ABSTRACT

Hypertension is a prevalent condition in the developed world and disease severity is directly correlated with additional cardiovascular complications. It is estimated that 30% of the adult population in the United States has hypertension, which is classified as a systolic blood pressure $\geq 140$ mmHg and/or a diastolic blood pressure $\geq 90$ mmHg. A prolonged increase in afterload ultimately leads to congestive heart failure in the majority of cases. Currently, medication designed to treat hypertension is inadequate, thus new therapies need to be explored. Blood pressure is tightly regulated by blood vessel radius, which is established by hormones and/or peptides binding to GPCRs (G-protein-coupled receptors). Catecholamines and peptide hormones, such as AngII (angiotensin II), are elevated in hypertension and, therefore, signalling by these GPCRs is increased. Their signalling is tightly controlled by a class of proteins, the GRKs (GPCR kinases). Elevated levels of either GRK2 or GRK5 in both the lymphocytes and VSM (vascular smooth muscle) are associated with human hypertension and animal models of the disease. The focus of the present review is on the role GRKs, and their regulation of GPCRs, play in high blood pressure.

INTRODUCTION

Hypertension, or high BP (blood pressure), is a prevalent risk factor in the development of ischaemic heart disease, leading to stroke, cardiac failure and renal failure, and its prevalence is increasing at an alarming rate [1,2]. BP homoeostasis is important for proper perfusion of the organs. Although the kidney and heart are important in BP control, another major contributor to the development of hypertension is the contractile state of VSM (vascular smooth muscle), which regulates the radius of blood vessels, thus modulating peripheral resistance. The radius of a blood vessel presents the largest contribution to resistance of blood flow, since resistance is inversely proportional to the radius to the fourth power ($\text{resistance} \propto \frac{1}{r^4}$). Therefore relatively small changes in blood vessel diameter can cause dramatic alterations in vascular resistance.

Blood vessel radius is maintained by a delicate balance of vasoconstrictor compared with vasodilatory inputs. There are various agonists that bind to plasma membrane receptors on VSM and endothelial cells that can modulate the contractile state of VSM. Many of these agonists transmit their ‘message’ via GPCRs (G-protein-coupled receptors). The GPCR superfamily is one of largest protein families and is the primary target of most pharmaceutical therapies [3]. GPCRs have a

Key words: $\beta$-adrenergic receptor, G-protein-coupled receptor (GPCR), G-protein-coupled receptor kinase (GRK), hypertension, vascular smooth muscle.

Abbreviations: AngII, angiotensin II; $\beta$ARK, $\beta$-adrenergic receptor kinase; AT1 receptor, AngII type 1 receptor; BKCa, large-conductance Ca$^{2+}$-activated K$^+$ channel; BP, blood pressure; CaM, calmodulin; DAG, diacylglycerol; eNOS, endothelial NO synthase; ERK, extracellular-signal-regulated kinase; ET-1, endothelin-1; GPCR, G-protein-coupled receptor; GRK, GPCR kinase; IP3, inositol 1,4,5-trisphosphate; Kv channel, voltage-gated K$^+$ channel; MLC, myosin light chain; MLCK, MLC kinase; MLCP, MLC phosphatase; NFAT, nuclear factor of activated T-cells; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PSD95, postsynaptic density 95; RGS, regulator of G-protein signalling; SHHF, spontaneously hypertensive heart failure; SHR, spontaneously hypertensive rat; TAC, transverse aortic constriction; VSM, vascular smooth muscle; VSMC, VSM cell.

Correspondence: Dr Andrea D. Eckhart (andrea.eckhart@jefferson.edu).
common seven-transmembrane span and the ability to activate heterotrimeric G-proteins. G-protein activation subsequently transduces the extracellular signal to intracellular effector molecules [4]. The classical downstream signalling events are often dictated by the G-protein α-subunit to which the GPCR is coupled. There are four major subclasses of G-proteins: G<sub>α</sub>s (stimulatory), G<sub>α</sub>i/o (inhibitory), G<sub>α</sub>q and G<sub>α</sub>12/13 [5]. The coupling of GPCRs to these different subclasses of G-proteins allows for very diverse intracellular signalling events. For example, activation of G<sub>α</sub>s stimulates adenylate cyclase (adenyl cyclase), which leads to the formation of cAMP and subsequent phosphorylation of intracellular targets by PKA (protein kinase A). PKA phosphorylation causes increased relaxation in smooth muscle cells ultimately leading to vasodilation. G<sub>α</sub>i/o, on the other hand, inhibits adenylate cyclase activity, decreasing cAMP formation and PKA phosphorylation, effectively decreasing relaxation and diminishing vasodilation. G<sub>α</sub>q activates PLC (phospholipase C) leading to the formation of IP<sub>3</sub> (inositol 1,4,5-trisphosphate) and DAG (diacylglycerol). IP<sub>3</sub> initiates Ca<sup>2+</sup> release from intracellular stores, which activates Ca<sup>2+</sup>-CaM (calmodulin), leading to contraction. Additionally, DAG activates PKC (protein kinase C), which also phosphorylates target proteins that facilitate smooth muscle contraction leading to vasoconstriction [6]. G<sub>α</sub>12/13 has been shown to activate Rho kinase, which phosphorylates and inhibits MLCP [MLC (myosin light chain) phosphatase] activity leading to smooth muscle contraction [7]. To add to the complexity, some agonists can initiate vasoconstriction or vasodilation depending upon which receptor is stimulated. For example, catecholamines bind to α-adrenergic receptors causing G<sub>αq</sub> activation and constriction; likewise, catecholamines also bind to β-adrenergic receptors activating G<sub>αs</sub> leading to cAMP formation and relaxation. This is also the case for other hormones such as serotonin and histamine [8].

