 31]. Moreover, Metaye *et al.* [34] and Santulli *et al.* [35] reported that GRK-2 could be a marker for the progression of HF after acute MI. It is important to point out that β-blocker therapy reduces the GRK-2 levels and then improves cardiac function [35]. Therefore, the levels of GRK-2 in early stages of acute MI might serve as a biological marker of progression

towards HF, and could be an indicator of β-AR blocker efficacy in patients with acute coronary syndrome. In addition, it has been shown that the application of β-ARKct could inhibit the GRK-2 and consequently improve β-AR signaling and contractile function of failing human myocytes [36]. In the study reported by Williams *et al.* [36] the β-ARKct was expressed *via* adenovirus-mediated gene transfer in ventricular myocytes isolated from hearts explanted from 10 patients with end-stage HF undergoing cardiac transplantation. Compared with uninfected failing myocytes, the velocities of both contraction and relaxation in transfected cells were increased in response to isoproterenol. The fractional shortening was enhanced, as was the activity of adenylyl cyclase [36]. This finding confirmed the results, previously reported from animal studies [33], that the expression of β-ARKct can also improve contractile function of failing human myocytes.

Role of G protein related kinase in arrhythmias and sudden death – evidence from human studies

Patients with HF have an increased plasma concentration of catecholamines, probably due to downregulation of β-AR and depression of the β-AR-mediated signal transduction axis [34]. Prolonged sympathetic stimulation could result in electrophysiological and metabolic disturbances of the myocardium due to disturbed intracellular calcium homeostasis, resulting in tachyarrhythmia and sudden death [37-39]. It seems that prolonged exposure to catecholamines induces changes in both expression and activity of components of the β-AR signaling pathway [34]. More detailed studies about the impact of GRKs in arrhythmias with a potential fatal outcome are still needed.

Role of GRKs in heart failure – evidence from human studies

Different diseases of the CV system (hypertension, CAD, cardiomyopathy) have a common clinical endpoint – HF [10, 23]. Dilated cardiomyopathy is a disease of left ventricular dysfunction accompanied by impairment of targets of the β1-AR signal cascade [10]. This disturbed β-AR function could be based on an elevated sympathetic tone observed in patients with HF [40, 41]. In these patients, the plasma concentration of catecholamines is elevated, providing evidence of sympathetic stimulation [42]. In patients with dilated cardiomyopathy β-AR responsiveness of the myocardium is diminished [42]. It has been reported [43] that in these patients the expression of β1-AR is reduced at the mRNA and protein level. In addition, the expression of the inhibitory G-protein Gi is found to be increased [43]. In addition, in HF patients the expression of GRK is elevated and it is upregulated [10, 44]. Taken together, studies reported by Ungerer *et al.* [44], Petrofski *et al.* [10] and Leineweber *et al.* [43] suggest that elevated levels of GRK-2 are an early, ubiquitous consequence of myocardial injury that leads, ultimately, to clinical HF. Also, GRK-2 upregulation often precedes the development of measurable HF and may represent both a novel indicator for cardiac injury and potential therapeutic intervention prior to clinical dysfunction [10]. In addition, GRK changes appear centered on GRK-2 although GRK-5 may also contribute to the pathophysiology seen in HF. GRK-5 expression and activity have been shown to be elevated in some animal models of HF, although its role in human HF remains unclear [45]. Restoration of β-AR signaling through selective myocardial GRK-2 inhibition represents a form of molecular ventricular assistance and a possible therapeutic approach to the failing heart. This may ultimately constitute a valuable treatment modality for patients with chronic HF [46].

Role of G protein related kinases in sympathetic neurocirculatory failure

Sympathetic neurocirculatory failure (SNF) features orthostatic hypotension and abnormal beat-to-beat blood pressure responses to the Valsalva maneuver [1]. This pathophysiological phenomenon is present in pure autonomic failure (or Bradbury-Eggleston syndrome or idiopathic orthostatic hypotension), Shy-Drager syndrome (multiple system atrophy with sympathetic neurocirculatory failure), and Parkinsonism with peripheral autonomic failure [1]. Several features of idiopathic orthostatic hypotension have been attributed to hypoactive or hyperactive states of adrenergic receptors of the sympathetic nervous system. Recent data indicate that autoimmune lesions of the β-AR signaling pathway could be one of the contributing factors to orthostatic intolerance [47, 48], but still there are no available data about the contribution of GRK to this syndrome.

