Abstract—G protein–coupled receptor kinase 2 (GRK2) is a ubiquitous serine/threonine protein kinase able to phosphorylate and desensitize the active form of several G protein–coupled receptors. Given the lack of selective inhibitors for GRK2, we investigated the effects elicited by GRK2 inhibition in vascular responses using global adult hemizygous mice (GRK2+/−). The vasodilator responses to acetylcholine or isoproterenol were increased in aortas and mesenteric resistance arteries from GRK2+/− mice compared with wild-type (WT) littermates. After angiotensin II (AngII) infusion, GRK2+/− mice were partially protected against hypertension, vascular remodeling, and mechanical alterations, even when resting basal blood pressures were not significantly different. AngII infusion also (1) increased GRK2 levels in WT but not in GRK2+/− vessels; (2) increased vasoconstrictor responses to phenylephrine in WT but not in GRK2+/− mice; and (3) decreased vasodilator responses to acetylcholine and vascular pAkt and eNOS levels more in WT than in GRK2+/− animals. Vascular NO production and the modulation of vasoconstrictor responses by endothelial-derived NO remained enhanced in GRK2+/− mice infused with AngII. Thus, GRK2+/− mice are resistant to the development of vascular remodeling and mechanical alterations, endothelial dysfunction, increased vasoconstrictor responses, and hypertension induced by AngII at least partially through the preservation of NO bioavailability. In conclusion, our results describe an important role for GRK2 in systemic hypertension and further establish that an inhibition of GRK2 could be a beneficial treatment for this condition. 

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Key Words: arteries ■ GRK2 ■ hypertension ■ nitric oxide

Different receptors and signaling molecules are involved in the development of hypertension by hyper-contracting or hypo-dilating blood vessels in a deleterious manner and by affecting the structure and mechanical properties of vessels. Among them, the G protein–coupled receptor (GPCR) family is of outmost importance. Adrenergic receptors and other GPCRs, such as angiotensin II (AngII), endothelin-1 (ET-1), dopamine, and vasopressin receptors, are key for vascular physiopathology.1 AngII is a master regulator of vascular tone, and many animal models of hypertension are based on the chronic elevation of AngII levels. GPCRs become inactivated to different extents when agonist signals are persistent in time, a process termed desensitization. This process is regulated by G protein–coupled receptor kinases (GRKs), a family of serine/threonine kinases able to phosphorylate intracellular domains of the receptors and initiate their uncoupling from the G protein, and thus signal termination.2 Among the 7 GRK isoforms, GRK2 is the most abundant in vessels together with GRK5 and plays a determinant role in the control of systemic vascular responses.3,4 The levels and activity of the GRK2 isoform are increased in animal models of hypertension and in lymphocytes from young patients with hypertension.5 In addition, GRK2 mRNA levels, but not those of GRK3 or GRK5, increase in correlation with systolic blood pressure addition, GRK2 mRNA levels, but not those of GRK3 or GRK5, increase in correlation with systolic blood pressure. Therefore, the increase in vascular GRK2 could have represented a protective mechanism for adaptation against a hypertensive phenotype. However, transgenic mice overexpressing GRK2 in the vascular smooth muscle cells (VSMC) show increased resting blood pressure.8 Moreover, elevated GRK2 levels impair vasodilator β adrenoceptors responses in different tissues and animal
models and, interestingly, in human patients treated with β-blockers GRK2 levels return to baseline.4,10

Classically, the preferential desensitization by GRK2 of vasodilator receptor subtypes versus vasoconstrictor ones has been invoked to explain the effect of upregulated GRK2 levels in human and in murine hypertension.8 However, novel results implicating GRK2 in non–GPCR-dependent pathways prompt to redefine the role of GRK2 in the regulation of vascular tone. One such example is the described interaction of GRK2 with Akt that inhibits Akt-dependent activation of NO synthase, thus impairing NO production.11 Another important emerging question is the relative importance of endothelial GRK2 as compared with VSMC-GRK2 because depletion of GRK2 in VSMC is unable to prevent portal hypertension.9 Our work tries to shed some light on these issues describing for the first time that a global reduction in GRK2 causes resistance to the development of systemic hypertension in adult mice. This antihypertensive effect of GRK2 downregulation prevails even when responses to both vasodilator and vasoconstrictor receptors are enhanced and this is because of the increased NO bioavailability detected in GRK2−/− mice. Although extensive research has been performed on the effects of GRK2 overexpression or deletion in cardiac phenotypes, few studies have addressed how systemic changes in GRK2 levels can affect vascular function and to our knowledge, this is the first report to address the effect of GRK2 in vascular structure and biomechanics.

Methods

The Materials and Methods are described in the online-only Data Supplement.