Importantly, signalling through GPCRs is under tight regulatory control. If GPCRs are subjected to prolonged or repeated stimulation, the receptor undergoes desensitization or down-regulation, which decreases the ability to activate appropriate G-proteins and initiate intracellular signalling cascades. This uncoupling is regulated in part by a series of serine/threonine GRKs (GPCR kinases) that phosphorylate agonist-bound GPCRs [9]. GRK phosphorylation then increases the affinity of the GPCR for the arrestin class of proteins. These receptors can be recycled back to the membrane following dephosphorylation or targeted for down-regulation and degradation [10]. The binding of arrestin initiates down-regulation and prevents the GPCR from associating with its G-protein, thus reducing the functional activity of classical signalling paradigms for that receptor up to 80% [5]. Maintenance of blood vessel radius is vital to the determination of peripheral resistance and it is regulated by many extracellular agonists binding to GPCRs. Therefore tight regulation of GPCR signalling is critical, and understanding the role of GRKs in modulating receptor signalling is important in understanding how GPCR signalling can change during disease states.

Although there are over 800 known GPCRs in the human genome, it is surprising that only seven GRKs (GRK1–GRK7) have been identified [11]. All GRKs have a similar basic structure with an N-terminal domain, a catalytic domain and a C-terminal domain. However, various post-translational modifications are responsible for their regulation within the cell. Furthermore, they are grouped functionally into three classes: GRK1-like (GRK1 and GRK7, otherwise known as rhodopsin kinases), GRK2-like [GRK2 and GRK3, otherwise known as βARK1 and 2 (β-adrenergic receptor kinases 1 and 2)] and GRK4-like (GRK4, GRK5 and GRK6). GRK1 and GRK7 are found almost exclusively in the retina and modulate opsins. GRK2 and GRK3 are widely expressed, although GRK2 is typically more abundant. GRK4 is found mostly in the testis and proximal tubule of the kidney. GRK5 and GRK6 are widely distributed among tissues [12]. Therefore most GPCRs in the body are regulated by four GRKs: GRK2, GRK3, GRK5 and GRK6.

GPCRs play a vital role in BP control. Stimulation of GPCRs can alter heart rate, vascular resistance and/or blood volume by changing the electrolyte balance. Heart rate is regulated by sympathetic and parasympathetic activation of adrenergic and muscarinic receptors. Likewise, vascular tone and peripheral resistance can be modulated by adrenergic regulation by the catecholamines noradrenaline (norepinephrine) and adrenaline (epinephrine), as well as other hormones, including AngII (angiotensin II), ET-1 (endothelin-1), histamine and adenosine. With the wide variety of GPCRs that are responsible for optimal BP control, it leaves no doubt that GRKs play a role in the development and/or maintenance of hypertension. Importantly, although it is a complex interaction among multiple systems in controlling BP, including central inputs, the heart, VSM, endothelial cells and the kidney, the focus of the present review is on alterations in molecules that regulate GPCRs in VSM and the possible implications in hypertension.

**ROLE OF GRKs IN HYPERTENSION**

**GRK2**

GRK2 is increased in human hypertension and animal models of the disease

Through agonists binding to GPCRs, BP can be increased by either enhancing constriction or impairing dilatory mechanisms of VSM. GRKs cause desensitization of GPCRs such as the β-adrenergic receptor. A decrease in β-adrenergic receptor signalling through increased...
GRK2 phosphorylation would decrease dilation, thus potentially increasing BP. Interestingly, there are findings that suggest that GRK2 expression and activity may be increased in subjects with hypertension. Using lymphocytes isolated from patients with normotension and hypertension, there was an increase in abundance and activity of GRK2 in young patients with hypertension compared with both young and old subjects with normotension [13]. This increase in GRK2 expression correlated with decreased isoprenaline (isoproterenol)-stimulated adenylate cyclase activity through β-adrenergic receptors [13]. Importantly, increased GRK2 levels also correlated with increased BP [14]. Subsequent animals studies using SHRs (spontaneously hypertensive rats) and Dahl salt-sensitive rats confirmed that GRK2 levels in VSM increased, consistent with the observation of increased lymphocyte GRK2 abundance and activity presented in human studies [15].