Hypertension

Although the pathophysiology of high blood pressure is undeniably complex, increased vascular resistance [49] and increased plasma norepinephrine [50] are likely to be involved. The pathophysiology of various stages of hypertension is different. In early hyperkinetic borderline hypertension, the sympathetic drive to the heart and blood vessels is increased, while the parasympathetic cardiac inhibition is decreased [49]. The elevated cardiac output, vascular resistance, and blood pressure at that stage can be fully normalized by

autonomic blockade [51]. The release of norepinephrine, the major sympathetic neurotransmitter, at the vascular level induces the activation of α1-AR, which mediates vasoconstriction, and β-ARs, which cause vasodilatation [52]. In hypertension, the balance between α1-AR and β-AR systems is shifted towards vasoconstriction, probably due to the defective vasodilatation in response to β-adrenergic stimulation. β-AR agonist administration in the human brachial artery causes vasodilatation, with this response attenuated in hypertension [49, 52]. GRK-2, the enzyme responsible for fast desensitization of β-AR, is abundantly expressed in the heart and vessels [53]. This significant increase has been observed in both heart and vessels of animal models of hypertension [54] and in genetically modified animals [55]. An increased level of GRK-2 was also observed in spontaneously hypertensive rats, as part of a more complex picture of the imbalance between vasoconstrictor and vasodilator systems of AR signaling [56]. In transgenic mice directing vascular smooth muscle expression of GRK-2, the mice showed elevated blood pressure with vascular thickening, hypertrophy of the myocardium and reduced vascular relaxation with the β-AR agonist isoproterenol [55]. These data imply that the primary abnormality of β-AR signaling pathway is GRK-2 overexpression in vascular smooth muscle causing β-AR desensitization and decreased vasodilatation [57]. Also, the desensitization of β-ARs and an increase in GRK-2 is confirmed in hypertensive patients [52, 58]. Although GRK-2 polymorphisms have not been associated with essential hypertension [59], increased GRK-2 expression (but not GRK-5) has been reported in lymphocytes of African Americans with hypertension, where hypertension occurs with higher prevalence and morbidity [60]. Whether increased GRK-2 expression is a predictor of increased CV risk remains to be determined, having in mind that GRK-2 upregulation occurs with progression of hypertension to HF [43]. Novel and optimistic data suggest that morphine-mediated reduction of GRK-2 expression as a hypertension-associated gene could open a new line of hypertension therapy [61]. One of the crucial physiological roles of the morphine-mediated molecular pathway is the maintenance of normal vascular tone [62].

Cardiac necrosis and cardiomyopathy

Stress (tako-tsubo) cardiomyopathy is a condition afflicting older women, characterized by acute left ventricular systolic dysfunction, triggered by emotionally and physically stressful events, and occurring without significant coronary obstruction [63]. Infusion of catecholamines, stimulation of the central nervous system, the combination of stress and steroids, myocardial reperfusion and pheochromocytoma, all could produce contraction band necrosis, observed in cases of sudden cardiac death [1]. The combination of subendocardial damage and arrhythmogenic action of catecholamines may explain the high frequency of sudden death in patients with stroke, epilepsy, head trauma, intracranial hypertension, and severe emotional distress [64]. Single nucleotide polymorphisms involving the β1-AR (amino acid positions 389 and 49) and α2C (deletion 322-325) have been investigated for susceptibility to stress cardiomyopathy [65], but the role of GRK in this necrotic process of cardiomyocytes is still not defined.

Role of G protein related kinases in neurally mediated syncope and vagally induced atrial fibrillation

While much is known about the sympathetic regulation of cardiac function in health and disease, less interest has been paid to the parasympathetic branch of the autonomic nervous system [66]. Parasympathetic control over the heart is performed by the vagal nerve, and the receptors for acetylcholine (AChR) at the heart level are of the muscarinic type [27]. These receptors are also GPCRs [66], which are desensitized by the phosphorylation by GRK. M1-, M2- and M3-ChR have been found to be phosphorylated *in vivo* by GRK-2, GRK-3 and GRK-5 [67] but only sparsely by GRK-1 and GRK-6 [68]. The role of receptor phosphorylation and β-arrestin binding in the internalization of M2 AChR and other muscarinic receptor subtypes remains controversial [66].