Results

GRK2 Deficiency Increases Vasoconstrictor Responses Without Influencing Receptor Levels

Partial deficiency of GRK2 increased vasoconstrictor responses to phenylephrine in aortas from male (Figure 1A; Table S1 in the online-only Data Supplement) and female (Figure S1A; Table S1) mice. ET-1 (0.1 μmol/L)–mediated vasoconstrictor responses were also increased in aorta from male GRK2−/− mice compared with WT littermates (Figure S2A; Table S1). After repeated exposure to ET-1, vasoconstrictor responses were reduced in WT and GRK2−/− mice (Figure S2A). However, contractile responses to the second and third ET-1 administration remained larger in GRK2−/− compared with WT mice (Figure S2A), suggesting that partial deficiency in GRK2 prevents, in part, ET-1–induced desensitization. GRK2 deficiency did not modify AngII (1 μmol/L)–induced vasoconstrictor responses (Figure S2B; Table S1) or changed the reduced vascular responses after repeated exposure to AngII (Figure S2B).

GRK2−/− mice displayed decreased vascular GRK2 gene and protein expression (Figures S2C and S3), but no differences in protein or gene expression of AT1, AT2, ETA, ETB, or α1D receptors were observed between WT and GRK2−/− mice in aortas, and a reduction of only AT2 receptor protein was observed in mesenteric resistance arteries (MRA) from GRK2−/− mice (Figures S2C and S3).

GRK2 Deficiency in Adult Mice Increases Endothelium-Dependent Vasodilator Responses and NO Release

The endothelium-dependent vasodilator responses induced by acetylcholine (ACh) or isoproterenol were increased in aorta from male and female GRK2−/− mice compared with WT mice (Figures 1B and 1C; Figure S1B and S1C). Accordingly, ACh-induced NO production was increased in aortas from GRK2−/− animals (Figures 1D; Figure S1D). In contrast, the endothelium-independent vasodilator responses induced by the NO donor diethylamine (DEA)-NO were similar in GRK2−/− and WT mice (Figures 1E; Figure S1E), suggesting that the observed differences are probably due to altered endothelium-mediated NO production. Similar results were observed in MRA from male and female mice (Figure S4A–S4F). ACh-induced NO production was also larger in MRA from male GRK2−/− mice (Figure S4G).

GRK2 Deficiency Reduces the Development of Hypertension and Prevents Vascular Remodeling After AngII Infusion

AngII infusion increased GRK2 levels in WT but not in GRK2−/− aortas (Figure 2A). Basal systolic blood pressure
In aorta, lumen and vessel diameters were similar in WT and GRK2+/− mice irrespective of AngII infusion (data not shown). However, media thickness (Figure 3D) and media/lumen ratio (Figure 3E) increased after AngII only in WT and not in GRK2+/− mice, suggesting that partial deletion of GRK2 protects against AngII-induced vascular remodeling.

**GRK2 Deficiency Improves Vascular Function and NO Signaling After AngII Infusion**

AngII treatment increased vasoconstrictor responses to phenylephrine in WT mice, as described, but not in GRK2+/− mice (Figure 4A and 4B). Because AngII infusion decreases NO availability in aorta, the lack of effect of AngII on phenylephrine responses in GRK2+/− arteries might be related to a lesser decrease in NO. As shown in Figure 4C and 4D, the NOS inhibitor N-nitro-l-arginine methyl ester (l-NAME; 100 µmol/L) enhanced phenylephrine contraction in GRK2+/− aortas more than in WT vessels after AngII (dAUC WT, 59±7; GRK2+/−, 237±31; P<0.05), suggesting that NO bioavailability after AngII was better preserved in GRK2+/− aortas. Interestingly, endothelium removal also increased phenylephrine contraction more in aorta from AngII-infused GRK2+/− than WT mice (Figure S9; dAUC WT, 149±17; GRK2+/−, 242±29; P<0.05), suggesting that in GRK2+/− mice there is an important contribution of the endothelium-derived vasodilator mediators, probably NO, on vascular contractile responses.

The endothelium-dependent vasodilator responses induced by ACh (Figure S10A and S10B) but not those triggered by the endothelium-independent vasodilator DEA-NO (Figure S10C and S10D) were reduced after AngII infusion. However, this deleterious effect of AngII infusion on endothelial function was less pronounced in GRK2+/− mice (% inhibition of maximal response induced by AngII; WT, 41.8±10; GRK2+/−, 8.15±3; P<0.05). In agreement, aortic NO production induced by ACh (10 µmol/L) was greater in GRK2+/− than in WT mice infused with AngII (Figure 4E).