Overexpression of GRK2 in VSM results in hypertension with a decrease in β-adrenergic receptor signalling

Previous results clearly show a potential role for GRK2 in desensitizing β-adrenergic receptor signalling, thus impairing dilation (Figure 1). To understand the consequence of elevated VSM GRK2, we have developed a transgenic mouse overexpressing VSM GRK2 to a similar extent as described in both human hypertension and animal models of the disease using a portion of the SM22α promoter [16]. The aim of these studies was not to address whether increased GRK2 is a cause or consequence of hypertension, but whether elevated VSM GRK2 affects BP. VSM GRK2 overexpression significantly increased resting mean arterial pressure approx. 20 % over controls [16]. Importantly, there was an attenuated response to β-adrenergic signalling in VSMCs (VSM cells) as determined through decreased cAMP accumulation as well as an increased BP response in vivo [16]. The increased BP due to GRK2 was also accompanied by vascular thickening and cardiac hypertrophy [16]. Interestingly and in keeping with the complexity of BP control, in vivo BP responses to increasing doses of AngII, which causes VSM constriction, were also attenuated in GRK2 mice [16]. This illustrates the intricate nature in which GRKs possess the ability to phosphorylate many different receptors and have affects on both constriction and relaxation. Overall, these findings suggest that decreased vasorelaxation due to impaired β-adrenergic receptor signalling is most probably the primary abnormality in GRK2-overexpressing mice and that desensitization of GRK2-sensitive GPCRs linked to vasoconstriction play less of a role in the development of hypertension, at least in this model. The reason for this is unclear and warrants further investigation. Importantly, the aforementioned results show that elevated GRK2 levels in VSM increases BP, and the processes that lead to increased

Figure 1 Overview of the GPCR signalling cascade resulting in either vasodilation or vasoconstriction

The classical role of GRKs is to phosphorylate agonist-bound GPCRs, thereby desensitizing them and preventing further activation of downstream signalling cascades. Within the present review, we describe the impact of elevated expression of both GRK2 and GRK5 in VSM. The role of GRK3 and GRK6 in VSM and hypertension remains to be determined. In GRK2 mice, desensitization of β-adrenergic receptors leads to decreased PKA activation, which has a direct impact on K+ channels and HSP-20 (heat-shock protein-20) and results in less vasodilation (VDil). Importantly, GRK phosphorylation also desensitizes GPCRs coupled to pathways that cause vasoconstriction (VC), such as through L-type calcium channels (LTCC), Rho kinase (RhoK), which inhibits MLCP, or PLC, which results in the accumulation of DAG activating PKC and IP3, leading to the activation of CaM which activates MLCK and phosphorylates MLC (MLC-P). The balance between vasodilation and vasoconstriction is under tight control and disruptions in either may lead to hypertension. Why the impact of decreased vasodilation takes precedence over decreased vasoconstriction warrants further investigation.

Overexpression of GRK2 in VSM expression in hypertensive states warrants further studies.

Overexpression of GRK2 in VSM results in hypertension with concomitant vascular and cardiac hypertrophy

In addition to GRK2 affecting the homoeostasis of blood vessel radius, it also affects structural aspects of VSM which could also have an indirect effect on BP status through narrowing of the blood vessel lumen due to increased vascular wall thickness. GRK2 overexpression in cultured VSMCs decreased mitogenic signalling and proliferation via ET-1, AngII, thrombin and PDGF (platelet-derived growth factor) [17]. Subsequent studies have shown reduced neointimal thickness after vein graft implantation using adenoviral delivery of GRK2 [18]. These studies suggest that increased VSM GRK2
could potentially be a therapeutic target in neointimal hyperplasia as well as hypertension. In contrast with these studies, we found that a 2-fold VSM overexpression of GRK2 in vivo increased wall thickness by approx. 30% [16]. Interestingly, more recent studies have shown that expression of an inhibitory peptide of VSM Gq signalling normalized this increased wall thickness, suggesting a potential link between GRK2 and Gq signalling [19]. Furthermore, studies using a peptide inhibitor of GRK2, which consists of the last 194 amino acids of GRK2 (βARKct) [20], decreased VSM proliferation in culture and reduced intimal hyperplasia in vivo [21]. Conflicting results between these studies could be due to differences in the models used (vein graft compared with injury), the cell type expressing increased GRK2 or the type of mitogenic signalling elicited. In our transgenic mice, GRK2 expression is increased only in VSM, whereas adenoviral expression undoubtedly leads to increased expression in endothelial cells, VSMCs and adventitia (primarily fibroblasts). Regardless of this minor discrepancy, all of these studies indicate the important role of GRK2 in regulating growth, proliferation and/or migration of VSM and investigation into the role GRK2 is playing in different cell types is warranted.

