G-Protein-Coupled Receptor Kinase 2 and Hypertension

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REVIEW ARTICLE

G-Protein-Coupled Receptor Kinase 2 and Hypertension

Molecular Insights and Pathophysiological Mechanisms

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Abstract Numerous factors partake in the fine-tuning of arterial blood pressure. The heptahelical G-protein-coupled receptors (GPCRs) represent one of the largest classes of cell-surface receptors. Further, ligands directed at GPCRs account for nearly 30 % of current clinical pharmaceutical agents available. Given the wide variety of GPCRs involved in blood pressure control, it is reasonable to speculate for a potential role of established intermediaries involved in the GPCR desensitization process, like the G-protein-coupled receptor kinases (GRKs), in the regulation of vascular tone. Of the seven mammalian GRKs, GRK2 seems to be the most relevant isoform at the cardiovascular level. This review attempts to assemble the currently available information concerning GRK2 and hypertension, opening new potential fields of translational investigation to treat this vexing disease.

Keywords Hypertension · GRK · Adrenergic receptors · Endothelium · Vascular tone

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1 Endothelial Dysfunction and Hypertension

Essential hypertension is a common condition that represents a well-known risk factor for the development of myocardial infarction, heart failure and cerebrovascular disease [1]. Increased peripheral vascular resistance to blood flow and vascular remodelling are common features of hypertension. Resistance arteries participate in the development of hypertension and may contribute to its complications [2, 3]. Vascular tone is mainly balanced between constriction and relaxation of vascular smooth muscle cells (VSMC). The endothelium plays a pivotal role in this regulation, affecting vascular function and remodelling [4, 5]. The endothelium is the active inner monolayer of the blood vessels, representing the largest organ in the body (approximately six tennis courts). It plays a critical role in vascular homeostasis [6–8]. Indeed, endothelial cells regulate vascular tone by releasing various relaxing and contracting factors including catecholamines, nitric oxide (NO), vasoactive peptides, arachidonic acid metabolites and reactive oxygen species (ROS) [9–11]. Therefore, the endothelium actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the inflammatory activity as well as cell proliferation. Thus, normal endothelial function depends on constant fine tuning and adjustment of opposing forces and effects [4]. Endothelial dysfunction is characterized by impaired vasomotor response (reduced vasodilation and increased endothelium-dependent contraction), cell proliferation, platelet activation, vascular permeability, and a proinflammatory and prothrombotic phenotype, including leucocyte-endothelial interactions that partake in vascular inflammation and increased adhesion and aggregation of platelets.

2 Adrenergic System and Vascular Tone

Several factors (structural and neurohormonal) participate in the modulation of arterial blood pressure, which is determined by a fine equilibrium between peripheral resistances and cardiac output. The sympathetic nervous system, because of its ability to control at the same time cardiac output and peripheral vascular tone, appears to play a fundamental role in this regulation [7].

Indeed, systemically circulating or locally released [12] sympathetic catecholamines lead to the activation of two main classes of adrenergic receptors (ARs): α_1AR and β_2AR, causing vasoconstriction and vasodilatation, respectively [8, 11]. Thus, under physiological conditions, vascular tone is tightly regulated by the adrenergic system, resulting from a balance between α_1AR-mediated vasoconstriction and βAR vasodilatation. In particular, β_2AR activation at the endothelial level induces the production and release of nitric oxide, causing vasodilation [8]. In the hypertensive state, this equilibrium is shifted toward increased vasoconstriction, likely because of a defective vasodilatation in response to βAR stimulation. Indeed, βAR agonist administration in the human brachial artery induces vasodilatation and, interestingly, this response appears to be attenuated in hypertensive patients [13, 14]. The role of β_2AR for the vasculature appears to be so critical that genetic variants of this receptor, causing excessive desensitization, may lead to reduced vasodilation [15, 16] and may also promote the occurrence of atherosclerosis [17, 18].

3 Vascular G-Protein-Coupled Receptors

There are many other receptors involved in the regulation of vascular tone, like the muscarinic receptor, the receptors for angiotensin II, endothelin, adenosine, thrombin, vasopressin and many others (see Table 1). Intriguingly, all these receptors belong to the same class of protein, called G-protein-coupled receptors (GPCRs). This family of heptahelical membrane sensors is one of the largest classes of cell-surface receptors, accounting for approximately 4 % of the entire protein-coding genome and represents the primary target of most pharmaceutical therapies. Indeed, ligands directed at GPCRs (agonists and antagonists) account for nearly 30 % of current clinical pharmaceutical agents available [19]. Several neurotransmitters, peptides, hormones, prostaglandins, leukotrienes, chemokines, lipids and nucleotides exert their effects on the cardiovascular system via GPCRs (the most important vascular ligands are reported in Table 1). Moreover, approximately 150 of the GPCRs found in the human genome have still unknown functions.