To determine whether the increase in NO bioavailability observed in aorta from GRK2+/− mice is a result of alterations of the eNOS-Akt pathway, we measured the activation of Akt (one of the most important upstream activators of eNOS) and eNOS levels. AngII infusion decreased Akt phosphorylation and eNOS protein expression in aortic homogenates from WT mice (Figure 4F and 4G). In contrast, we did not detect any statistically significant decrease in pAkt levels after AngII infusion in GRK2+/− mice and the reduction on eNOS expression was lower than that observed in WT mice (Figure 4F and 4G).

**Discussion**

Vascular responses of adult GRK2+/− mice were characterized as a surrogate model of the effects exerted by a long-awaited pharmacological GRK2 inhibitor. GRK2+/− mice show enhanced vasodilator and vasoconstrictor responses, with only particular differences toward certain contractile agonists. However, these mice are resistant to AngII-induced systemic hypertension, vascular remodeling, and
Partial GRK2 deficiency is not enough to overcome the overt hypertensive phenotype completely achieved by chronic AngII infusion. However, results in diabetic and ob/ob mice showed a full reversal of these types of mild hypertension by the use of a nonselective GRK2 inhibitor. This discrepancy might be explained by differences in the hypertensive models, the use of a wide spectrum GRK2 inhibitor as opposed to a reduction in of GRK2 levels in GRK2−/− mice, and by the lower blood pressure implicit to the diabetes mellitus/obesity study. Also, our mice have been aged until an adult stage (9 months), a period more related to the human clinical setting of hypertension occurrence.

An apparent discrepancy exists between the fact that upregulation of GRK2 in mice models or hypertensive subjects causes elevated blood pressure and results establishing that increased GRK2 protein impairs vasoconstrictor signals. In fact, when GRK2 levels are decreased, desensitization of certain GPCRs is impaired in vascular beds, but, in turn, GRK2 could be regulating vasodilator preferentially over vasoconstrictor receptors. We observed that changes in vasoconstrictor responses induced by GRK2 deficiency depend on the agonist studied, whereas vasodilator ones are always increased in both sexes and in both conductance and resistance arteries.

![Figure 3](image-url)

Figure 3. Partial G protein–coupled receptor kinase 2 (GRK2) deficiency protects against angiotensin II (AngII)–induced vascular remodeling and stiffness. Wall/lumen ratio (A and B) and stress–strain relationship (C) in mesenteric resistance arteries from untreated and AngII-infused wild-type (WT) and GRK2−/− mice. Media thickness (D) and media/lumen ratio (E) in aorta from WT and GRK2−/− mice untreated or treated with AngII. Representative photographs of hematoxylin-eosin aortic sections are shown. Data represent mean±SEM. n=5 to 11. *P<0.05, **P<0.01 vs untreated animals. +P<0.05 vs WT in the presence of AngII.)
GPCR-derived routes could be playing a role. For instance, GRK2 was described to interact with Akt, to impair eNOS activation, and to decrease NO bioavailability and GRK2 silencing reducing renal portal hypertension by increasing the Akt-NOS route, and also a role for GRK2-mediated regulation of NOS in maintaining vascular responses, and during diabetic- or obesity-triggered changes in blood pressure has been reported. Our results represent the first demonstration that NO bioavailability is key to explain the antihypertensive phenotype derived from GRK2 down-regulation because NO production is increased basally in GRK2+/− aortas and mesenteric arteries, and adult GRK2+/− mice are capable of attenuating the AngII-induced drop in NO production much more efficiently than WT littermates. Accordingly, endothelium removal or l-NAME incubation enhanced phenylephrine contraction more in AngII-infused GRK2+/− than in WT aortas. Also, GRK2+/− mice are partially protected from AngII-induced vascular stiffness and remodeling, important determinants of high blood pressure, both in the resistance and in the conductance vasculature. Of note, an increase in GRK2 protein levels in hypertensive WT but not GRK2+/− vessels is observed. This could be ascribed to GRK2+/− aortas and mesenteric arteries, and adult GRK2+/− mice are capable of attenuating the AngII-induced drop in NO production much more efficiently than WT littermates.

Figure 4. Partial G protein–coupled receptor kinase 2 (GRK2) deficiency protects against angiotensin II (AngII)–induced increased vasoconstriction by increasing endothelial-derived NO production. Effect of AngII infusion on the concentration–response curve to phenylephrine (Phe) in wild-type (WT) and GRK2+/− aortic segments (A and B). Effect of l-NAME (C and D) on the concentration–response curve to Phe in aortic segments from WT and GRK2+/− treated with AngII. E. Quantification of acetylcholine (ACh)-induced NO release in aortic segments from AngII-treated mice. Densitometric analysis and representative blots of phosphoAkt (pAkt) and Akt (F) and eNOS and GAPDH (G) in WT and GRK2+/− aortas from mice untreated or treated with AngII. Results are expressed as ratio of phosphoAkt to total Akt or between eNOS to GAPDH and normalized to values obtained for untreated WT mice. Data represent means±SEM. n=4 to 13. *P<0.05, **P<0.01, ***P<0.001 vs the corresponding control. +P<0.05 vs WT infused with AngII.
the lower hypertension detected in GRK2+/− animals and supports a pathological role of elevated vascular GRK2 levels in the hypertensive phenotype.