There have been limited studies focusing on the role of GRKs in endothelial cells, although GPCR regulation of these cells is critical to the maintenance of vascular tone and should initiate many potential studies. In a study of portal hypertension, it was shown that GRK2 could physically interact with Akt, inhibiting its activity [22]. Importantly, Akt is able to phosphorylate and activate eNOS (endothelial NO synthase), thus GRK2-mediated Akt inhibition would shift the vasculature toward constriction in the setting of less eNOS activity. Importantly, GRK2 expression was increased in rats with portal hypertension and, when the expression was knocked-down, there was restoration of Akt activity and NO production [22]. Although these findings occur in a relatively specific form of hypertension due to liver damage, it provides important results regarding the potential role of GRKs in endothelial cell GPCR regulation.

**GRK5**

Hypertension or haemodynamic stress has also been shown to increase levels of GRK5 in VSM. Initial reports have shown that GRK5 expression was up-regulated in cultured VSMCs after 1 h of stimulation with 100 nmol/l AngII, with peak expression occurring at 16 h [23]. In this study, Ishizaka et al. [23] determined that Ca2+ influx was necessary for this GRK5 up-regulation. This was confirmed in vivo using osmotic mini-pumps to infuse either AngII or noradrenaline to increase BP in rats. GRK5 mRNA was measured in aorta removed from these rats and was shown to be increased at 5 days following AngII infusion (0.7 mg·kg−1·day−1). Subpressor doses of AngII (0.3 mg·kg−1 of body weight·day−1), losartan [an AT1 receptor (AngII type 1 receptor) antagonist] and hydralazine did not increase BP at day 5 and did not increase mRNA levels of GRK5. These findings suggest that hypertension itself was the contributing factor to AngII-induced GRK5 up-regulation [23].

**Overexpression of GRK5 in VSM results in Gq-dependent hypertension without concomitant vascular or cardiac hypertrophy**

To elucidate further the role of increased VSM GRK5 following hypertension, we made transgenic mice overexpressing GRK5 specifically in VSM using a portion of the SM2α promoter [24]. The 2-fold increase in mRNA levels of VSM GRK5 was similar to increases following AngII infusion [23]. GRK5 increased BP, and the increase in BP was shown to be gender-dependent. Interestingly, male mice with GRK5 overexpression had a much larger increase in BP (approx. 45 mmHg) compared with female GRK5 mice (approx. 17 mmHg) [24]. BP was restored to control values in both male and female GRK5 mice when treated with pertussis toxin, indicating a dependency on Gq signalling compared with Gq signalling such as is important in the setting of increased GRK2. Interestingly, impaired β1-adrenergic receptor signalling was shown to be altered in males, whereas females had enhanced AngII constriction [24]. Finally, unlike VSM overexpression of GRK2, transgenic overexpression of GRK5 did not alter vascular or cardiac hypertrophy [16]. Taken together, these findings indicate that different GRKs may possibly phosphorylate similar substrates, yet have differing consequences on G-protein signalling pathways. Additionally, these results offer insight into the differential role of GRKs based upon gender. Further investigation into the role that GRKs play in defining non-classical signalling paradigms within hypertension is warranted.

**GRK3**

GRK3 is also expressed in VSM, although, at least at the mRNA level, its expression level is approximately one-tenth of the amount of GRK2 or GRK5 (H.I. Cohn and A.D. Eckhart, unpublished work). Similar to GRK2 and GRK5, GRK3 also has unique in vivo substrates and has affinity for the α1B-adrenergic receptor [25]. Surprisingly, recent studies have found that inhibition of cardiac GRK3 causes hypertension due to increased cardiac output, which the authors attribute to hyper-responsive α1B-adrenergic receptors [26]. The role of GRK3 in the vasculature and, in particular, VSM is unappreciated and warrants further study, especially given the potential impact GRK3 may have on such a critical vasoconstricting GPCR.
GRK4

GRK4 and the kidney
GRK4γ plays an important role in the renal proximal tubules controlling water and electrolyte transport through regulation of the dopamine D1 receptor (Gβγ-coupled). Patients with essential hypertension have decreased dopamine D1 signalling and decreased adenylate cyclase activity, which lends to decreased natriuresis [27,28]. Genetic analyses from human subjects showed that essential hypertension was linked to the GRK4γ locus [29] and that three SNPs (single nucleotide polymorphisms) were present in the GRK4γ cDNA from patients with hypertension: R65L variant (nucleotide 448), A142V variant (nucleotide 679) and A486V variant (nucleotide 1711) [30,31]. Subsequent findings have shown that transgenic mice overexpressing the A142V variant under the control of the ubiquitously expressing CMV (cytomegalovirus) promoter caused hypertension, whereas the wild-type GRK4 mice were normotensive [30]. More recently, it has been shown that the hypertension in the A142V transgenic mice was due to altered dopamine signalling and not to an increase in ROS (reactive oxygen species), which are increased in essential hypertension [32]. Importantly, it is thought that the desensitization of the dopamine D1 receptors due to GRK4 causes AngII receptors to predominate in proximal tubule cells leading to antinatriuretic functions [33]. Whether the results seen in these studies are specifically due to renal proximal tubule GRK4 activity or because GRK4 is expressed in some other cell type remains to be determined, but these studies clearly illustrate a potentially important role for yet another GRK in hypertension.