Neurally mediated syncope (vasovagal syncope, carotid sinus hypersensitivity)

Vasovagal syncope (VVS) is a common clinical problem, characterized by transient episodes of loss of consciousness due to abnormal autonomic activity [69]. Classical VVS is mediated by emotional and/or orthostatic stress. The pathophysiology of VVS is still unclear [70]. G-protein signal transduction pathways play a basic role in CV reflexes [71]. The predisposition to reflex-mediated syncope is associated with genetic variations in G-protein genes, but the relevance of GRK in this pathophysiological condition still remains hypothetical [71].

Vagally mediated atrial fibrillation

Experimental studies have indicated that in atrial fibrillation vagal activity may have a decisive influence on the electrophysiological properties of the atrial myocardium [72]. It is considered to represent a form of atrial fibrillation particularly affecting males aged 40 to 50 years. The arrhythmic episodes manifest themselves most often during the night, when parasympathetic predominance occurs [73]

preceded by bradycardia, is not triggered by stress and lasts from minutes to hours [74, 75]. It has been reported that vagally mediated atrial fibrillation is present in normal, pathologically unchanged heart, whereas patients with structural heart diseases more often have sympathetically induced atrial fibrillation [73, 76]. Pathogenesis of vagally induced atrial fibrillation is probably through downregulation of the inward potassium current [72] but the role of GRK in the proposed molecular mechanism is still not defined.

Therapeutic implications of G protein related kinases molecular signaling pathway

Neurocardiovascular diseases, such as hypertension, HF, and CAD, represent common causes of morbidity and mortality in developed countries "and will remain so by the year 2020" [77]. Currently used therapy is unable to cure these diseases [57, 78]. Therefore, research efforts should be focused at the molecular level and explore GRK molecular signaling pathways for treatment purposes. β-ARKct is a potential therapeutic candidate, especially for hypertension, HF and CAD [20].

β-ARKct is designed from the carboxyl-terminus of GRK-2, which is the domain that physically interacts with the βγ subunits of the activated G protein [25, 57]. β-ARKct is an effective inhibitor of AR phosphorylation and a potential option to prevent HF, MI and hypertension [57].

The available literature also suggests that GRK-2 could be beneficial in the setting of acute ischemic injury and hypertension. In contrast to the global knockout of GRK-2 that was found to be embryonically lethal [28], when GRK-2 was ablated in the heart after post-MI HF was recognized, the function of cardiac muscle was significantly improved [31]. In addition, 3 months after MI, β-AR responsiveness was preserved and cardiac hypertrophy was diminished [31]. These data support the fact that GRK-2 is important in the development of post-MI and HF. Elevated levels of GRK-2 are present both in human hypertension [52, 58] and animal models of hypertension [54, 56]. Therefore, the inhibition of GRK may prove to be a therapeutic approach for the treatment of hypertension [57], opening new perspectives for modern hypertension management [79].

GRK-2 is among potential candidate molecules for future gene therapy of HF [78]. The fascinating possibility offered by inhibiting GRK-2 activity is the reduction of sympathetic nervous system hyperactivity specifically acting on the adrenal gland [78]. This approach was successful in animal models of HF [80], where specific inhibition, *via* adenovirus mediated β-ARKct adrenal gene delivery, reduced circulating catecholamine levels, downregulating adrenal function and improving cardiac function [78, 80]. These results offer a promising therapeutic strategy for all neurocardiological diseases where sympathetic nervous activity is increased (e.g. HF, CAD and hypertension). Also, interesting results have come from a study of the interaction of the sympathetic nervous system and immune system in pathogenesis of HF [81]. Molecular structures, such as Toll-like receptors, their biochemical pathways and their potential influence on GRK-2 might shed light on the interactive role of the neural and immune system in the genesis of HF [82].

In addition, recently new inhibitors of GRK-2 were designed by Takeda Pharmaceuticals with the specific intention of being a new therapeutic agent for HF [76]. These compounds induce slight closure of the kinase active site, to a degree that corresponds to the level of inhibitory capacity of these agents [76]. Both approaches, gene therapy and GRK-2 inhibitors, open promising lines for treatment of neurocardiological diseases.

Conclusions

In this review we present the latest data from recent animal and clinical studies, which focus on the role of GRKs in neurocardiovascular physiology and pathophysiology. It is essential to understand the molecular mechanisms underlying the regulation of GRKs, during both normal function and pathological states. The role of GRKs in pathogenesis of neurocardiological diseases provides a new insight into potential therapeutic options.

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