An increased basal blood pressure is found in mice over-expressing GRK2 in VSMC, whereas mice deficient in muscular GRK2 show a lack of resistance to renal-induced hypertension. Thus, the restoration of vasodilation achieved by VSMC-GRK2 targeting may not be sufficient to overcome the blunted relaxation responses elicited by endothelial cells. Moreover, Cohn et al.9 described no changes in vasoconstrictor AngII-induced vascular responses in endothelial-deprived vessels or in AngII-induced acute increases in blood pressure in VSMC-GRK2−/− mice. In contrast, our results using global GRK2+/− mice demonstrate that these animals are partially resistant to AngII-induced hypertension. Comparison of these results clearly establishes that to achieve a therapeutic effect, endothelial GRK2 should be targeted, and that the control of NO bioavailability by GRK2 is crucial. GRK2 directly binds and inhibits Akt, and we detect a maintenance of Akt activation after AngII treatment only in GRK2+/− mice, whereas WT animals efficiently inhibit this route. This preserved activation of Akt is also detected in other tissues in GRK2 homozygous mice during aging-induced, high fat diet-induced, or mals efficiently inhibit this route. This preserved activation of Akt, together with the lesser deterioration of vascular structure and mechanics, explains why GRK2+/− animals are partially resistant to AngII-induced hypertension. Our results provide novel evidences suggesting that a global decrease could become an efficient treatment for hypertension, associated with blood pressure in black Americans. Hypertension. 2009;54:71–76.

In conclusion, a partial deficiency of GRK2 differentially alters vasoconstrictor responses to different agonists. Nevertheless, vasodilator responses seem to be homogeneously increased in GRK2+/− arteries what probably explains the lack of changes in basal blood pressure observed in GRK2−/− mice. In addition, vascular structure and mechanics are similar in both genotypes. However, after an AngII challenge, lower GRK2 levels help to maintain the activation of eNOS through the Akt pathway, thereby leading to preserved NO bioavailability. In this situation, NO-dependent vasodilation overcomes constriction which, together with the lesser deterioration of vascular structure and mechanics, explains why GRK2−/− mice are resistant to hypertension (Figure S11) and further highlights GRK2 as a therapeutic target for hypertension.

Perspectives

Our results provide novel evidences suggesting that a global GRK2 decrease could become an efficient treatment for hypertension and further highlight the importance of targeting endothelial GRK2 for an effective control of this condition, thus establishing that GRK2 targeting needs to include the endothelial compartment if reversal of hypertension is to be achieved.

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Disclosures

None.

References

What Is New?

- Partial deficiency in G protein–coupled receptor kinase 2 (GRK2) affects vasoconstrictor responses and desensitization in a differential manner depending on the agonist studied without apparent changes in receptor levels. However, endothelium-dependent vasodilator responses are homogeneously increased in adult GRK2+/− conductance and resistance arteries via an increased NO availability.
- After AngII challenge, the lower GRK2 levels in adult GRK2+/− mice maintain the activation of eNOS by preserving phosphorylation of Akt. This better maintains NO bioavailability and protects vascular function in GRK2+/− mice. These facts, together with a less deteriorated vascular structure and mechanics after AngII challenge in GRK2+/− mice, might explain their resistance to the development of hypertension.
- This is the first report to characterize the effect of a systemic reduction in GRK2 levels on vascular structure and biomechanics, both in basal and in AngII-treated adult mice.

What Is Relevant?

- Our results provide new evidences for a novel therapeutic effect of lowering GRK2 levels/activity through the modulation of vascular function and NO bioavailability.

Summary

Partial deficiency of GRK2 differentially alters vasoconstrictor responses to different agonists, whereas vasodilator responses are homogeneously increased. After an AngII challenge, GRK2+/− mice maintain endothelial function, exhibit diminished vasoconstrictor responses, and display improved vascular structure and vessel stiffness compared with age-matched wild-type littermates. Moreover, GRK2+/− mice display an impaired AngII-induced decline in both the activation of the Akt-eNOS route and in total levels of the eNOS protein, thus leading to a preserved NO availability and a resistance to the development of hypertension.