ROLE OF RGS (REGULATOR OF G-PROTEIN SIGNALLING) PROTEINS IN HYPERTENSION

The N-terminal portion of GRK2 can act as an RGS; therefore it is also important to understand the role that RGS proteins may play in hypertension to better appreciate the additional influences GRKs may be having on BP control. GPCR signalling consists of more than the receptor, a G-protein and the activated classical signal pathway. The discovery of RGS molecules offers new insight into the complex network of molecules governing vascular GPCR signalling and the extent to which GRKs may be additionally influencing signalling. There are currently >30 known RGS or RGS-like proteins, with many (>20) expressed in the vascular system [34]. Proteins sharing this homologous RGS domain serve as GTPase-activating proteins, which initiate GTP hydrolysis and thus terminate signalling [35]. There are currently six subfamilies of RGS signalling proteins and in the present review we will focus on the limited number of studies regarding RGSs and RGS-like proteins and their role in vasculature GPCR signalling.

RGS2

One subfamily of RGS signalling proteins is the ‘small’ or RGS4-like RGS proteins, which appear to be regulators of G11G and Gq signalling [36]. These molecules provide an interesting potential therapeutic in diseases such as hypertension and cardiac hypertrophy due to the role of enhanced Gq signalling that occurs in these disorders from elevated levels of AngII, ET-1 and catecholamines binding to α-adrenergic receptors. Importantly, induction of RGS2 mRNA was increased the most when a panel of aorta RGS proteins was considered (RGS1, RGS2, RGS3 and RGS4) following stimulation with agonists important for vascular function [e.g. AngII, PDGF, TGF-β (transforming growth factor β)] [37]. Additionally, Cho et al. [37] found that RGS1, RGS2 and RGS4 attenuated AT1 receptor signalling, where as only RGS3 attenuated ET-1 signalling. RGS2 is one of the most studied family members and mice that were deficient in rgs2−/− had a hypertensive phenotype with increased resistance in the vasculature [38]. The hypertension associated with rgs2−/− could be decreased using an AT1 receptor antagonist, suggesting that the hypertension is AngII-dependent [38]. Additionally, rgs2−/− mice had increased sensitivity to ATP compared with wild-type mice, which resulted in higher peak Ca2+ and decreased relaxation kinetics and could contribute to vascular constriction [38]. Subsequent studies suggest that RGS2 is critical in regulating vasodilatory mechanisms through NO [39]. In VSM, NO normally inhibits smooth muscle contraction by activating PKG1α (cGMP-dependent kinase 1α), which activates MLCP to dephosphorylate MLC [39]. Genetic variations in RGS2 have been observed in Japanese subjects with hypertension and normotension, with an insertion/deletion variant in 1891–1892 in the non-coding region of the gene in patients with hypertension [40]. This insertion/deletion variant was confirmed by Riddle et al. [41], although, in their studies, this variant was only present in black patients with hypertension. In addition, as noted in a commentary by Feldman and Gros [42], further results are needed to determine whether there is any physiological role in function as these changes are located within the non-coding region. More recently, vascular microarrays performed from two hypertensive rat models, SHR and ACTH (adrenocorticotropic hormone)-induced rats, showed a decreased expression of both RGS2 and RGS5 [43]. Taken together, these findings suggest that RGSs could be a potential therapeutic target as they can essentially terminate maladaptive signalling through Gq-coupled receptors. Furthermore, if GRK2 is capable of acting as an RGS, it has the potential to have similar effects, although this remains to be determined.

RGS3

Another subfamily of RGS proteins consists of the RGS3 proteins. These RGS proteins possess an extended N-terminal domain, the function of which remains
unknown. There are two versions that are truncated within this N-terminus RGS3S and RGS3L [44,45]. Interestingly, RGS3 and its truncated forms have been shown to attenuate both inositol phosphate and cAMP production following CGRP (calcitonin gene-related peptide) receptor stimulation, suggesting that it can play a role in both Gs and Gq signalling, at least in kidney cells [44]. RGS3 has also been shown to bind G_{i/0} and inhibit its activity, which leads to decreased MAPK (mitogen-activated protein kinase) and PI3K (phosphoinositide 3-kinase) activities [46]. Another isoform of RGS3, PSD95 (postsynaptic density 95), has recently been identified and possesses an N-terminal PDZ domain, which plays a role in the assembly of protein complexes [47,48]. PSD95 has been shown to be in a complex with β_{1}-adrenergic receptors, and GRK5, also an RGS-like protein, can disrupt the physical association of PSD95 and the β_{1}-adrenergic receptor, thus altering receptor function [49] and suggesting another potential mechanism whereby GRK5 may be influencing GPCR signalling in hypertension.