Table 1 Vasodilators and vasoconstrictors acting through G protein-coupled receptors (GPCRs)

GPCR mediating vasodilation	Ligand	GPCR mediating vasoconstriction
M1, M3	Acetylcholine	
A2A, A2B	Adenosine	A1
Beta	Adrenaline	Alpha1
AM, CGRP1	Adrenomedullin	
CGRP1	Amylin	
	Angiotensin II	Angiotensin II1A-R
	Apelin	APJ
B1/2	Bradykinin	
CGRP1	CGRP	
D1	Dopamine	
	Endothelin-1	ETA
Ghrelin	Ghrelin	
H2	Histamine	H1
	Leukotriene B4	BLT1
	Leukotriene D4	CysLT1
	Motilin	GPR38
	Neurokinin A	NK2
	Neuromedin	NMU1 (GPR66)
	Neuropeptide Y	Y1
NOP (ORL1)	Nociceptin	
	Noradrenaline	Alpha1
P1Y	Nucleotides	P2Y
PAF	PAF	
IP1	Prostacyclin	
DP	Prostaglandin D2	
EP4	Prostaglandin E2	EP3
5-HT7	Serotonin	5-HT2A
	Somatostatin	sst2
S1P1	Sphingosine-1-phosphate	S1P2
NK1	Substance P	
	Thrombin	PAR1
	Thromboxane A2	TP
CRF2	Urocortin 1-2-3	
	Urotensin-II	UT (GPR14)
V2	Vasopressin	V1A
VPAC	VIP	

Despite the lack of sequence homology between classes, all GPCRs share a common structure and mechanism of signal transduction.

How GPCRs do work? GPCRs couple their ligand binding to heterotrimeric G-protein activation, which subsequently transduces the extracellular signals to intracellular effector molecules [20]. A possible mechanism underlying the impaired vasodilatation in hypertension could be a decreased production of downstream second

messengers involved in the signal transduction pathway, as observed in several tissues from patients with essential hypertension [21]. Another hypothesis evokes the desensitization of the receptor, defined as the loss of functional response that occurs after exposition to continuous agonist stimulation [22]. This process is associated with phosphorylation of the receptor followed by uncoupling from its signal pathway [23].

Furthermore, G-protein signalling is terminated by phosphorylation of the intracellular domains of the receptor by the family of G-protein-coupled receptor kinases (GRKs) [23, 24]. GRK-mediated phosphorylation increases the affinity of GPCRs for the arrestin class of proteins, which uncouples the phosphorylated receptor from the G-protein and subsequently targets the receptor for internalization [20, 25]. Downregulation of GPCRs reduces the functional activity of classical signalling paradigms up to 80 % [26]. Given the wide variety of GPCRs involved in blood pressure control (see Table 1), it is reasonable to speculate a possible role of GRKs in the regulation of these mechanisms, especially if we consider that there are over 800 known GPCRs in the human genome and so far only seven GRKs have been identified [27]. Recent data indicate that GRKs are also able to modulate other receptor families, such as tyrosine kinase receptors [28]. Furthermore, many non-GPCR substrates have been identified for GRKs, ranging from tubulin to phosducin, ezrin, radixin, synuclein and p38MAPK, which broaden the horizon of their potential cellular functions [29–33].

4 The GRK Family

GRKs are 60–80 kDa serine/threonine kinase that are able to phosphorylate GPCR, thereby preventing the further coupling of that receptor to its G protein. As mentioned in Sect. 3, seven mammalian genes encoding GRKs have been cloned (Table 2). These genes display a similar structure consisting of an aminoterminal domain containing protein-binding sequences, a highly conserved central catalytic domain (of approximately 270 amino acids) and a carboxyl terminus, considered the most important determinant of subcellular localization and agonist-dependent translocation. This family has been subdivided into three different groups: visual or rhodopsin-specific GRKs (GRK1 and 7), βAR kinases (βARK 1 and 2, corresponding to GRK2 and 3, respectively) and the GRK4 subfamily (GRK4, 5 and 6).

Of interest, in addition to phosphorylation-dependent processes, GRKs regulate cellular responses in a phosphorylation-independent manner (non-catalytic actions), due to their ability to interact with several proteins involved in trafficking and signalling [34, 35].