Many of the studies mentioned above have provided interesting results for the potential role of RGS proteins and regulation of GPCRs in hypertension, although limited studies have been performed in VSM. The fact that most of these receptors have the ability to attenuate receptor signalling provides an exciting avenue to potentially ‘turn off’ signalling due to pathological stimuli. However, G_{i}-coupled receptors initiate cAMP formation and vasorelaxation, and attenuating signals through these receptors might be detrimental. Obviously, more studies are needed to elucidate the role of RGS proteins, and the ability of GRKs to act as RGS proteins, in GPCR signalling and hypertension.

**ROLE OF ADENYLATE CYCLASE IN HYPERTENSION**

One of the major findings consistent throughout a multitude of studies on hypertension is impaired vasodilation through G_{i}-coupled receptors (β_{1}-adrenergic and dopamine D_{1}), and studies done by us and others suggest that this is most probably due, at least in part, to desensitization of these dilatory GPCRs by GRKs. As early as 1980, it was shown that inhibition of adrenergic stimulation by adenosine in the mesenteric vascular bed was decreased in SHRs [50]. Impaired relaxation to histamine, a G_{s}-coupled inflammatory mediator, was present in mesenteric arteries of SHRs, although this was attributed to endothelium dysfunction [51]. Ultimately, alterations in G_{i} signalling lead to attenuation of adenylate cyclase activity and decreased cAMP formation, which is critical for VSM relaxation. Studies have shown that isoprenaline-stimulated adenylate cyclase activity was decreased in lymphocytes obtained from subjects with borderline hypertension [52]. Interestingly, this defect was normalized when the patients with hypertension were put on a low-sodium diet, indicating that, at the very least, salt intake may be responsible in the early stages of hypertension [52]. Reductions in adenylate cyclase activity have additionally been shown in rat models of hypertension with various G_{i}-coupled-receptor agonists. Adenylate cyclase activity in both aorta and hearts from SHRs in response to stimulation of numerous by G_{i}-coupled GPCRs (isoprenaline, glucagon and dopamine) was decreased with increased G_{i} levels [53]. These findings demonstrate the possibility of both defective G_{i} signalling and/or enhanced G_{i}, signalling in decreasing adenylate cyclase activity. Many studies showing decreased adenylate cyclase activity have been performed in rat models of hypertension, which are outlined elegantly in a review by Feldman and Gros [8] and will not be discussed further here. Importantly, however, changes in GRKs and the GPCRs they regulate have profound influences on BP, and understanding molecules altered downstream of GRKs is also important in appreciating the full impact of GRKs in BP control.

**G-PROTEINS, PLC AND HYPERTENSION**

In addition to decreases in relaxation elicited through decreased adenylate cyclase activity, it also appears that there is a facilitation of proteins within the pathways leading to vasoconstriction. Activation of G_{i} inhibits adenylate cyclase activity and, interestingly, G_{i} is increased in both the VSM and heart of SHRs compared with control normotensive rats, whereas there is no change in expression of G_{i} in rats or humans [54,55], but an apparent decrease in G_{i} activity which would also attenuate vasodilatation and increase peripheral resistance [55]. PLC activation leads to increased PKC and Ca^{2+}, which ultimately increases MLCK (MLC kinase) through Ca^{2+}-CaM and inhibition of MLCP through CPI-17 (Figure 1). Protein levels of different isoforms of PLC and PLC activity are up-regulated in the vasculature of SHRs [56]. This suggests that the signalling pathways activated by GPCRs that use PLC may be augmented during the development and/or progression of hypertension [56]. What GPCRs are activating G_{i} and PLC and whether their increased expression leads to enhanced signalling in hypertension and the role that GRKs play in the regulation of this signalling needs to be investigated further.