Table 2 The family of G-protein-coupled receptor kinases (GRKs)

Name	Tissue expression	AA	Size (kDa)	References
GRK1	Retina (rods), pineal gland	563	63	[71, 72]
GRK2	Ubiquitous (leukocytes, cerebral cortex, heart, endothelium, vascular smooth muscle, liver, adrenal)	689	79	[42, 43, 73–75]
GRK3	Ubiquitous (spleen, brain, olfactory tubercle)	688	80	[76, 77]
GRK4	Kidney, testis, brain, lung	578	66	[78, 79]
GRK5	Ubiquitous (heart, lung, skeletal muscle, liver)	590	68	[80–82]
GRK6	Spleen, lung, brain, adrenal, kidney, muscle, T lymphocytes	576	66	[83–85]
GRK7	Retina (cones)	553	62	[86, 87]

AA number of amino acids in *Homo sapiens*

4.1 GRK2 Plays a Pivotal Role in Essential Hypertension

Of the seven mammalian GRKs, GRK2 seems to be the most important isoform, as judged by the embryonic lethality of homozygous GRK2-deficient mice [36]. On the other hand, gene ablation for the other GRKs resulted in relatively subtle phenotypes [36–40].

The physiological relevance of GRK2 is further confirmed by the fact that it partakes in several essential cellular processes, like cell cycle progression, migration and differentiation [32, 41]. Moreover, as mentioned in Sect. 3, GRK-mediated desensitization does not always rely on its catalytic activity. It also depends on its capacity to establish selective protein–protein interactions [34, 35], occurring not only at the cell membrane [42].

Alterations in GRK2 expression and activity have been found in several diseases, including heart failure [43], thyroid gland pathologies [44], opioid addiction [45], cystic fibrosis [46], Alzheimer's disease [47], multiple sclerosis [48], rheumatoid arthritis [49] and ovarian cancer [50]. Different animal models of genetically modified mice proving in vivo regulation of GPCRs by GRK2 are reported in Table 3.

Importantly, GRK2 levels in peripheral blood lymphocytes have been reported to mirror changes in kinase expression in other organs under several physiopathological circumstances [43]. In particular, GRK2 levels and activity have been found increased in lymphocytes from hypertensive patients [51]. Since a generalized impairment of β-adrenergic mediated vasodilation has been shown both in animal models of hypertension [52] and in human hypertensive subjects [5, 13], this alteration has been related to the increased GRK2 abundance and activity [13].

Table 3 Genetically modified mice proving in vivo regulation of G-protein-coupled receptors (GPCRs) by GRK2

GPCR	Genetic manipulation	Effect	References
α_1-Adrenergic receptor	Myocardial overexpression	No cardiac effect compared to control	[88]
	Vascular smooth muscle ablation	Enhanced α_{1D}-adrenergic mediated vasoconstriction	[89]
β-Adrenergic receptor	Myocardial overexpression	Decreased myocardial contractile response to a β-agonist	[90]
	Vascular smooth muscle overexpression	Attenuation of β-agonist-stimulated vasodilatation	[54]
	One allele deletion	Enhanced myocardial contractile response to a β-agonist	[91]
	Myocardial ablation	Improved calcium handling and prevention of maladaptive postinfarction remodelling	[92]
Angiotensin II-1A receptor	Myocardial overexpression	Attenuation of contractility and heart rate in response to angiotensin II	[93]
Parathyroid hormone (PTH)/PTH-related peptide receptor	Osteoblast expression of GRK inhibitor	Anabolic effects by an increase in bone density and trabecular bone volume	[94]
Sphingosine-1-phosphate receptor 1 (S1PR1)	Deletion in blood B and T cells	Reduced ability to enter lymphonodes	[73]

Indeed, a decrease in β-adrenergic signalling through increased GRK2 phosphorylation would decrease the vasodilatative response, thereby increasing the blood pressure. This point of view is supported by the fact that GRK2 expression inversely correlates with blood pressure as well as β-adrenergic mediated adenylate cyclase activity [51]. Other experiments performed in Dahl salt-sensitive rats and spontaneously hypertensive rats (SHR) confirmed the increased levels of GRK2 in VSMC, consistent with the observations in peripheral lymphocytes [53].