**VSM GPCR SIGNALLING AND ION CHANNELS**

Other molecules downstream of GPCR activation that are important in VSM constriction and play a key role in BP regulation are ion channels. Normal GRK-mediated desensitization of GPCRs helps to maintain the delicate balance of ion channel activation. Although it has not yet been investigated, disruptions in GRK expression and/or
activity during hypertension may potentially affect ion channel activation thus further upsetting the interplay of inputs that establish BP. Intracellular Ca^{2+} dictates the amount and length of smooth muscle contraction, and the opening of L-type Ca^{2+} channels determines the influx of Ca^{2+}. Intracellular Ca^{2+} also activates K^{+} channels inducing K^{+} efflux, causing repolarization of the membrane and subsequent relaxation. Alterations in the expression or activity of these channels can strongly tilt the balance of forces and GPCRs and, therefore, GRKs may have a direct impact on ion channel activity. Consistent with many of the molecular studies on hypertension, defects in relaxation parameters induced by ion channel changes appear to predominate over defects causing increased contraction. Previous studies have shown that SHR s have decreased levels of the β1 subunit of the BK<sub>Ca</sub> channel (large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channel) compared with control rats [57]. Many agonists bind to VSM GPCRs triggering downstream signalling, which results in elevated intracellular Ca<sup>2+</sup> and vasoconstriction through activation of ion channels. AngII, for example, is one of the most potent vasoconstrictors that increases intracellular Ca<sup>2+</sup> by activating IP<sub>3</sub>-sensitive Ca<sup>2+</sup> stores as well as inhibiting the activity of BK<sub>Ca</sub> and Kv (voltage-gated K<sup>+</sup>) channels by PKC activation [58]. Ultimately, this leads to prolonged membrane depolarization and contraction. Chronic AngII infusion led to decreased expression of the Kv2.1 subunit via a calcineurin/NFATc3 (nuclear factor of activated T-cells c3)-dependent pathway [59]. In addition, activation of NFATc3 by AngII infusion led to the down-regulation of the BK<sub>Ca</sub> β1 subunit [60]. In contrast, other studies have found that the BK<sub>Ca</sub> β1 subunit was increased in arteries of SHR s [61], although this could be due to the different tissue types tested (cerebral compared with mesenteric arteries). Although significantly fewer studies have been able to show alterations in Ca<sup>2+</sup> channels, abnormal vascular tone has also been attributed to alterations in the expression of L-type Ca<sup>2+</sup> channels. A study by Rusch and co-workers [62] found that mesenteric and skeletal arteries from SHR s had increased levels of the pore-forming α<sub>1C</sub> subunit of the L-type Ca<sup>2+</sup> channel. Furthermore, they went on to show that increased BP itself was the mechanism behind the up-regulation [63]. These studies indicate that hypertension itself, increased neurohormonal factors associated with it, such as AngII, and potentially GRK regulation of GPCRs can alter the expression levels of critical ion channels.

HYPERTENSION LEADING TO ALTERATIONS IN CARDIAC GRKs IN THE PROGRESSION OF HEART FAILURE

Much of the work regarding GRKs in cardiovascular disease has been performed in cardiac tissues. Increases in GRK levels and activities are associated with heart failure [64,65]. Hypertension is a well-established risk factor for cardiac hypertrophy and failure, although molecular mechanisms underlying the transition to heart failure are not well defined. An attempt to clarify some of the molecular mechanisms underlying the transition from hypertrophy to failure due to hypertension was addressed in a study using SHHF (spontaneously hypertensive heart failure) rats [66]. The purpose of that study was to address differences in the β-adrenergic system with regards to hypertension. Interestingly, although changes in cardiac β-adrenergic responsiveness were not apparent until decreased cardiac function was detected, increased GRK2 expression was detected earlier [66]. These findings suggest that the up-regulation of GRK2 could potentially play a role in the transition of the heart from a hypertrophic stage to failure. Importantly, no changes in GRK3 or GRK5 expression levels were detected, although it does appear that there may be nuclear translocation of GRK5 in failing myocytes from SHHF compared with age-matched control rats [67]. Another model of heart failure is TAC (transverse aortic constriction). This model is used as a way to increase afterload or pressure overload similar to hypertension. Interestingly, very few studies have examined GRK levels following TAC; however, one study found that, 7 days following TAC, cardiac extracts had a 3-fold increase in GRK activity [68]. Another study found that there was also increased GRK2 activity at 8 weeks following TAC [69]. According to the AHA (American Heart Association), approx. 90 % of cases of congestive heart failure are preceded by hypertension. Whether changes in cardiac GRK levels are due to hypertension itself or increased neurohormonal activation associated with hypertension remains to be determined.

ROLE OF GRK-MEDIATED NON-CLASSICAL SIGNALLING IN HYPERTENSION

Previous findings have suggested that the signalling implications of GRK modification of GPCRs are more complex than originally thought [70,71]. Depending on which GRK phosphorylates a GPCR, differential signalling cascades can be activated, potentially having profound implications to the cell and organism [72–75]. Generally, it appears that GRK2 phosphorylation leads to the classically described desensitization, whereas phosphorylation of GRK5/GRK6 leads to the recruitment of arrestin and the activation of non-classical GPCR signalling pathways (Figure 2). In addition, results suggest that whether ERK (extracellular-signal-regulated kinase) is activated by a G-protein or an arrestin (recruited by the GRK) can have compartmentalization implications helping to shuttle ERK to the nucleus or to keep it within the cytoplasm, depending on the cell type and GPCR agonist used [76]. Whether this occurs within the...
There is a decrease in Gq-agonist-infused BP [77]. This Gq-coupled receptors, providing a window of such as catecholamines, AngII and ET-1, activate Many vasoconstrictors up-regulated in hypertension, [78,80]. In the heart, inhibition of Gq signalling decreases amount of neointima formation following vascular injury [79,80]. Figure 2 Classical and non-classical roles of GRK in GPCR signalling Recent findings suggest that, depending on the GRK that phosphorylates a given GPCR, there can be profound signalling consequences within the cell. It appears that GRK2 may lead to the classically appreciated desensitization of GPCR signalling. In contrast, GRK5/GRK6 phosphorylation may facilitate arrestin-mediated receptor tyrosine kinase (RTK) transactivation and subsequent activation of PI3K and ERK, ERK and/or Ca<sup>2+</sup> accumulation in a more non-classically appreciated signalling event. The role of GRKs in classical and non-classical signalling in VSM and hypertension remains to be determined.

**POSSIBLE THERAPEUTIC STRATEGIES TARGETING VSM GPCRs**

GPCRs remain the primary target of pharmaceutical research [3]. Importantly, only one-third of people with hypertension have it under control, which obviates the necessity for novel therapeutic strategies to improve this statistic. Common therapies for the treatment of hypertension include β-adrenergic receptor antagonists, α-adrenergic antagonists and AT<sub>1</sub> receptor antagonists. Many vasoconstrictors up-regulated in hypertension, such as catecholamines, AngII and ET-1, activate G<sub>q</sub>-coupled receptors, providing a window of opportunity for class-specific inhibition of G-protein signalling. Studies have shown that when a 54-amino-acid peptide from the C-terminus of G<sub>q</sub> is targeted to VSM, there is a decrease in G<sub>q</sub>-agonist-infused BP [77]. This peptide was also successful in lowering BP in a renal-derived model of hypertension (two kidney, one clip) and a genetic model (VSM GRK2-overexpressing mice) [78]. Interestingly, other studies using a G<sub>q</sub>-inhibiting compound, YM-254890, showed an attenuation in the amount of neointima formation following vascular injury [79,82]. In the heart, inhibition of G<sub>q</sub> signalling decreases cardiac hypertrophy following pressure overload [81]. These studies provide evidence for the potential role of class-specific inhibition of G<sub>q</sub> signalling in treating vascular injury leading to cardiac hypertrophy, especially when GRK2 expression is increased.

As outlined within the present review, a common aspect of hypertension is impaired relaxation due to alterations in G<sub>q</sub>-coupled signalling. β-Adrenergic receptors play a critical role in the vasodilatory mechanisms of VSM and, therefore, resensitization of this pathway may provide an avenue for potential therapies. GRK2 phosphorylates β-adrenergic receptors essentially diminishing the signalling cascade leading to relaxation and, therefore, development of a GRK2 inhibitor molecule could lead to enhanced β-adrenergic signalling. Koch et al. [82] have developed an inhibitor peptide of GRK2, composed of the last 194 amino acids of the GRK2 C-terminus termed βARKct. They found that cardiac overexpression of βARKct increased cardiac contractility through enhancement of β-adrenergic receptor signalling [82]. This molecule represents a possible target to prevent the uncoupling of β-adrenergic receptor signalling that occurs in VSM; however, caution must be warranted as it must also be noted that GRK2 phosphorylates and desensitizes other receptors, such as the AT<sub>1</sub> receptor, and desensitization of the AT<sub>1</sub> receptor may be beneficial in the development/progression of hypertension.

**CONCLUSIONS**

The dogma regarding hypertension has been that kidney dysfunction is the sole underlying cause; however, more recently, it has been appreciated that VSM also plays an important role in the development of hypertension. Recent studies outlined within the present review illustrate some of the molecular defects in VSM observed in models or subjects with hypertension, including increases in GRK2 and GRK5. Increased GRK activity will desensitize and potentially down-regulate GPCRs. Because blood vessel radius is regulated tightly by a delicate balance of vasoconstrictive and vasodilatory inputs through GPCRs, any alteration within this complex highly regulated signalling cascade can initiate a domino effect and tilt the balance in either direction. A decrease in GPCR signalling can ultimately lead to increased BP most likely, at least in the setting of increased GRK2, via a decrease in dilation mediated through β-adrenergic receptors. Much of the work regarding VSM and hypertension has focused on genetically hypertensive rats. Recent technological advances will hopefully open new doors for investigators to utilize transgenic mouse technology to dissect the contributions of various proteins within specific tissues to hypertension. Elucidating cardiovascular GPCR function, including receptors on endothelial cells, VSM and adventitia, will increase our understanding of
their contributions to hypertension and hopefully offer promising therapeutic strategies in the future.

ACKNOWLEDGMENTS

We are funded by the NHLBI (National Heart, Lung and Blood Institute), the WW Smith Charitable Trust (A.D.E.) and an American Heart Association Predoctoral Fellowship (H.I.C.). In addition, our laboratory is also funded, in part, under a grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

REFERENCES

43 Grayson, T. H., Ohms, S. J., Brickenbury, T. D. et al. (2007) Vascular microcirculation in two models of hypertension identifies Cav-1, Rgs2 and Rgs5 as antihypertensive targets. BMC Genomics 8, 404


Received 11 December 2007/17 January 2008; accepted 13 February 2008
Published on the Internet 2 July 2008, doi:10.1042/CS20070442