The role of GRK2 in VSMC was explored in an elegant transgenic animal model, consisting of mice with VSMC-targeted overexpression of GRK2 [54]. These mice displayed significantly increased resting mean arterial pressure (by approximately 20 %) accompanied by an attenuated response to βAR signalling compared with non-transgenic littermates. The increased blood pressure due to GRK2 overexpression in VSMC was also accompanied by cardiac hypertrophy and vascular thickening, two hallmarks of the hypertensive phenotype [54]. Based on these findings, it is tempting to speculate that elevated levels of GRK2 in hypertensive arterial smooth muscle may promote VSMC proliferation and migration, contributing to the atherosclerotic modifications reported in hypertensive vessels [55, 56].

Another significant study focused on the endothelial GRK2 in the determination of portal hypertension, relying on the physical interaction between GRK2 and Akt [57]. Since Akt is able to activate endothelial nitric oxide synthase (eNOS), thereby mediating vasodilation, the GRK2-mediated inhibition of Akt has been shown to shift the vascular tone toward constriction, in the setting of endothelial dysfunction due to decreased eNOS activity [57, 58]. Noticeably, endothelial modulation of the contractile state of vascular smooth muscle has been shown to be impaired in atherosclerosis and in several conditions known to be associated with the premature development of atherosclerosis. Two different reports, both showing that reducing GRK2 activity can reduce the endothelial dysfunction observed in the hypertensive vasculature, support the role of GRK2 in the pathogenesis of arterial hypertension. The first study demonstrated in the aged rat, a model of impaired βAR signalling, that chronic exercise may lead to a decrease of GRK activity in the vasculature, in particular in the endothelium, and that this reduction mirrors an ameliorated vasodilatation [59]. The second work showed the preventive effect of a specific GRK2 inhibitor on vascular dysfunction in diabetic mice, ameliorating Akt/eNOS impairment and improving the translocation of β-arrestin to the plasma membrane [60]. Consistent with these data, we recently showed that acute inhibition of GRK2 using a non-selective inhibitor, heparin, determined in normotensive subjects the inhibition of the desensitization of the vasodilatation to the βAR agonist isoproterenol [13]. Interestingly, heparin administration in hypertensive patients was able to restore the impaired βAR vasodilation, ameliorating local resistance [13].

Remarkably, the correlation between GRK2 abundancy and hypertension is also present in other conditions characterized by increased blood pressure including the above-mentioned portal hypertension [57] and pre-eclampsia [61]. In gestational hypertension, the increase in GRK2 in the fetal placental vasculature seems to be compensatory rather than causative of increased blood pressure, in order to balance the excessive vascular tension, since the lack of protective effect of elevated GRK2 expression levels negatively affected the outcome of the hypertensive state [61]. In this case, a potential explanation could rely on the

metabolic effect of GRK2, which is able to place the cell in a low energy state that might favour survival in stress conditions [42, 62, 63].

Indeed, a mounting body of evidence suggests that elevated GRK2 levels may imply metabolic alterations and, in particular, lead to insulin resistance, a common feature of the hypertensive state [64, 65]. It has been reported that increased GRK2 expression mediates insulin resistance in myoblasts via a mechanism that involves sequestration of Gq and the insulin receptor substrate-1 (IRS-1) [66]. In addition, we have recently shown that GRK2 is able to bind and phosphorylate IRS1 and that the inhibition of GRK2 action is able to ameliorate the insulin sensitivity [67, 68]. We have also demonstrated that GRK2 negatively affects cardiac glucose uptake and that lowering GRK2 after ischaemic injury will contribute to restoring cardiac metabolism and prevent the development of subsequent heart failure [67]. In line with these data, we verified that lymphocyte GRK2 levels significantly increase in patients with myocardial infarction and are associated with worse systolic and diastolic function [69]. Moreover, early revascularization and β-blocker therapy influenced GRK2 levels. Noteworthy, at 2-year follow-up, patients with higher GRK2 levels at admission had worse systolic function and cardiac remodelling [69], strongly suggesting that GRK2 levels may reflect haemodynamic impairment and might have a prognostic value after myocardial infarction.

5 Conclusions

As with the reports of other cellular defects described in hypertension, the association between GRK2 levels and hypertension does not represent a clear causal relationship [70]. Additionally, whether other mechanisms regulating GRK2 stability, including ubiquitination, contribute to the phenotype of increased GRK2 expression in hypertension, remains to be elucidated. Finally, why GPCRs linked to vasodilatory mechanisms are preferentially affected by GRK2 overexpression has yet to be established. It is tempting to speculate that a possible mechanism underlying such 'selectivity' could be dependant on the stoichiometry of coupling of G_s-linked vasodilatatory systems versus the G_q-linked vasoconstrictor system.

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